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Volume 528
ADAMANTIADES-BEHÇET'S DISEASE
Edited by Christos C. Zouboulis

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Edited by

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Dedication

This book is dedicated to Gundula, Konstantinos, and Viktor-Alexandros for their patience and enormous support.

Foreword

It is with great pleasure that I write this Foreword to the Proceedings of the 10th International Conference on Behçet's Disease which was held in Berlin in June 2002. This was the first International Conference held under the auspices of the International Society for Behçet's Disease which was founded in 2000 in Seoul.

First, I congratulate our colleagues in Berlin, led by Professor Christos Zouboulis of the Department of Dermatology at the Free University of Berlin, for having organised a most successful conference and for having compiled these proceedings so rapidly. It will be realised immediately on scanning the contents of this book that the conference was truly international with 210 participants from 26 countries, as Professor Zouboulis has noted in his preface. These included basic scientists, epidemiologists, pathologists, clinicians and, importantly, representatives from patient organisations. The latter held their own conference alongside the scientific-medical conference to mutual benefit. The combined session of patients and doctors (abstracts on pp 601 – 626) gave the opportunity for an exchange of information and fruitful discussion.

The wide ranging scope of the communications is evident from the index and it was most encouraging to see their origin – from all parts of the world, from senior and junior colleagues and, from many different disciplines. Many communications may be regarded as preliminary reports of research in progress and we look forward to seeing the definitive publications in appropriate journals in due course.

The programme of the conference proved to be very busy arising from the large number of abstracts received. The latter indicates the progressive development of these conferences, and the increasing interest and research into this condition. This inevitably led to a discussion about the format of future conferences; to schedule more time for open discussion, specialised workshops, and symposia.

The historical background of the description of the disease was again reviewed. As with so many eponymously named diseases one can debate who was the first to describe it and thus whether the eponym is appropriate. As Zouboulis and Keitel note in their historical review (pp 7 – 14), Hippocrates described the syndrome in the 5th century BC and many other physicians from both eastern and western countries described aspects of the syndrome in the first half of the 20th century. Also the debate whether this should be regarded as a disease or a syndrome continues. The most widely recognised name, thus used by the majority of physicians and scientists, is Behçet's Disease; the use of the eponym Adamantiades represents a regional variation which might be confusing. Only continued research will lead to a resolution of this confusion of nomenclature – the identification of the cause, whether this is a single disease with variable manifestations or a syndrome, an agreed name based on aetiology and pathology, etc.

The description of the manifestations of Behçet's Disease and its epidemiology continue to appear from various countries. There are also important and encouraging accounts of therapeutic advances. However, the way ahead seems to depend on:

- an agreed definition of the disease and Diagnostic Classification for research (not routine clinical) purposes,
- an agreed method of assessing and quantifying activity of disease, and
- multicentre / multinational studies including basic laboratory research and masked therapeutic trials.

It is planned that the International Society for Behçet's Disease will progressively facilitate such a programme and be the catalyst for increased international co-operation.

Dr. Colin G. Barnes

President
International Society for Behçet's Disease

Preface

Dear colleagues and friends,

It is a great pleasure to present the Proceedings of the 10th International Conference on Behçet's Disease, held June 27-29, 2002 in Berlin. We have been proud to host this outstanding event, which was the first thematic conference to be organized under the auspices of the young International Society for Behçet's Disease.

We have included 129 manuscripts which reflect the high quality of the 200 communications presented in the conference and the great amount of knowledge gained through hard scientific work over the years. For the first time, all abstracts submitted to the conference have been peer-reviewed in an anonymized way to improve the quality of the Conference. In addition, selected invited lectures have been presented by experts of world-wide reputation who have never been involved before in Adamantiades-Behçet's disease but may serve as nuclei of vivid, valuable collaborations in the future.

The book is put together in a manner to present the state-of-the-art in historical perspectives, epidemiology, diagnostic criteria, prognostic parameters, methods for assessment of disease activity and quality of life, clinical investigation, etiopathology including the genetics and immunology of the disease, basic research, therapeutics, and physician-to-patient relations. Our attention in the collection of manuscripts was enhanced by the knowledge that this book may become the only source of information on this rare disease in the next years in a number of countries.

Another innovation was the organisation of the 2nd International Convention for Patients with the Silk Road Disease (Behçet's Disease) at the same time and place with the 10th International Conference on Behçet's Disease. The manuscripts of a common patients / physicians session have been included in our book. The intensive exchange among expert physicians and patient representatives on scientific and personal levels which took place during the conferences may become of great advantage for our patients and their families.

We have been honoured to host 210 participants from 26 countries around the globe, from Austria, Chile, Denmark, Egypt, France, Germany, Greece, Iran, Iraq, Israel, Italy, Japan, Jordan, Korea, Morocco, The Netherlands, Portugal, Rumania, Russia, South Africa, Switzerland, Syria, Tunesia, Turkey, UK, and the USA. One hundred sixty six individuals came to Berlin from abroad. This publication appeals not only to all these wonderful people but also to all those professionals including clinicians, scientists, patients' representatives and representatives from the pharmaceutical industry who have not been able to come to Berlin. We hope that it will be highly informative for newcomers but also for those already involved providing a comprehensive survey of clinical subjects and research on this multisystemic rare disorder.

We entitled the book "Adamantiades-Behçet's Disease" to honour both pioneers, Benediktos Adamantiades and Hulûsi Behçet, who had studied patients with the disease in the first half of the 20^{th} century and have published their data with the conviction of describing signs of a new disorder.

Hoping that you will find "Adamantiades-Behçet's Disease" informative, interesting, and stimulating, I wish you a pleasant reading.

January 2003

Prof. Dr. med. Christos C. Zouboulis

President
The 10^{th} International Conference on Behçet's Disease

Acknowledgments

I take the opportunity to acknowledge the invaluable help and contribution of the members of the Executive Committee of the International Society for Behçet's Disease, in particular Dr. Colin G. Barnes and Prof. Hasan Yazici, the International and National Scientific Committees, the Programme Evaluation Committees, and the Young Investigator Awards and Poster Prize Selection Committees.

I am extremely pleased to recognize the charity Deutsches Register Morbus Adamantiades-Behçet e.V., the Deutsche Forschungsgemeinschaft (DFG), the Investitionsbank Berlin (IBB), the Berliner Stiftung für Dermatologie (BSD), and 3M Medica for their financial support towards the organization of the 10th International Conference on Behçet's Disease.

I also acknowledge the donations of the pharmaceutical companies Galderma, Pfizer, Hermal, and Janssen-Cilag to the Deutsches Register Morbus Adamantiades-Behçet e.V. which facilitated the work of the charity and the publication of this book.

I express my sincere thanks to all my co-workers in Berlin, and especially to the members of the Local Organizing Committee Prof. Dr. Beate Tebbe, Dr. Regina Treudler, Dr. Lothar Krause, Dr. Anett Boschnakow, and Dr. Julia R. Turnbull. I also wish to cordially thank Prof. Dr. Constantin E. Orfanos, Director of the Department of Dermatology, for his support.

The project "Adamantiades-Behçet's Disease" was realized with the help of Mr. André Gronau at the Congress Secretariat, Mrs. Antonia Akmann being responsible for linguistic revision of the manuscripts, and Mr. Jens Fischer and Mr. Theodosios Alestas who brought the manuscripts in their final form.

We express our sincere thanks to Kluwer/Plenum Publishers and Ms. Joanna Lawrence for publishing the final result under most favourable conditions.

Last but not least, all our thanks belong to our international manuscript contributors. We hope that this book will satisfy their efforts.

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Initial Contributions of Adamantiades and Behçet

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1. INTRODUCTION

The translation of the original works by Adamantiades and Behçet are provided to those attending the 10th International Conference on Behçet's Disease, so that the power and insight of these scientists, through their writings, may be available to all. This task is done with the appreciation of physicians and investigators today, as well as the thanks of patients with Morbus Behçet, for if it were not for the energy and skills of these two pioneering physicians, precious time would have been lost in the battle against the disease.

2. BENEDIKTOS ADAMANTIADES

Benediktos Adamantiades was born in Prousa, Asia Minor (now Turkey), in 1875. He was raised by an uncle, the Bishop of Thrace. He graduated with distinction from secondary school at the "Illustrious School of the Generation" in Istanbul. He received his medical degree at the University of Athens. He studied ophthalmology in Paris at Hotel Dieu and Quinze Vignts. In his practice in Athens, he was a tireless investigator, writing over 150 articles, making several first observations.

Adamantiades presented "On a case of relapsing iritis with hypopyon" at the meeting of the Medical Association of Athens in November 1930. This case was published that year in the Annals of the Medical Society of Athens and in 1931 in *Annales d'Oculistique.* His patient, a 20 year-old male, also

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had findings of oral and genital aphthosis, pyoderma, and arthritis. Adamantiades' work continues to be recognized by many current investigators through the faithful use of the dual eponym "Adamantiades-Behçet Disease." In addition to his initial observations, Adamantiades is credited with the recognition of retinal and peripheral thrombophlebitis as part of this disorder. He presented the first classification of the disease and diagnostic criteria; he recognized the poorer prognosis in males.

3. HULÛSI BEHÇET

Hulûsi Behçet was born in Istanbul in 1889. He was trained in medicine and dermatology at the Gülhane Military Academy and later received additional training in Budapest and Berlin. Behçet spent most of his career at the Guraba Hospital in Istanbul, which became part of the University of Istanbul in 1933. There he established the Department of Dermatology and Venerology where he remained until his death in 1948. He was recognized as an expert in a number of diseases, including syphilis, leishmaniasis, parasitic diseases, and fungal disorders. He frequently presented his work at international meetings and was a prolific writer, with 196 medical articles to his credit.

In spite of earlier publications, it was the report of Hulûsi Behçet in 1937 that sparked the excitement and widespread interest in a new syndrome. Behçet published 6 papers on the disorder between 1937 and 1940 that included 7 patients and early efforts to identify a cause. The proliferation of his ideas in reports in four languages and the rapid recruitment of new cases by other writers was responsible for the attention received. In 1941 the ophthalmologist Jensen reported a case and was the first to refer to this condition as "Syndrome Behçet". In 1947, at the International Congress of Dermatology held in Geneva, the trisymptom complex was recognized as a distinct disorder and officially eponymized as "Morbus Behçet" at the suggestion of professor Mischner of the University of Zürich.

Behçet possessed the necessary powers of observation, together with scientific curiosity and fortitude to recognize a disease affecting different organ systems over a long interval. His observations on the triple symptom complex began with a patient in the mid 1920s whom he followed for more than 20 years. A second patient with similar symptoms came to his attention in the early 1930s. Behçet recognized a third patient in 1937, reported in the following year. His belief that these patients may have a new disease was first presented at a meeting of the Association of Dermatological and Venereal Diseases in Istanbul in 1936 and published that same year in the Turkish Archives of Dermatological and Venereal Diseases. In 1937, his

most famous work was published in the international journal *Dermatologische Wochenschrift* and focuses on the description of his first two patients, one male and one female.

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A Historical Review of Adamantiades-Behçet's Disease

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1. BENEDIKTOS ADAMANTIADES

During the annual meeting of the Medical Society of Athens on November 15, 1930 Benediktos Adamantiades (1875-1962) (Fig. 1), Greek ophthalmologist from Prussa, Asia minor (nowadays Bursa, Turkey)[1-3], presented in a lecture with the title *"A case of relapsing iritis with hypopyon",* a 20-year-old male patient with the three cardinal signs of the disease. The disease had begun at the age of 18 with edema and ulcerations at the left leg diagnosed as thrombophlebitis. During the following 2 years (1928-1930) the patient was developing recurrent iritis with hypopyon in both eyes which led to blindness and atrophy of the optic nerve, scrotal ulcers healing with scars, oral aphthous ulcers, and a sterile arthritis of both knees. The latter three signs were recurrent. Bacterial cultures of knee and anterior eye chamber punctures were sterile and the inoculation experiments in animals were negative while staphylococci had grown in cultures from scrotal ulcers and a tonsilar abscess. In the same year, the lecture was published in the Proceedings of the Medical Society of Athens[4] (Fig. 2) and in the French journal *Annales d'Oculistique* a year later[5]. Adamantiades put the genital ulcers, the arthritis and the ocular signs together as signs of one single disease. He referred to important previous publications[6,7] describing similar cases and backed the hypothesis of a bacterial, staphylococcal, focal illness which had been initiated by Gilbert[8].

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Figure 1. Benediktos Adamantiades (1875-1962)

Η ΕΝ ΑΘΗΝΑΙΣ

ΙΑΤΡΙΚΗ ΕΤΑΙΡΕΙΑ

ΠΡΑΚΤΙΚΑ

ΤΩΝ ΣΥΝΕΔΡΙΩΝ ΤΟΥ ΕΤΟΥΣ 1930

593

Ἀφθησαῦ μικροοργανισμός, ἐὰν ἐγένοντο μικροβιολογικαὶ ἐξετάσεις εἰς τὸν ἐξουσθέντα ὀφθαλμόν.

Β. ΑΔΑΜΑΝΤΙΑΔΗΣ. Ἤθελον νὰ παρατηρήσω πρὸς τὸν Κον Συνάδελφον ὅτι αἱ νόσοι τὰς ὁποίας ἀνεφέρει ὁ Gilbert ὡς αἰτιολογικὰς δὲν διαφοροῦν εἶμαι τῆς ἰρίτιδος, ἀλλὰ μόνον τὴν ἐν λόγῳ ἰρίτιδα μετ' ὀλιγωρίαν ἑκατονταετηρίδος ἤτις ἀποτελεῖ ἴδιον πλέον κλινικὴν ὀντότητα.

ΕΝ ΑΘΗΝΑΙΣ
ΤΥΠΟΓΡΑΦΕΙΟΝ Σ. Κ. ΒΛΑΣΤΟΥ
1930

Figure 2. Original text of B. Adamantiades at the meeting of the Medical Society of Athens on November 15, 1930 proving his belief that his reported case constituted a new clinical entity.

In 1946, Adamantiades reported on 2 farther patients and defined thrombophlebitis as 4th cardinal sign of the disease[9]. Later on, he presented the first classification of the disease by describing the ocular, mucocutaneous, and systemic forms in a review work[10]. He pointed out that the disease could occur for years as a monosymptomatic or oligosymptomatic disorder and that eye involvement and severe prognosis were more common in men than in women. In this work he also proposed the first diagnostic criteria. In 1958, Adamantiades published his last work on the neurological complications of the disease[11].

2. HULÛSI BEHÇET

Hulûsi Behçet (1889-1948) (Fig. 3) was the first director of the Department of Dermatology and Syphilology of the medical faculty of the University of Istanbul, which was established in 1933. He was named "Professor" in 1939[12,13]. Between 1918-19 he specialized in dermatology in Budapest (Prof. Sellei) and Berlin (Prof. Arndt). Together with Prof. Braun, Director of the Institute of Microbiology, University of Istanbul, he presented a 34-year-old female patient suffering from recurrent oral aphthous ulcers, genital ulcers, and ocular lesions for 7 years at the meeting of the Dermatological Association of Istanbul on May 11, 1937. This patient together with a 40-year-old man with a disease history of over 20 years was published by Behçet in the same year[14]. The microscopic Giemsa preparation from an oral ulcer of the first patient showed structures whose size corresponded to smallpox elementary bodies and, therefore, Behçet initiated the hypothesis of the viral etiology of the syndrome[15]. During the following 3 years he published on 5 further patients in different languages[15-19]. In these publications he added periodontitis, jaw cysts, acneiform skin lesions, erythema nodosum, and arthralgia to the so-called "triple symptom complex". He was convinced of the autonomy of this multi-symptomatic illness as well as of its viral etiology, finally drawing the attention of the scientific community to this puzzling disease.

Figure 3. Hulûsi Behçet (1889-1948)

3. FIRST DESCRIPTION AND OTHER OBSERVATIONS

The first description of the disease is neither by Adamantiades nor by Behçet. Hippokrates of Kos (460-377 B.C.) described an illness whose manifestations resembled very well the cardinal signs of Adamantiades-Behçet's disease. Already in the 5th century before Christ in his 3rd "Epidemion" book, case 7 (Fig. 4) he stated: *"But there were also other fevers, as it will be described. Many had their mouths affected with aphthous ulcerations. There were also many defluxions about the genital parts, and ulcerations, boils (phymata), externally and internally about the groins. Watery ophthalmies of a chronic character, with pains; fungous excrescences of the eyelids, externally and internally, called fici, which destroyed the sight of many persons. There were fungous growths, in many other instances, on ulcers, especially on those seated on the genital organs. (Many carbuncles grew in the summer as well as other lesions, which were septic, large ecthymata and many large herpetic lesions)"*[20]. It was the ophthalmologist A. Feigenbaum who paid attention to the context of the text quoted above in correspondence with Adamantiades-Behçet's disease (ABD)[21].

Ἦσαν δὲ καὶ ἄλλοι πυρετοί, περὶ ὧν γε-
γράψεται. στόματα πολλοῖσιν ἀφθώδεα, ἑλκώδεα.
ῥεύματα περὶ αἰδοῖα πολλά, ἑλκώματα, φύματα
ἔξωθεν, ἔσωθεν· τὰ περὶ βουβῶνας. ὀφθαλμίαι
ὑγραί, μακροχρόνιοι μετὰ πόνων. ἐπιφύσιες βλεφί-
ρων ἔξωθεν, ἔσωθεν, πολλῶν φθείροντα τὰς ὄψιας,
ἃ σῦκα ἐπονομάζουσιν. ἐφύετο δὲ καὶ ἐπὶ τῶν
ἄλλων ἑλκέων πολλὰ καὶ ἐν αἰδοίοισιν. ἄνθρακες
πολλοὶ κατὰ θέρος καὶ ἄλλα, ἃ σὴψ καλεῖται.
ἐκθύματα μεγάλα. ἕρπητες πολλοῖσι μεγάλοι.

Figure 4. Original description of the disease by Hippokrates of Kos (text written in Greek, Epidimion book III, case 7)

Already since the 18th century there are reports in international literature describing patients with a symptom complex that resembles ABD. In the historic review presented by Zouboulis and Keitel[22] only those case reports or individual cases from larger publications were included which described an illness that can be considered as ABD due to the close similarity of the clinical picture or the obligatory relapsing character of the signs. All 17 patients who have been reported in 10 publications by ophthalmologists presented ocular lesions. Seven of them (41%) presented oral aphthous and genital ulcers while in 12 publications by dermatologists 24 out of 25 patients presented oral aphthous and genital ulcers (96%) but only 3 patients had ocular lesions (12%; $p<0.001$, chi square test with Yates correction). It is interesting that distinct phenotypes had been reported on male and female patients at that time. While on all male patients ocular lesions (15/15, 100%) were reported, only 5 of the 27 female patients had developed ocular involvement (18.5%, $p<0.001$, chi square test with Yates correction). On the other hand, in female patients oral aphthous and genital ulcers (25/27, 93%) were more common than in male ones (6/15, 40%, $p<0.001$). Cutaneous lesions (male 60%, female 63%), mostly erythema nodosum, and arthritis (male 47%, female 15%) were almost equally found in both groups. Indeed, among the 17 patients who had been reported by ophthalmologists were 14 males and 3 females whereas out of 25 patients being presented by dermatologists one was male and 24 female. This finding may explain why ABD was recognized so late as a separate disorder. Ophthalmologists of that time who had mostly followed-up male patients with ocular lesions initially described the disease as "iritis with hypopyon" whereas dermatologists who had followed-üp female patients with bipolar mucosal aphthous ulcers used the term "aphthosis". In addition to the term "triple symptom complex" used by Behçet[18], Dascalopoulos proposed the term "Uveitis recidivans aphthosa"[23]. Between 1940 and 1950, several cases from different countries

were described for the first time[22]. The Parisian dermatologist A. Touraine described 274 patients with not-infectious oral aphthosis – among them also patients with recurrent iritis with hypopyon -[24] in 1941. For the latter cases, Touraine used the term "aphtose généralisée" or "grande aphtose".

Behçet was aware of the publication of Adamantiades and included it in his references. In 1941, Jensen from Denmark, being unaware of Adamantiades' merit, introduced the term "Behçet's syndrome" to describe a patient with the triple symptom complex and ulcerous haemorrhagic colitis and established the pathergy test as a diagnostic criterium[25,26]. In 1944, Berlin and Ephraim simultaneously described the disorder in necropsy material of a patient from Tel Aviv[27] and in a patient from Haifa[28], both referring to the important work of Jensen and mentioning the name of Behçet in the titles of their publications. The term "Behçet's disease" was first used in 1946 by Feigenbaum und Kornblüth who considered it a manifestation of a chronic septic condition connected with a constitutional disorder in their description of 4 additional patients from Jerusalem[29]. Ollendorff Curth, former vice chair of the Department of Dermatology, Virchow Hospital in Berlin (Prof. Buschke), and immigrant to the United States knew the work of Behçet since she had met Behçet in person in Berlin. Her two publications which reported on two American patients[30,31] finally made the term "Behçet's syndrome" popular. By reading the first publication of Ollendorff Curth[30] it becomes obvious that she had gone through the publications of Jensen, Berlin and Ephraim (which were written in English) but did not have detailed knowledge of Adamantiades' work (written in French) although she included him in her references. Later on, many authors who got access to the work of both authors added the name of Adamantiades to the one of Behçet[22].

4. CONCLUSION

The term *Adamantiades-Behçet's disease* honors both scientists who first described – in modern times - several manifestations constituting an autonomous disease and is therefore advisable to be used for naming this disorder.

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EPIDEMIOLOGY

Multicentre Clinical Registries

Establishment of multicentre clinical registries as a basis for comparative evaluation of rare diseases

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1. THE NEED FOR MULTICENTRE CLINICAL REGISTRIES IN RARE DISEASES

The study of diseases, whether in the framework of controlled clinical trials or observational clinical studies faces the problem of heterogeneity of subjects. Heterogeneity usually is tackled by the application of statistical methods which lead to general results valid for populations but not for single individuals. In statistical models, the true heterogeneity is viewed as a random variation produced by a stochastic mechanism. This approach has been proven successful in many medical applications concerned with frequent diseases in the second half of the twentieth century.

However, apart from systematic errors, for rare diseases, the statistical error produced by random variation of results may be considerable. In the following tableau, we demonstrate the statistical precision of an observed proportion (e.g. a specific finding among a group of patients) depending on the number of subjects in the study. Results are based on the statistical convention that erroneous inferences from samples to populations should be limited to maximal one of 20 studies.

We present results for observed proportions of 50% and observed proportions of 10%. Due to the mathematical symmetry, proportions of 90% lead to equivalent results to those of 10%, as one can simply exchange the labels of "finding" and "absence of finding" (Table 1).

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Table 1. 95% Confidence Limits for proportions

Number of subjects	10	20	50	100	400	10000
			Observed proportion: 50%			
Upper bound [%]	82	73	65	60	55	51
Lower bound [%]	18	27	35	40	45	49
			Observed proportion: 10%			
Upper bound [%]	45	32	22	18	14	10.5
Lower bound [%]	<1	1	3	5	7	9.5

From this table it can be clearly seen that in most clinical questions, sample sizes below 100 will not allow valid conclusions. For specific problems, especially in the multifactorial setting, even sample sizes of thousands of patients will be necessary to obtain sufficient evidence.

Thus, the need of multicentre approaches for rare diseases is obvious. This, however, is not only true for controlled clinical trials but also for clinical epidemiology working with data from hospital based registries.

2. CHALLENGES TO THE IMPLEMENTATION OF A MULTICENTRE CLINICAL REGISTRY OF ADAMANTIADES-BEHÇET'S DISEASE

2.1 Definition of manifest Adamantiades-Behçet's disease according to clinical findings

Still there is no commonly accepted diagnostic scheme for Adamantiades-Behçet's disease. This fact seems to provide a major problem for the establishment of a multicentre registry. However, the different diagnostic schemes for Adamantiades-Behçet's disease are essentially composed of the same manifestations with different weighing or different hierarchical classifications. Among others, these manifestations are ocular, oral, genital, or skin-related. Additionally, the pathergy test is used as a valid diagnostic criterion[1,2].

In an analysis from the German Registry of Adamantiades-Behçet's disease, the comparison of different diagnostic schemes leads to less than 10% "inconsistent" cases, i.e. the diagnosis of manifest Adamantiades-Behçet's disease was identical in more than 90% of cases. Additionally, the sheer information needed to classify patients according to different definitions of Adamantiades-Behçet's disease should be available in many centres, working with one specific diagnostic scheme.

Thus, even if for the individual patient there might be considerable disagreement about how to classify him or her, from the statistical point of view the different definitions of Adamantiades-Behçet's disease do not provide a really striking problem. As long as in case of doubt patients are documented in databases and not completely excluded, the different populations referring to one or another definition of Adamantiades-Behçet's disease can easily be extracted from registries using simple techniques of data management.

2.2 Assessment of clinical signs

The definition of Adamantiades-Behçet's disease according to some diagnostic rule however is only the last step in a process, which uses the results obtained from different diagnostic evaluations. For a multicentre registry the establishment of clinical standards concerning diagnostic procedures is thus indispensable. The installation of referral centres – if feasible – could be one way to solve this problem.

Three different sources of bias or random error in diagnostic evaluations have to be noted: Centre effects, inter observer variability and intra observer variability. In this framework, systematic errors might be excluded more easily than random errors. Calibration methods will help to obtain a quality of diagnostic assessment which leads to identical results in different centres. However, random errors of intra and interobserver variability might be excluded to a lesser extent.

One very important challenge to a multicentre registry might be the presence of true differences between patients in different centres. These centre "effects" are in fact population effects which have to be adjusted for in the statistical analysis.

2.3 Assessment of disease severity

For the comparison of several populations, the assessment of disease severity is indispensable. Consequences for conclusions referring to disease frequency, prognostic value of clinical findings, and validity of diagnostic devices are obvious. Disease severity has to be included as a relevant covariate in many statistical analyses.

However, from the statistical point of view, the "concept" of disease severity will never be measurable without error. Different instruments referring to "hard" (e.g. laboratory values) or "soft" (e.g. questionnaires or subjective judgements) criteria will always be affected by distinct causes of error. Thus, the exploration of these criteria for disease severity should be performed for two different reasons: First, comparability of populations has

to be ensured but, second, these explorations using e.g. psychometrical methods like factor analysis will enhance insight in the pathogenic mechanism underlying the disease.

2.4 Time

The natural course of the disease might be divided into several different points or intervals in time: Etiological factors provoke the onset of a pathogenic process which leads to the manifestation and long term course of the disease. However, in general it is not feasible to study this process. This is due to two different reasons: At the beginning of the process, the subject has not yet been identified, patient history has to be obtained by interviews or inspection of clinical charts. At the end of the process, the patient will be treated and thus only the follow-up under therapy will be available. This imposes natural and inevitable limitations to findings from clinical epidemiology.

3. PROPOSAL FOR AN INTERNATIONAL MULTICENTRE CLINICAL REGISTRY OF ADAMANTIADES-BEHÇET'S DISEASE

3.1 The current status of the German Registry

The German Registry of Adamantiades-Behçet's disease was founded in 1990 in Berlin. During the last 12 years approximately 500 patients have been included from more than 30 institutions. The patients are completely anonymized, thus no follow-up is possible within the framework of this registry.

The registry has been the basis of many clinical studies. Additionally, it provides support to other European registries and works on reports of German, European and worldwide etiology of Adamantiades-Behçet's disease.

3.2 A European multicentre registry for Adamantiades-Behçet's disease

Several aims motivate the installation of a European multicentre registry for Adamantiades-Behçet's disease: The observation of manifestations and courses of the disease (under therapy), the generation of epidemiological data referring to incidence and prevalence of the disease, the development of

standards with respect to diagnostic evaluation, therapeutic management, follow-up and, of course, documentation of findings. Clinical and experimental research should be supported. Diagnostic and therapeutic procedures in the centres participating in the registry should be improved. The final consequence should be the transfer of these improvements to centres not participating in the registry.

3.3 Documentation and follow-up in the multicentre registry

The following Table 2 gives a proposal for the documentation and follow-up in a multicentre registry for Adamantiades-Behçet's disease.

Of course, this documentation only provides a core panel of variables to be documented in every centre for every patient. Some centres may routinely include additional findings or will shorten intervals between the visits. Moreover, in the framework of clinical trials, specific variables and/or visits will be included for a specified time period.

If relational data bases were used, these potential projects could benefit from the pool of patients with a valid diagnosis, and from the established documentation system.

Table 2. Proposal for documentation and follow-up in the multicentre registry

	Recruitment	Follow-up each 4 to 8 weeks	Follow-up each 6 months	Follow-up each year
Demography	X			
Anamnesis/ Katamnesis	X	X	X	X
Clinical manifestations	X	X	X	X
Pathergy test	X			X
HLA typing	X			
Serology	X	X	X	X
Skin histology	X			
Eye examination	X	X	X	X
Ongoing therapy	X	X	X	X

The database should include as primary key patient identification and the number of regular follow-up. If e.g. a patient did not meet a specific follow-up visit with number 4, than the next visit should be labelled by number 5, and no record should be stored with follow-up number 4 for this patient. The calendar date of each visit should be stored additionally. Visits not within the regular time schedule should be labelled by a specific value of follow-up

number (e.g. 0 or 999), the course of time for these visits could be extracted from the database using the calendar date.

3.4 Quality assurance in the multicentre registry

Only those centres should be included in the registry, which fulfil minimum standards of equipment and experience. It is desired to include primarily registries which comprise unique centres for Adamantiades-Behçet's disease in their region. This would allow to retrieve epidemiological and not only clinical data from the registry. However, it is not intended to exclude centres from the registry which do not meet this specific criterion. Besides these criteria referring to structure and process of the centres, for specific findings referral centres should be established to guarantee a common high standard of diagnosis and therapy. Statistical monitoring should complement quality assurance.

3.5 Technical tools for a multicentre registry

Using defined inclusion and exclusion criteria, the recruitment of patients should be performed via Fax in the documentation centre of the registry. A patient may only be included in the registry after a positive response from the documentation centre. This procedure is similar to the method used in multicentre trials with the exception that no result of randomisation or stratification has to be communicated. A professional translation of case report form, optimally using the method of translation and retranslation until coherence is preliminary to the work of the registry. Case report forms could be received via pdf files centrally using the internet. In the future, a remote data entry (RDE) system should be developed. Nevertheless, documentation on paper should be parallelly offered to all centres.

3.6 Patients' rights and benefits

Like in clinical studies, the rights of the subjects documented in the multicentre registries have to be respected. The situation is less critical as e.g. in randomised or uncontrolled therapeutic or diagnostic studies, as the patient will not receive specific diagnostic examinations or therapeutic procedures solely as consequence of being in the registry.

However, the right of informational self-determination leads to the same consequences as in interventional studies: Every subject has to sign an informed consent form. to confirm knowledge about the rationale of the registry and the use of his or her data. In contrast to the German Registry,

anonymisation will not be feasible as follow-up data have to be matched. However, techniques of pseudonymisation may be applied which allow a high level of data protection.

Local ethic committees should be informed about the installation and work of the registry. Specific protection should be given to genetic data. Especially the use of individual genetic patient data has to be specified in advance. If regulatory conditions are different in participating countries, local, non centralised storage of a subset of data might be indicated.

Basically, all patients should benefit from the participation in the registry. The improved quality of diagnostic examinations and therapeutic interventions should improve the prognosis of these patients.

4. STATISTICAL CONSIDERATIONS

4.1 Power

In the following Table 3 we demonstrate detectable effects in terms of odds ratios for risk factors with the usual convention of a two-sided significance level of 0.05 and a power of 80%. We refer to the comparison of two study arms, which may arise from randomisation (therapy) or mere observation (prognosis). It depends on the clinical relevance assigned to the size of odds ratios which number of patients will be required in a specific study.

Table 3. Detectable odds ratios in clinical studies

Number of subjects	20	50	100	400	1000	10000
	Proportion in the control group: 50%					
Detectable odds ratio	5.1	3.3	2.3	1.5	1.29	1.09
	Proportion in the control group: 10%					
Detectable odds ratio	9	4.4	3	1.8	1.47	1.14

4.2 Studies and pitfalls

In the following Table 4 we present a list of possible studies using data from the registry and our own view of how valid these data may be for the specific scientific questions associated with these studies.

However, some possible pitfalls should be mentioned: Lead time bias may lead to seemingly improved prognosis of patients only due to the fact that they were identified earlier during the course of the disease. Spurious correlations may be found due to the heterogeneity of populations within the registry. This may affect disease-specific or demographic characteristics.

Finally, cultural differences in the assessment and interpretation of clinical signs may even lead to self-fulfilling prophecies as e.g. specific subtypes of the disease which are very rare in some regions will be underdiagnosed in exactly these areas for medical doctors will not take into account these subtypes in their diagnostic decisions.

Table 4. The usefulness of clinical registries for rare diseases

Epidemiology	Prevalence proportions	(+)
	incidence rates	-
Etiology – pathogenesis	Interviews	+
	chart reviews	+
Natural course	prospective? ethical problems	(+)
Diagnosis	Sensitivity, specificity + predictive values	+
Severity measures	comparison of different instruments	+
Therapy	direct use for cohort studies	+
	basis for multicentre controlled clinical trials	(+)
Prognosis	direct use for prognostic studies	++
Outcome	survival, disease severity, life quality	++

5. CONCLUSIONS

In this paper we discussed the usefulness and problems concerning the implementation of a multicentre clinical registry for Adamantiades-Behçet's disease. Of course, this paper was written from the perspective of a statistician. Thus, the concepts described in the paper have to be filled with life by clinical experts of Adamantiades-Behçet's disease. However, the necessity of such a registry and the benefit obtained from the cooperation of different centres is obvious and may improve patient care and research in this medical field.

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Epidemiology of Behçet's Disease in Asian Countries and Japan

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1. INTRODUCTION

As Ohno estimated in 1986, patients with Behçet's disease (BD) are distributed endemically along the ancient Silk Route from Mediterranean countries to Eastern Asian countries[1]. The highest prevalence of the disease is reported for Turks living in Anatolia with 370 patients per 100,000 inhabitants but in northern European and north and south American countries, BD is a rather rare disorder[2,3]. More than 60% of BD patients are supposed to have HLA-B51 in their genetic background[2] and the world-wide relative risk of HLA-B51 individuals to develop BD is also estimated to be high in the prevalent regions along the "ancient Silk Route"[3].

However, little is known about the prevalence of BD in the middle and northern areas, along the "ancient Silk Route" in China. We would like to report on the recent prevalence and distribution of BD in Asian countries and Japan.

2. PREVALENCE AND DISTRIBUTION

Although BD is suspected to be distributed along the ancient Silk Route as stated above, its prevalence is not known in the areas around the Silk Route of China. Jiang et al.[4] demonstrated in 1998 that the highest

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prevalence districts with 120 patients per 100,000 inhabitants were in the area of the Ningxiahei auto-governed province located in the middle of China where ethnically mixed Aravian and Asian people are living, and in the north area of Heilonyjiang (110 per 100,000) which is near the boarder of North Korea (Fig. 1). BD seems to occur frequently at latitudes between 30° and 45° in Asian populations. In South Korea a number of BD patients have been reported by Korean investigators[5,6]. Although BD patients are rare, 33 were diagnosed at the National Skin Centre in Singapore, from 1990 to 1997[7].

Figure 1. High prevalence districs of BD in Asian countries. In the area of Ningxiahei auto-governed province and Heilonyjiang in China, 120 and 110 patients per 100,000 inhabitants are reported, respectively. In South Korea and the northern part of Japan, the high prevalence is cleared.

In Japan, BD patients have been registered for their medical service with the Ministry of Health, Welfare and Labor since 1972 when they were diagnosed according to the Japanese Diagnostic Criteria (revised in 1987)[8]. The number of BD patients who were registered every year has increased and reached 180,000 last year. The highest prevalence is in Hokkaido (22 per 100,000) and the North-East Honshu islands (more than 18.0) located in the northern part of Japan[9]. The sex ratio is 1.1 with females being dominant. The age distribution of the patients registered exhibits a peak between 50 and 60 years indicating that elderly people seem to be involved (Fig. 2),

though the ages of onset are different. The clinical manifestations are similar to those reported from other countries[10].

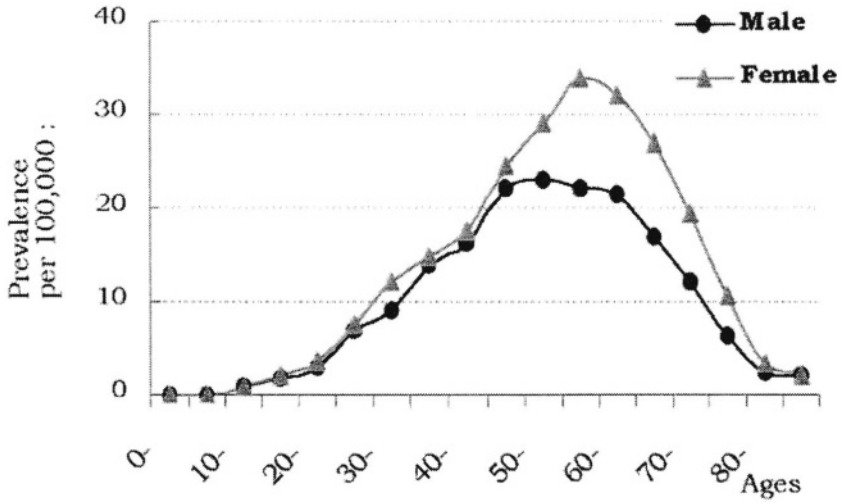

Figure 2. Age distribution of BD patients registered in Japanese Ministry of Health, Welfare and Labor in 1997. The age distribution exhibits a peak between 50 and 60 years indicating elderly people seem to be involved.

3. PROBLEMS OF TREATMENT WITH CYCLOSPORINE A (CsA)

It is important to investigate the quality of life (QOL) of BD patients, so we evaluated questionnaires from 239 BD patients who were treated during 6 months in 2000 (Table 1)[11]. Among them were 59 patients (25%) treated with CsA whose eyes were severly involved. During and after the treatments, nervous system problems, such as tremor, neuralgia, dizziness, etc., appeared in 33 of 59 patients, and these symptoms were distinctly noted in 29 (50%) patients after CsA treatment. Since these patients did not have these symptoms before CsA treatment, they even might have been induced by the CsA treatment. The duration of CsA treatment which might have caused the symptoms was 59±34 months (mean±SD). However, further investigation concerning this observation is necessary.

Table 1. Nervous system involvement and cyclosporin A (CsA) therapy in BD patients

Questionnaire collection		239
CsA treatment	Yes	59 (25%)
	No	180
Nervous system	Yes	33 (14%)
Involvement occurrence after CsA treatment		29 (50%)
	Before	4 (12%)
Duration of CsA treatment		59±34 (mean months)

Questionnaire system was performed and the answers were obtained from 239 BD patients (71%) who were treated during March 1 to September 30, 2000 (Nishimori et al.[11]).

4. COMMENTS

It is clear that BD patients are distributed in the Mediterranean countries and in the districts around the ancient Silk Route from China to Japan. HLA-B51 is strongly associated with BD, and the occurrence of its clinical manifestations seems to correspond with those reported on other parts of the world[2,6,10]. The pathogenesis of BD is still obscure, and generally anti-inflammatory agents, immunomodulators including CsA and interferon α, and anti-chemotactic agents are used for treatment on a case by case basis. Especially in cases with eye involvement, CsA is frequently used as an immunosuppressive treatment. It is important to investigate the quality of life of BD patients who are treated with CsA. Further investigation is needed in the near future.

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Recent Epidemiological Data on Behçet's Disease in Iran

The 2001 Survey

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1. INTRODUCTION

Behçet's disease (BD) is a multi-systemic disease classified among vasculitides[1]. It has been reported from many countries all over the world, but it occurs most commonly in countries along the silk route[2,3]. Besides the different distribution of the disease, its clinical manifestations also differ throughout the world[2,3].

Iran is among the countries with a rather high prevalence of the disease[4]. The epidemiological survey, providing data on different aspects of the disease, has been carried out continuously in our center since 1975. According to the previous surveys, a tendency toward milder forms of the disease was noticed[5].

The recent nation-wide survey was conducted in 2001. We here present its data, and compare them with the previous surveys.

2. MATERIALS AND METHODS

It was a cohort study on registered patients with the diagnosis of BD referred to our BD unit during the past 27 years (from 1975 to December 2001). These were over 95% of all diagnosed cases in Iran. All patients were

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seen in a multidisciplinary clinic by the same team of doctors comprising rheumatologists, ophthalmologists, and dermatologists. Patients were seen by affiliated neurologists and gastroenterologists when needed. Diagnosis was based on the clinical picture of the disease instead of a particular diagnostic criterion. The majority of cases however, was classified by at least more than two of the major sets of diagnosis criteria.

A computerized form, including 100 clinical and paraclinical parameters, was used to collect different manifestations of the disease and fed into an electronic database. It was updated every week after each visit of the patients.

A confidence interval (CI) at 95 percent for each item, and a standard deviation (SD) for the means and the percentages were calculated. The comparisons were made by chi square test.

3. RESULTS

3.1 Incidence, disease duration, family history

The annual incidence rate of BD in the past 5 years was around 285 patients per year. The mean disease duration was 9.2 years (SD:7), and the mean follow-up was 2.9 years (SD:3.8).

Positive familial history for BD was present in 5.9% (CI:1) of the patients, mostly (66.9%, CI:7.8) in their first degree relatives (parents, children, or siblings). In 50.2% (CI:2) of the patients a positive history of oral aphthosis was also present, 89.4% (CI:1.8) in the first degree relatives.

3.2 Sex and age distribution

Fifty-four percent of our patients were male (CI:1.4). The male to female ratio was 1.14/1. It is interesting that the sex difference was only significant during the third and forth decade of life.

The disease onset was mainly in the third decade of life, but with a range between 1 to 70 years. The mean age at onset of the disease was 26 years (SD:9.7, CI:0.3). Most of the patients (85.7%, CI:1) were in the adult group. In the remaining patients although the disease onset was before the age of 16, the majority completed their disease in adulthood (9.7%, CI:0.8). Among those who completed their disease in childhood, 1.5% (CI:0.3) were diagnosed in adulthood. Only in 3.1% (CI:0.5) the diagnosis of BD was made during the childhood.

3.3 Presenting signs

As the first manifestation, oral aphthosis was the most frequent one, presenting in 80.2% (CI:1.1) of the cases. Genital aphthosis in 10.3% (CI:0.9), ocular lesions as uveitis in 9.7% (CI:0.8), retinal vasculitis in 0.3% (CI:0.2), joint involvement in 5.3% (CI:0.6), and other signs (mostly skin lesions) in 8.1% (CI:0.8) were the other initial manifestations of the disease.

3.4 Major manifestations

The mucous membrane involvement, either oral or genital, was present in 96.9% (CI:0.5) of the patients. Oral aphthosis was the most frequent symptom, seen in 96.6% (CI:0.5) of the patients. Genital aphthosis was seen in 65.3% (CI: 1.4).

Skin lesions were present in 70.3% (CI:1.3) of the patients, pseudofolliculitis in 62.1% (CI:1.4), and erythema nodosum in 22.3% (CI:1.2) of cases. Other skin lesions were seen rarely (6.3%, CI:0.7).

Ocular lesions were seen in 55.9% (CI:1.4) of our patients, anterior uveitis in 41.1% (CI:1.4), posterior uveitis in 44.9% (CI:1.4), and retinal vasculitis in 30.8% (CI:1.3) of cases.

3.5 Minor manifestations

Joint involvement was seen in 34.8% (CI:1.4) of the patients. Asymmetric oligo-arthritis, usually in the large joints of lower limbs, was seen in 17.1% (CI:1.1). Inflammatory arthralgia with morning stiffness lasting not longer than 1 hour, was reported by 15.3% (CI:1) of the patients. Monoarthritis, mainly involving the knee joints, was seen in 7.6% (CI:0.8), and ankylosing spondylitis in 1.5% (CI:0.3) of our cases.

Neurologic manifestations were fortunately rare in Iranian patients. It was seen only in 3.2% (CI:0.5) of cases, and most of them were due to central involvement (3%, CI:0.5). Peripheral nervous system lesions were present only in 0.2% (CI:0.l).

Large vessel involvement was seen in 8.7% (CI:0.8) of the patients. Venous involvement was seen more frequently: deep vein thrombosis in 6.2% (CI:0.7), superficial phlebitis in 2.3% (CI:0.4), and large vein thrombosis in 1% (CI:0.3) of cases. Arterial involvement was rare (0.6%, CI:0.2), and aneurysm was more common than thrombosis.

Gastrointestinal manifestations were uncommon, with overall prevalence of 7.9% (CI:0.8). Gastroduodenitis was seen in 2.9% (CI:0.5), peptic ulcers in 1.6% (CI:0.4), diarrhea in 2.1% (CI:0.4), rectal bleeding in 0.8% (CI:0.3),

and abdominal pain mimicking a surgical acute abdomen in 1.7% (CI:0.4) of the patients.

Cardiopulmonary involvement was rare. We have seen pulmonary involvement in 37 patients (0.8%, CI:0.3), and cardiac manifestations in only 23 patients (0.5%, CI:0.2). Only vasculitis and perhaps serositis seem to be related to the disease.

Among the other manifestations, epididymo-orchitis was the most important, seen in 10.5% (CI:1.2) of males. Headache was reported by 7.3% (CI:0.7). Hepatosplenomegalia was rarely seen (0.5%, CI:0.2). In 1.7% (CI:0.4) of the patients an overlap or association with another autoimmune or collagen vascular disease was present.

3.6 Laboratory findings

Erythrocyte sedimentation rate (ESR) was normal (<20) during the disease course in most of the patients (46.2%, CI:1.5). It was between 20 and 49 in 36.1% (CI:1.4), between 50 and 100 in 16% (CI:1.1), and >100 in 1.7% (CI:0.4) of the patients.

Urinary abnormalities were detected in 10.8% (CI:0.9) of the patients. Hematuria was seen in 5.1% (CI:0.6), proteinuria in 2.3% (CI:0.4), leukocyturia in 5.7% (CI:0.6), and urinary casts in 0.3% (CI:0.2). They were transient in most of the cases, and only in 14 cases kidney biopsy was needed. Amyloidosis was present only in 2 of our patients.

Pathergy test was positive in 58% (CI:1.4), HLA-B5 in 53.4% (CI:1.5), and HLA-B27 in 9.4% (CI:0.9) of our patients. False positive reaction for syphilis (VDRL or RPR test was seen in 1.6% (CI:0.4) of the patients.

3.7 Disease classification

The most sensitive diagnosis criteria in Iranian patients was the classification tree (97.3%, CI:0.5). The sensitivity of other sets of diagnosis criteria were: Mason & Barnes criteria 68.2% (CI:1.3), O'Duffy criteria 72.4% (CI:1.3), International criteria 82.4% (CI:1.1), Dilsen criteria 86.6% (CI:1), Japan criteria 87.9% (CI:0.9), and Iran criteria (traditional format) 93% (CI:0.7).

4. DISCUSSION

Comparison of these data with the previous two surveys in 1991 (on 1822 patients)[6] and 1996 (on 3153 patients)[4] showed a decrease in the

annual incidence rate of BD, whereas the male to female ratio and the mean age of onset showed no significant changes (Table 1).

Table 1. Comparison (demographic pattern)

	1991	1996	2001	p
No. of patients	1822	3153	4704	-
Mean age of onset	25.9	26.2	26	0.56
Male/female ratio	1.22	1.13	1.14	0.35
Incidence rate/yr	-	345	285	-

As the first manifestation, oral aphthosis was seen more frequently, while uveitis and joint involvement were less encountered (Table 2).

Table 2. Comparison (presenting signs)

	1991	1996	2001	P
Oral aphthosis	70	76.5	80.2	<0.000001
Genital aphthosis	11	10.2	10.3	0.68
Uveitis	-	11.2	9.7	<0.03
Joint involvement	-	7	5.3	<0.002
Others	-	8.5	8.1	0.55

Among the clinical manifestations, the prevalence of skin lesions, notably pseudofolliculitis, and ocular lesions was lower. The same decrease was seen for deep vein thrombosis, joint and GI involvements (Table 3).

Table 3. Comparison (clinical manifestations)

	1991	1996	2001	P
Oral aphthosis	95	95.7	96.6	<0.006
Genital aphthosis	65	63.9	65.3	0.68
Skin lesions	77	73.9	70.3	<0.000001
Pseudofolliculitis	70	66	62.1	<0.000001
Erythema nodosum	23	23	22.3	0.68
Ocular lesions	66	58.8	55.9	<0.000001
Joint involvement	52	41	34.8	<0.000001
Thrombophlebitis	11	7	6.2	<0.000001
CNS involvement	3.1	3.3	3	0.80
GI involvement	11	9.2	7.9	0.0008
Epididymo-orchitis	9	11	10.5	0.23

In laboratory findings, the decrease in the positivity rate of pathergy and VDRL test was significant (Table 4).

Table 4. Comparison (laboratory findings)

	1991	1996	2001	p
Positive pathergy test	60	60.9	58	<0.000001
High ESR	-	56	53.8	0.06
Urine abnormality	-	11	10.8	0.81
False positive VDRL	-	2	1.6	<0.03
HLA-B5	-	55	53.4	0.16
HLA-B27	-	9.6	9.4	0.76

5. CONCLUSION

Recent nation-wide survey in Iran revealed a remarkable decrease of the incidence rate of BD and a tendency toward milder forms of the disease as was noticed in previous studies[5]. This may have many explanations such as changing patterns of the disease, inclusion of milder forms of the disease, and finally the impact of new treatments on the course of the disease.

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Prevalence of Behçet's Disease among Iraqis

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1. INTRODUCTION

Behçet's disease is a multi-system inflammatory disorder of unknown aetiology, now classified as vasculitis[1]. It runs a chronic progressive course that is clinically characterised by recurrent oral ulcers, genital ulcers, and iritis with hypopyon[2,3]; other systemic and cutaneous manifestations have been reported[4].

The area of distribution of the disease has been associated with the Silk Road, began in Venice, ended in Peking, stopping short before Japan and completely missing North Africa, two regions where BD is commonly seen[5].

Now, it is recognised that BD is prevalent in people around the Mediterranean basin especially its Eastern part, and in the Eastern rim of Asia, but less prevalent in other parts of the world[6].

We aim to estimate the prevalence of BD among Iraqis.

2. PATIENTS AND METHODS

A cross sectional survey for BD was carried out during August 1999 to April 2000 in two stages in Saglawia, town in Al-Anbar province with a total population of 35,125 persons.

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2.1 Stage 1

In each town in Iraq, there is a main food distribution centre, which distributes food rations every month through some agents in the area using the ration ticket system. The rations of the main food items reach every individual within its family at nominal prices which are supplemented by the state. Mouth ulcer questionnaire was distributed to each family with their food ration. Family members aging 16-45 years were registered, which accounts for 14, 155 persons.

The questionnaire inquires for the presence of mouth ulcers at present and/or during the past. Literate people were encouraged to fill in the forms (self administered), while the forms for illiterate people were filled in during an interview by one of the health officers working in the local medical centre serving that geographical area, after approval of local administrative and health authorities for conduction of the survey.

2.2 Stage 2

All people with mouth ulcers were seen by a physician in the main health centre of the area. Full history was taken from all individuals regarding frequency, types of ulcers and their size, whether they are painful or painless. The same was asked for genital ulcers. History for eye symptoms, skin lesions, joint pain and other complaints relevant to BD were recorded.

Complete clinical examination was performed with special concentration on signs of BD to confirm their satisfaction to the International Study Group Criteria (ISGC)[7] for diagnosis of BD.

Pathergy test was done (according to the methods described in the ISGC) for all suspected cases.

Statistical analysis was done using descriptive statistics [frequency, percentage and mean ± standard deviation (SD) and chi square] as indicated.

3. RESULTS

The completion rate for the 14,155 people (age 16-45 years) questioned in the first stage of the survey was 89%.

The demographic distribution of Saglawia population according to the information available at the department of statistics, food ration distribution centre of the area is shown in Table 1. There were 372 individuals (209 males, 163 females) with mouth ulcers out of 12,617 people interviewed (2.9%).

Table 1. Demographic distribution of Saglawia population

Total population	35,125 inhabitants
Age distribution	(%)
0-15 year-old	47.4%
16-45 year-old	40.3% (14,155 individuals)
>45 year-old	12.3%
Sex distribution	
Male	50.4%
Female	49.6%

On examination during stage 2 of the survey only six patients (4 males, 2 females) fulfilled ISGC for diagnosis of BD.

So the estimated prevalence for 10,000 inhabitants was 1.7 as shown in Fig.1 (in comparison to different countries).

The clinical features of six patients with BD are mouth ulcers in 6 (100%), genital ulcers in 5 (83.3%), skin lesions in 3 (50%), eye lesions in 2/5 (40%), arthritis in 2 (33.3%), headache (migraneous) in one (16.6%), gastro-intestinal involvement in one (16.6%), and pathergy test was positive in 5 patients (83.3%). The mean age of patients at onset was 24.16±6.99 years and at presentation was 33.16±5.7 years. The mean duration of disease was 9±5.17 years (range 3-15 years).

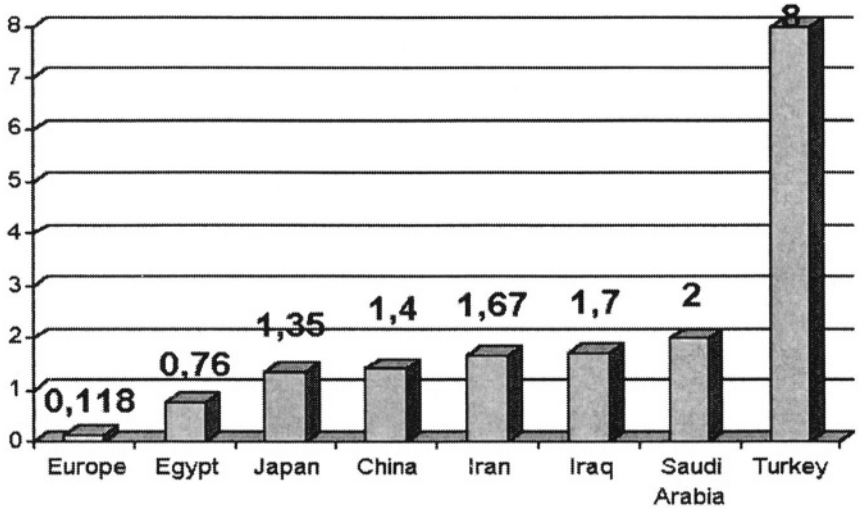

Figure 1. The prevalence of BD among different countries in comparison to Iraq

4. DISCUSSION

Our observed frequency of 1.7 BD patients for 10,000 inhabitants is more or less similar to the prevalence of the disease in other Mediterranean and Far East countries.

A recent estimated prevalence for the same number of inhabitants (10,000) was: Japan 1.35[8], China 1.4, Iran 1.67, Egypt 0.76, Saudi Arabia 2.0[5]. The highest prevalence was reported from Turkey 8.0-37.0[9] and the lowest from Europe 0.027-0.25 whilst only few cases were reported from the United States and Australia[6].

The clinical manifestations and positivity of pathergy test in this study are comparable to those of earlier Iraqi BD patients reported in 1986[10], and to studies from the same geographical areas[11,12].

Recurrent oral aphthous ulceration which is considered now mandatory for the diagnosis of BD according to ISG criteria is a warning signal for BD. It is shown in 20% of normal western healthy population[13],and is much less frequently reported among Iraqi population (2.9%, $P<0.001$). This low prevalence of recurrent oral ulcers among our population (290 per 10,000 inhabitants), with relatively high prevalence of BD (1.7 per 10,000 inhabitants) compared to high prevalence of recurrent oral ulcer in western healthy population (2,000 per 10,000 inhabitants), with low prevalence of BD among them (the highest reported was from Italy with 0.25 per 10,000 inhabitants)[6] raises the risk for developing BD 47 times more in our population with recurrent oral ulcers compared to individuals with oral ulcers from western populations, so the presence of recurrent oral ulcers among Iraqis is a significantly higher risk factor ($P<0.001$) for developing BD compared to its presence among western individuals. This will elicit the significance of the presence of recurrent oral ulcers as a predictor for BD especially during prevalence studies.

The pathergy test has been more standardised internationally, during the last decade. The test shows high prevalence in Middle East populations with BD, and because the pathergy phenomenon was added to the ISG criteria for diagnosis of BD, we can conclude that pathergy skin test is an easy, simple and very valuable tool for the diagnosis of the disease.

5. CONCLUSION

The estimated prevalence of 1.7 BD patients for 10,000 Iraqi population is more or less similar to the prevalence in other Mediterranean and Far East countries, excluding Turkey.

The presence of recurrent mouth ulcers is a high predictor for developing BD among Iraqis compared to European individuals.

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Current Epidemiological Data from the German Registry of Adamantiades-Behçet's Disease

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1. INTRODUCTION

The German Registry of Adamantiades-Behçet's disease – which was founded in Berlin in 1990 and has received the status of a charity in 2000 - provides data on epidemiology, clinical manifestations, and course of the disease in Germany on a continuous basis. In this work current epidemiological features of the disease in Germany are presented.

2. PATIENTS AND METHODS

Patients have to be permanent residents in Germany to be included in the German Registry. Until December 2001, 415 patients with Adamantiades-Behçet's disease (245 male, 170 female) have been reported to the registry. Demographic data, frequencies of clinical manifestations, onset signs, prognosis of the disease, and prognostic markers were determined. In addition to analysis of the entire number, the both largest ethnic groups being represented which were German (n=151, 36.4%) and Turkish (n=196,

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47.2%) were also investigated. The prevalence of the disease was evaluated in Berlin-West because of the previously isolated situation of the city which is unique for epidemiological studies. A retrospective evaluation was performed for the years 1984-89, while the study is a prospective one for the subsequent years. As development of the complete clinical picture the fulfilment of the criteria of the International Study Group for Behçet's disease[1] was determined.

3. RESULTS

3.1 Demographic data

The prevalence of the disease in Berlin-West was 0.65/100,000 inhabitants in 1984 and raised to 2.26/100,000 in 1994. The 3.5-fold increase was assessed in patients of both German and foreign origin. The prevalence in Berlin citizens of foreign origin (92% from Turkey) was 20-fold higher than that in German patients. On the other hand, the prevalence of the disease in Berlin citizens of German origin was similar to that reported from other northern European countries and from the U.S.A.[2]

The median age of onset of the disease in Germany was 26 years (range 0 to 72 years) been 27 years for German and 25 years for Turkish patients. A peak age prevalence in German individuals for developing Adamantiades-Behçet's disease was calculated between 20 and 40 years, while individuals of Turkish origin developed the disease mostly between 20 and 30 years of age. The complete clinical picture developed at an average of 29 years (range 5 to 72 years), with 30 years in German and 28 years in Turkish patients. Juvenile disease (complete symptom complex developing at $\leq$16 years of age) was recorded in 7.1% of the patients. The interval between onset of the disease and diagnosis with 17 months was being significantly longer than the duration of development of the complete clinical picture (8.5 months, $p<0.007$). The disease was diagnosed later in German (25 months) than in Turkish patients (11 months, $p=0.002$). While German patients presented an equal male-to-female ratio, a male predominance was shown in Turkish patients (M:F 2:1, $p=0.001$). Familial occurrence was detected in 4.5% of the German and 18% of the Turkish patients ($p=0.001$).

3.2 Clinical manifestations

The frequencies of the major clinical manifestations were: oral aphthous ulcers 98%, skin lesions 74%, genital ulcers 65%, arthritis 53%, and ocular

manifestations 51%. Papules and sterile pustules (53%) as well as erythema nodosum (39.5%) were the most common skin lesions. A positive pathergy test was detected in 38% of the patients. German patients presented more often lung (6.3% vs. 1.1%, p=0.02) and gastrointestinal (20% vs. 11%, p=0.02) involvement as well as prostatitis/epididymitis (19% vs. 8.6%, p=0.03) and lethal outcome (4.5% vs. 0.6%, p=0.04) than Turkish patients. Male patients, of both German and Turkish origin, developed more often vascular involvement (25%) than female ones (8%, p<0.001), especially thrombophlebitis (18% vs. 4%, p<0.001). Female patients of Turkish origin developed more often neurological manifestations (19% vs. 6%, p=0.008) than male patients.

3.3 Onset signs of the disease

Skin and mucosal lesions represented 86% of the onset features of the disease. Oral ulcers with 79% were the most common onset sign followed by arthritis (6%), erythema nodosum (5%), uveitis (5%), genital ulcers (4%), superficial thrombophlebitis (1%), and papules/sterile pustules (1%). Uveitis (n=18) and erythema nodosum (n=3) as onset signs shortened the median interval to diagnosis (4 months and immediately, respectively).

3.4 Prognosis of the disease and prognostic markers

A severe course occurred in 16% of the patients; irreversible retinal vasculitis to blindness in 10%, sterile meningoencephalitis in 6%, severe arthritis in 4%, lethal outcome in 2%, hemoptysis in 1%, and bowel perforation in 1%. Five of the 7_3 patients of the German Registry who died due to complications of their disease were of German origin.

Fifty-one of 121 German patients evaluated (42%) were HLA-B51-positive vs. 196 of 1415 controls (14%, p<0.00l). Among the 51 HLA-B51-positive German patients, 28 (of 59) were male (47.5%; controls 86/691, 12%) and 23 (of 62) female (37%; controls 110/735, 15%). The calculated odds ratio concerning the development of the disease in HLA-B51-positive individuals of German origin was 4.5-fold higher in males (6.4) than in females (3.4). On the other hand, 121 of 161 registry patients of Turkish origin evaluated (75%) were HLA-B51-positive vs. 83 of 268 controls[4] (31%, p<0.00l, odds ratio 6.7).

HLA-B51-positivity was found to be associated with a worse prognosis in the patients of the German Registry: Ocular manifestations (86% vs. 46%, p=0.027), vascular involvement (31% vs. 13%, p=0.008), superficial thrombophlebitis (18% vs. 3%, p=0.003), and cutaneous lesions (88% vs. 64%, p<0.001) were more frequent in HLA-B51-positive patients than in

HLA-B51-negative ones. In addition, HLA-B51-positive patients developed the complete clinical picture earlier (at 26 years) than HLA-B51-negative patients (at 33 years, p=0.017).

Thirty-seven of 87 patients evaluated (43%) had elevated levels of circulating cardiolipin autoantibodies, 13 of the IgG isotype (15%), 18 of the IgM (21%), and 6 had both isotypes (7%). In most positive patients moderate increases were assessed; mean IgG levels were 14.45±1.30 GLP/ml (normal values <10 GLP/ml) in 18 patients with increased values while only one patient presented values over 100 GLP/ml, and mean IgM levels were 15.07±8.38 MLP/ml (normal values <6 GLP/ml) in all 24 patients with increased values. Cardiolipin autoantibodies of the IgM type were associated with the presence of central nervous system involvement (48% with positive vs. 12% with negative levels, p<0.001), cutaneous vasculitis (70% vs. 34%, p=0.004), erythema nodosum (58% vs. 26%, p=0.006), thrombophlebitis (25% vs. 7%, p=0.02), and retinitis (54% with positive vs. 28% with negative levels, p=0.02).

Associations of clinical signs and demographic data could be detected, namely oral aphthous with genital ulcers (p=0.02), erythema nodosum with superficial thrombophlebitis (p=0.01), ocular with systemic vascular involvement (p=0.03), and male gender with systemic vascular involvement (p<0.001).

4. DISCUSSION

Adamantiades-Behçet's disease is a rather rare disorder in ethnic Germans, however, there is a continuous increase in diagnosed cases. The prevalence of the disease evaluated in Berlin citizens of German origin was similar to that reported from other northern European countries and the U.S.A.[2] In contrast, the prevalence of the disease in residents of Turkish origin in Germany is high, although it is 5-fold lower than the prevalence determined in the European part of Turkey and 18-fold lower than in Anatolia[2]. This markedly different prevalence of the disease in Turks dependent on the geographic area of residence in association with similar data reported from Japan and Hawaii for individuals of Japanese origin leads to the suggestion of an unknown environmental factor possibly influencing the development or onset of the disease[5].

A median age of onset in the third decade of life as well as cases with early and late onset of the disease were also reported from other European countries[6]. The rate of German patients with juvenile disease reported in this study (7%) is similar to those reported from Turkey (6%), Iran (3%), Morocco (3%), Tunis (2%), and Japan (2%)[2]. These observations are

important regarding the better prognosis reported for German patients than for those from endemic areas[6] and also the better prognosis detected in juvenile than in adult patients[7]. The delay of diagnosis in ethnic Germans compared to those of Turkish origin may be due to the supposition that Adamantiades-Behçet's disease is an eastern Mediterranean disorder which is still wide-spread in western Europe.

In contrast to the classical Japanese and Turkish reports of an androtropism, more recent epidemiological studies registered an approx. 1:1 male-to-female ratio in Japan, Korea, China, Iran, Turkey, Brazil, and Europe[2]. An androtropism is still observed in some countries around the eastern Mediterranean area (Saudi Arabia, Israel, Egypt, Morocco, Greece, and Italy)[6] whereas gynaecotropism is evident in northern European countries.

The familial occurrence in patients of German origin was as low as in other European countries, namely Greece (0%), Italy (1-3%), Great Britain (4%), Spain (5%), and Portugal (3%). Familial occurrence was as high in patients of Turkish origin in Germany as in patients from endemic geographic areas (12-13% in Israel, Korea and Tunisia)[2].

The high frequencies of oral aphthous ulcers, genital ulcers, skin and ocular lesions in European patients and their almost exclusive occurrence as onset signs confirmed the importance of these clinical features for diagnosis. Higher rates of ocular lesions could be detected in south-eastern European patients (Italian and Greek) compared to south-western as well as northern European patients, while all further manifestations were overall similar[6]. Highly recurrent oral aphthosis, the most frequent onset sign, is a warning signal for Adamantiades Behçet's disease. In Korea, fifty-two percent of 67 prospectively evaluated patients with recurrent oral aphthosis (averagely 10 recurrences per year) developed Adamantiades Behçet's disease 8 years after onset of oral aphthous ulcers[8]. The high prevalence of oral aphthous ulcers as onset sign in German patients was compatible with reports on several groups of native European patients[6].

The potentially severe prognosis of the disease (10-year mortality[9] in 5%) was confirmed in our study. Retinal vasculitis leading to blindness, central nervous system and vascular involvement are the most disabling and/or life-threatening features. The disease led to a lethal outcome in German patients probably due to delayed diagnosis and treatment.

The close association of HLA-B51 with the disease is well established. The confirmation of this association in several ethnic groups[2,6] led to the assumption that HLA-B51 may be directly involved in the development of the disease. However, more recent data indicate that the pathogenic gene(s) responsible for the disease is not HLA-B51 itself but other gene(s) around the HLA-B locus[10]. Our data support the alternative suggestion that HLA-

B51 is a marker of severe prognosis[11], being especially associated with an earlier development of the complete clinical picture, ocular lesions, vascular involvement, and cutaneous lesions. However, HLA-B51-positive individuals of German origin as well as patients from other northern European countries presented a lower odds ratio regarding the development of the disease compared to HLA-B51-positive southern Europeans, especially patients from south-eastern European countries[6]. On the other hand, the present study confirms our previous data concerning the association of moderately increased cardiolipin autoantibodies with cutaneous vasculitis[12].

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Heterogenity of Clinical Manifestations in Behçet's Disease among Different Ethnic Groups

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1. INTRODUCTION

Behçet's disease (BD) mainly spreads in geographical regions where the great silk route passed. It is less often seen in European countries. The prevalence of the disease, HLA associations and clinical manifestations vary in different countries[1-4]. The aim of this study was to analyze the frequency of BD manifestations and their HLA B5 association in patients (pts) of various ethnic groups.

2. MATERIALS AND METHODS

52 pts fulfilling the ISG criteria for BD were included in the study [36 men and 16 women, mean age 31±7.8 years (y) ranged from 23-45 y]. All pts were typed for HLA-B5. The ethnic origin of pts was as follows: Russian –13, inhabitants of Caucasus (IC)- 35, Asians – 4.

3. DISCUSSION

We compared our data with literature references: 77% of the IC pts were HLA-B5 positive which corresponds to data reported from Saudi Arabia, p=0.71, while in Russians HLA-B5 positivity was lower and thus close to

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the frequency in German pts, p=0.83. Male predominance was found among IC, which again is similar to findings in Saudi Arabia, while female predominance was noted among Russians which is close to the ratio hi Germany.

Table 1. Frequency of BD manifestations

	Russian n=13	IC n=35	P
Recurrent Oral Ulcers	100	100	NS
Genital ulcers	77	77	NS
Skin lesions	69	83	NS
Eye lesions	30	42	NS
Pathergy test	53	74	NS
HLA B5	11	77	<0.0001
M:F	0.4 : 1	4.8 : 1	<0.0001

77% of male pts were HLA-B5 positive vs. 23% of the female pts (P <0.0001)

4. CONCLUSION

No statistical differences were noted in clinical manifestations among Russians and IC pts with BD. The principle features of pts from Caucasus were HLA B5 positivity and male sex, which is close to pts from Saudi Arabia.

Table 2. The frequency of clinical manifestations of BD pts in different countries

	Saudi Arabia N=119	Germany n=49	Iran n=2068	Japan n=335	Russia n=13	IC n=35
Eye lesions	56	30	65	90	30	42
Pathergy test		52	60.3		53	74
Thrombophlebitis	38	23	10	10	23	40
Arthritis	56	62	60	52	69	83
GI involvement	4	28	10	52	46	29
HLA B5 +	72	18.4	53		11	77
M:F	3.4:1	0.88:1		0.77:1	0.4:1	4.8:1

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Behçet's Disease in Patients of German and Turkish Origin – A Comparative Study

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1. INTRODUCTION

Behçet's Disease (BD) is most frequent in countries along the ancient silk route from Japan to the Middle East and the Mediterranean basin, but is rarely encountered in Northern Europe and North America[1]. Ethnic origin and environmental factors are supposed to modulate the prevalence and expression of BD, as the expression of BD has been reported to differ between various geographical areas. Thus, pathergy reaction is considered highly sensitive and specific for BD in patients originating from Turkey, the Middle East, Japan and Korea[2], but is often negative in patients from Western European countries or the USA[3]. Gastrointestinal involvement occurs in one third of Japanese patients, but is rare in Mediterranean countries[4]. Regional differences also have been described for disease severity, as for example patients from the UK and the USA are reported to have more widespread disease with arthritis, CNS vasculitis and other vascular problems than patients from Middle Eastern or Mediterranean countries[5]. In Israeli Jewish patients, BD was reported to be most severe for those originating from North African countries when compared to those originating from Iran/Iraq or Turkey, with a higher rate of ocular disease[6]. Eye disease was more severe in Japanese patients when compared to patients

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from the UK[7]. The aim of our study was to evaluate if the ethnic origin of German or Turkish patients with BD living in Germany influences the expression of the disease.

2. MATERIALS AND METHODS

Between 1995 and 2000, 32 patients of German and 33 patients of Turkish origin living in Germany were evaluated with respect to the entire spectrum of disease manifestations, HLA associations, gender, age at manifestation, and time by diagnosis. All patients fulfilled the International Study Group Criteria[8]. Patients were interviewed by two rheumatologists (IK, IG), one of them also speaking Turkish (IG) concerning the occurrence of any of the symptoms and the time at which the respective manifestations had occurred. Data relating to the spectrum of disease manifestations were also collected from the medical files. Additionally, a complete physical (IK, IG) and ophthalmological examination (NS) was performed at two different times to reveal symptoms possibly not recognised by the patients. Pathergy test was performed in each untreated patient according to the standard procedure, an intracutaneous needle prick on the forearm, considered positive if a sterile papulopustule occurs after 24-48 hours. HLA-typing was performed by oligonucleotide sequencing. With the exception of the symptoms at onset of BD, manifestations were only considered for further analysis when seen and documented by a physician or, in case of neurological or gastrointestinal involvement, proven by MRT, analysis of cerebrospinal fluid, electrophysiological methods or endoscopy.

2.1 Statistical analysis

For differences between manifestations of the disease and ethnic origin, sex, and association to HLA-B51, the odds of developing a symptom (and other binary traits) were compared between groups by odds ratio (OR), for which approximative 95% confidence intervals (CI) were computed along with P-values for the hypothesis that the odds ratio is 1. Considering a dozen symptoms, adjustment for multiple testing was performed by the Bonferroni-Holm procedure, if any relation was significant. Age at first manifestation, diagnosis and time by diagnosis were compared by Wilcoxon Rank Test.

3. RESULTS

In the German patient group, 12 were female and 20 male, whereas in the Turkish group 7 were female and 26 male (male to female ratio 1.7:1 and 3.7:1, respectively, 63% males in the German versus 78% males in the Turkish patient group). However, sex ratios did not differ significantly between patients of German descent and Turkish descent. In both patient groups, the most common primary manifestation of the disease was oral aphthous ulcers (90%), mostly in association with cutaneous symptoms (60%). There was no statistically significant difference with respect to the rate of oral aphthous ulcers, genital ulcers, skin lesions, ocular disease, positivity of pathergy reaction, arthritis, vascular lesions, gastrointestinal manifestations or epididymitis. The frequency of CNS manifestations was too low to be statistically analysed. There was also no difference concerning the association to HLA-B51, which was positive in 75% of the Turkish and 72% of the German patients. Furthermore, there was no significant association between any of the manifestations of BD, gender or the presence of HLA-B51.

At time of the first manifestation of the disease, the median age of the German patients was 26 years (range: 14 to 48), of the Turkish patients 25 years (4 to 43), at diagnosis, the Germans were 31.5 (20 to 52), the Turkish 26 (4 to 49) years old (not significant). The median time from the first manifestation to final diagnosis of BD was 0 (0 to 17) years in the Turkish and 3.5 (0 to 21) years in the German patients (p=0.0005).

In the German group, 4 patients had been diagnosed as seronegative spondyloarthropathy before the diagnosis of BD was made (12%), whereas BD was correctly diagnosed in all Turkish patients.

4. DISCUSSION AND CONCLUSIONS

Today, more than 4 Million people of Turkish origin are living in Germany. As BD is very rare in people of German origin (estimated prevalence 0.6/100.000)[4,9] and endemic in Turkey (prevalence 80/100.000-370/100.000)[10,11], the Turkish patients with BD living in Germany represent a very unique population for comparative studies. The non-ethnical differences are minimised in this comparison, as both patient groups share the same environment, although there still may be some differences in diet or lifestyle.

Our study revealed no difference in the male to female ratio or other variables such as age at first manifestation, age at final diagnosis, association to HLA-B51, pathergy phenomenon or frequency of the various disease

manifestations (mucocutaneous, articular, ocular) between both patient groups.

The expression of the various manifestations of BD for Turks living in Turkey was reported as follows: oral aphthae 100%, genital ulcers 73 to 88%, papulopustules 54 to 94%, erythema nodosum 42 to 54%, ocular involvement 28.9%, arthritis 16 to 47.4%, neurological 2 to 8%, phlebitis 10 to 38%, pulmonary 1 to 18%, and gastrointestinal 3 to 5%[12]. The distribution of symptoms is the same as in the Turkish patients living in Germany analysed in the present study, except for the frequency of ocular involvement, which was considerably higher in the Turks living in Germany (present study 75%) than that reported for Turks living in Turkey (28 to 47%). In contrast to the results of the present study, ocular manifestations were significantly more frequent in Turks than in Germans (66.3% vs. 47.6%) in the German Registry[1]. This difference may be due to the lower number of patients in our study, or to the fact that we performed ophthalmologic examinations in all patients, irrespective of whether or not they reported ocular symptoms, which might have revealed cases of hitherto undetected ocular involvement. The higher frequency of epididymitis, neurological and gastrointestinal involvement in the German Registry when compared to the present evaluation can only be explained by a low specificity of the questionnaire, which is used for registration of the patients, not requiring objective signs and examinations.

Although the rate of positive pathergy reaction was reported to be significantly higher in Turkish patients compared to patients from the UK or the USA[3,13], this is not true for comparison with German patients (German Registry: Turks and Germans 51.8%; here: Turks 55%, Germans 39%). The pathergy reaction is reported to be positive in 57 to 65% of the Turkish patients living in Turkey,[14-16] which is similar to that in Turkish patients living in Germany.

The association of BD to HLA-B5 or its split antigen HLA-B51 is well known and has been described for most of the populations where it was examined [17,18], varying from 54% in Lebanon[19] to 77% in Turkey[20], with the possible exception of Great Britain, where it is reported to be only 15%[21]. For German patients it ranges between 75 to 76% with the suballeles HLA-B*5101 and B*5108 being most common[17]. We could not find significant differences in the association of BD to HLA-B51 between Turkish and German patients. Furthermore, the present study did not reveal an association of HLA-B51 with any of the manifestations of BD or gender, which supports the data of Müftüoglu et al.[20] and Gül et al.[22] but contrasts those of the German Registry, where ocular manifestations, vascular

involvement, and skin lesions appeared to be associated with HLA-B5, or those of Azizleri et al. who reported that HLA-B5 was associated with a higher frequency of genital ulcerations [9,23]. It is possible that we would have found differences in the association of individual disease manifestations to HLA-B51 if we had analysed both patient groups separately, which for statistical reasons would have required a much larger number of patients.

The main difference between both patient groups was found when the time from first manifestation of BD to final diagnosis was analysed, resulting in a median of 0 years for the Turks and 3.5 years for the Germans ($p=0.0005$). Furthermore, in 4 German patients (12%) the symptoms of BD were misdiagnosed as spondyloarthropathy, whereas no misdiagnoses occurred in the Turkish patients. We assume that this is due to the rarity of BD in Germans; consequently the physicians in Germany are not familiar with its features. Also, even if they consider BD as differential diagnosis, they believe that it is less probable in people of German origin than for example spondyloarthropathy, thus misdiagnosing it especially in German patients.

In conclusion, ethnic differences seem to play a minor role in the expression of BD, as the frequency of the various manifestations of the disease, association to HLA-B51, and age at onset were the all same in Turks living in Germany, Turks living in Turkey as reported in the literature, and Germans. There may be environmental influences concerning the frequency of ocular manifestations which were more common in Turkish patients in Germany, and the male to female ratio, which was considerably higher in the Turkish patients in Germany compared to those in Turkey. The striking difference in the time from the first manifestation to final diagnosis of BD in the German patients compared to Turkish patients also living in Germany, and the considerable rate of misdiagnoses (exclusively spondyloarthropathy) in the German patients suggest that BD may be underestimated in patients of German origin. Further studies are required to evaluate the real frequency of BD in the German population, for example by performing field studies with experts on BD, following an example from Turkey[10] or "Zero-Patient-Design"-studies, as proposed by H. Yazici[24], thereby taking advantage of the structures of the Cooperative Regional Rheumatology Centres in Germany.

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Influence of Sex on Patients with Behçet's Disease in Korea

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1. INTRODUCTION

Behçet's disease (BD) is a chronic systemic disease which consists of varying combinations of mucocutaneous, ocular, neurologic, cardiovascular, pulmonary, renal, gastrointestinal and other manifestations. Several aspects of the immunopathogenesis are considered but its accurate etiology and pathogenesis are still unknown.

The influence of sexual difference on BD is a well-known fact and there are several reports suggesting more severe course of the disease among males[1,2].

This aspect has never been studied in Korea before. The purpose of our study is to determine sex ratio of patients with BD in Korea and the effects of gender on the severity and clinical features of BD patients from Korea.

2. PATIENTS AND METHODS

The study included 1,904 patients with BD who visited the BD Speciality Clinic of Yonsei University College of Medicine and Ajou University School of Medicine. The diagnosis of BD was based on the criteria of International Study Group for BD and on the revised criteria of BD Research Committee of Japan.

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Epidemiological and clinical data of patients were analyzed according to the sexual difference. Statistical analysis was performed using chi-squared test for comparison. A value of $p<0.05$ was accepted as statistically significant.

3. RESULTS

The mean age of onset was 33.2 years and the male to female ratio was 0.61:1 with female predominance. There was no statistically significant difference between males and females in the mean age at onset (Table 1). The frequency of the major and minor clinical manifestations in 1,904 patients was in decreasing order as follows: oral ulcer, genital ulcer, skin lesion, ocular lesion, articular symptom, gastrointestinal symptom, vascular symptom, and neurologic symptom. Among those symptoms, ocular symptom and vascular symptom were more frequently observed in male patients while oral ulcer, genital ulcer, skin lesion, articular symptom, and neurologic symptom were more in female patients (Table 2). Further detailed analysis of skin lesions revealed that females had higher frequency of erythema nodosum, whereas males presented more often with furuncle, erythema multiforme, and abscess (Table 3). Further investigation into the details of ocular involvement revealed that males had higher frequency of uveitis, cataract, iritis, chorioretinitis, and retinal hemorrhage (Table 4). Ocular and vascular symptom as clinical features with severe complications or mortality were shown more frequently in men than in women (Table 5). The mean age at the onset of patients with the worst prognosis such as ocular, gastrointestinal, neurologic, and vascular involvements was significantly lower in male than in female patients ($p<0.05$)(Table 6).

Table 1. Age and sex distribution at the onset

Age (years)	Male	Female	Total
	Number (%)	Number (%)	Number (%)
Under 10	6 (0.9)	15 (1.4)	21 (1.2)
11 - 20	47 (7.0)	93 (8.4)	140 (8.0)
21 - 30	231 (34.6)	317 (28.8)	548 (31.0)
31 - 40	247 (37.0)	442 (40.1)	689 (38.9)
41 - 50	101 (15.1)	177 (16.1)	278 (15.7)
51 - 60	32 (4.8)	43 (3.9)	75 (4.2)
Over 61	4 (0.6)	14 (1.3)	18 (1.0)
Total	668 (100)	1101 (100)	1769 (100)
Mean Age*	33.9±9.9	33.2±10.4	33.2±10.1
M:F ratio	0.61	1	0.61:1

*Values are mean±SD

Table 2. Clinical features of male and female patients with Behçet's disease

Symptom	Male (n=722) Number (%)	Female (n=1182) Number (%)	*p*-value
Major:			
Oral ulcer	717 (99.3)	1181 (99.9)	0.022
Genital ulcer	537 (74.4)	987 (83.5)	0.001
Skin lesion	531 (73.5)	991 (83.8)	0.001
Eye lesion	466 (64.5)	508 (42.9)	0.001
Minor:			
Articular	237 (32.8)	465 (39.3)	0.004
Vascular	24 (3.3)	8 (0.7)	0.001
Gastrointestinal	26 (3.6)	47 (4.0)	ns
Neurologic	3 (0.4)	25 (2.1)	0.003

ns: not significant

Table 3. Analysis of skin lesions in patients with Behçet's disease according to sex

Skin lesion	Male (n=722) Number (%)	Female (n=1182) Number (%)	*p*-value
Erythema nodosum	385 (53.3)	795 (67.3)	0.001
Folliculitis	26 (3.6)	54 (4.6)	ns
Furuncle	43 (5.9)	37 (3.1)	0.003
Erythema multiforme	34 (4.7)	32 (2.7)	0.021
Ulcer	7 (1.0)	11 (0.9)	ns
Abscess	10 (1.4)	6 (0.5)	0.042
Pustule	6 (0.8)	7 (0.6)	ns
Acneiform eruption	2 (0.3)	5 (0.4)	ns
Thrombophlebitis	4 (0.6)	2 (0.2)	ns
Others	29 (4.0)	59 (4.9)	ns

ns: not significant

Table 4. Analysis of eye lesions in patients with Behçet's disease according to sex

Eye lesion	Male (n=722) Number (%)	Female (n=1182) Number (%)	*p*-value
Uveitis	241 (33.4)	179 (15.1)	0.001
Conjuctivitis	32 (4.4)	57 (4.8)	ns
Cataract	44 (6.1)	39 (3.3)	0.004
Iritis	29 (4.0)	22 (1.9)	0.005
Chorioretinitis	17 (2.4)	10 (0.9)	0.007
Retinal detatchment	9 (1.3)	11 (0.9)	ns
Retinal hemorrhage	14 (1.9)	5 (0.4)	0.001
Glaucoma	11 (1.5)	8 (0.7)	ns
Retinal degeneration	1 (0.1)	4 (0.3)	ns
Others	134 (18.6)	216 (18.3)	ns

ns: not significant

Table 5. Comparison between male and female patients with clinical manifestations of Behçet's disease which cause disability, mortality or severe sequelae

Symptom	Male (n=722)	Female (n=1182)	Total (n=1904)	*p*-value
	Number (%)	Number (%)	Number (%)	
Ocular	316 (43.8)	254 (21.5)	570 (29.9)	0.001
Gastrointestinal	26 (3.6)	47 (4.0)	73 (3.8)	ns
Neurologic	3 (0.4)	25 (2.1)	28 (1.5)	0.003
Vascular	24 (3.3)	8 (0.7)	32 (1.7)	0.001

ns: not significant

Table 6. Age and sex distribution at the onset in Behçet's disease patients with the worst prognosis such as ocular, gastrointestinal, neurologic and vascular involvements

Age (years)	Male	Female	Total
	Number (%)	Number (%)	Number (%)
Under 10	4 (1.2)	8 (2.7)	12 (1.9)
11 - 20	19 (5.7)	26 (8.7)	45 (7.1)
21 - 30	127 (38.3)	84 (28.2)	211 (33.4)
31 - 40	123 (37.0)	108 (36.2)	231 (36.6)
41 - 50	44 (13.3)	45 (15.1)	89 (14.3)
51 - 60	13 (3.9)	17 (5.7)	30 (4.8)
Over 61	2 (0.6)	10 (3.4)	12 (1.9)
Total	332 (100)	298 (100)	630 (100)
Mean Age*	32.6±9.7**	33.9±12.2**	33.2±10.9

*Values are mean±SD, **$p<0.05$

4. DISCUSSION

Behçet's disease in Korea has a female predominance. This is similar to the reports from European countries and the U.S.. However, most other countries have reported male predominance[3,4].

Skin lesions were observed in 79.9% of patients, of whom 77.5% had erythema nodosum which was more frequent in females corresponding to data reported from Turkey[1,4]. Eye lesion was more common in males showing the higher frequency of uveitis, cataract, iritis, chorioretinitis, and retinal hemorrhage.

The result reveals that in Korea, severer clinical features appeared to occur more frequently in men at an earlier age than in women. Yazici et al.[1] also associated male gender and lower age of disease onset(<25 years) with a severer course of disease. Dilsen et al[5] reported that male patients with earlier onset(<25 years) had the higher prevalence of vital organ involvement. Moreover, Madanat et al.[4] reported that male patients with BD had a severer course of the disease with higher frequency of severe eye disease and neurologic involvement. However, Demiroglu and Dundar[6]

reported that younger patients with BD had a severer course, but clinical severity was not influenced by gender. Krause et al[7] reported that there was no significant difference in the expression of BD between males and females.

In conclusion, this study elucidated the influences of sexual difference on BD in Korea. In future, further researches are anticipated on the factors related to sexual difference such as hormones or race.

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The Influence of Gender on the Frequency of Clinical Symptoms in Behçet's Disease

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1. INTRODUCTION

It is well known for quite a long time that some of the manifestations of Behçet's disease (BD) are seen more frequently in men than in women[1-2]. The aim of this study was to investigate this aspect and answer particularly to two questions. The first question is: In case of a difference, is the difference statistically significant? The second question is: In case of a statistically significant difference, is it of any clinical relevance?

2. MATERIALS AND METHODS

The study design was a cohort study. The diagnosis was clinical (not based on diagnosis criteria). All Behçet's patients of the Behçet's Disease Registry (4717 at January 20, 2002) were included in the study. The incidence of 105 symptoms and signs were compared systematically in men and women.

The statistical comparison was made by the chi square test (fourfold table). When required, the Yates correction formula was applied to the calculations.

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3. RESULTS

Due to the large number of patients, many symptoms or signs show statistically significant differences in men and women. A 2% difference between men and women in our large cohort was considered a statistically significant difference. For clinical relevance, we report only those symptoms with a difference exceeding 5%.

Genital aphthosis was seen in 62% of men versus 69% in women. Behçet's pustulosis was seen in 69% of men versus 53% in women. Anterior uveitis was seen in 46% of men versus 36% in women. Posterior uveitis was seen in 51% of men versus 38% in women. Retinal vasculitis was seen in 36% of men versus 24% in women. Joint manifestations were seen in 37% of men versus 32% in women. Phlebitis was seen in 9% of men versus 3% in women. Complete form of Japan classification criteria was seen in 28% of men versus 18% in women.

Some of the symptoms were seen in overall less than 5%. Those being significantly more frequent in men were: ankylosing spondylitis, proctorrhagia, central nervous system manifestations, aneurysm, large vein thrombosis, superficial phlebitis, normal ESR, and HLA-B5 positivity. Those symptoms that were significantly more frequent in women were headache and high ESR.

4. CONCLUSION

The only symptom that was seen more frequently in women was genital aphthosis (7% difference). The major differences between male and female patients were in Behçet's pustulosis (16%), ocular manifestations (13%), and the complete form of Japan criteria (10%). The difference in phlebitis was 6%, and 5% in joint manifestations.

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The Influence of Gender on the Severity and the Outcome of Ocular Lesions in Behçet's Disease

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1. INTRODUCTION

It is widely accepted that in Behçet's disease (BD) some manifestations are seen more frequently in men than in women. In our last analysis of 4717 BD patients (also included in this book), ocular manifestations were seen more frequently in men than in women (62% men, 49% women, chi square: 105.950, p<0.00000l). It is also widely accepted that men suffer from the severer course of disease than women, and their outcome is worse[1-2].

The aim of this study was to investigate if men had more severe ocular manifestations, and if their responsiveness to the treatment, and their outcome were worse than in women.

2. MATERIALS AND METHODS

All BD patients having ocular manifestations and being treated consequently, are registered in the Behçet's Disease Ocular Treatment Registry (BDOTR). In January 2002, there were 1009 patients who received 1274 different treatment protocols. Patients resistant to one treatment protocol were switched to another one, and further, if still non-responding. These treatment protocols were: High dose pulse cyclophosphamide (PCP) 359 patients, low dose pulse cyclophosphamide (LDP) 181 patients, oral cyclophosphamide (OCP) 41 patients, methotrexate (MTX) 348 patients,

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chlorambucil (CHL) 89 patients, cyclosporine A (CyA) 24 patients, azathioprine (AZA) 106 patients, combination with LDP-MTX (LDP-MTX) 91 patients, high dose MTX (HMTX) 23 patients, and combination of LDP and AZA (LDP-AZA) 12 patients.

It was previously demonstrated that all different protocols had approximately the same efficacy on ocular lesions in BD[3-5]. Therefore, all patients of the registry were enrolled in this study.

Patients were divided into male and female. In each group the visual acuity (VA) before and after the treatment was calculated by the Snellen chart on a scale of 10/10. An activity index was calculated for anterior uveitis (AU), posterior uveitis (PU), and retinal vasculitis (RV) upon the inflammatory state of each eye. Each of the inflammatory parameters was graded from zero (none) to 4 (highest degree of inflammation). These indices were determined as follows. For AU: cells, flares, keratic precipitates, and hypopyon. For PU: cells, snow ball and snow banking. For RV: periarteritis, periphlebitis, edema of disk, edema of macula, edema of retina, papillitis, and active peripheral lesions.

Student t test was used to compare the mean VA and different activity indices.

The mean VA, AU, PU, and RV before the treatment were compared in male and female patients to investigate if males presented a more severe involvement than females. Then, the mean improvement for each parameter was compared in male and females patients to find if males had the more resistant disease to treatment than females.

3. RESULTS

3.1 Group comparison at the entry

Both groups were similar regarding the mean duration of the eye disease (33 months for men versus 32.9 months for women) and the mean treatment time (18.5 months for men versus 20.6 months for women).

3.2 Severity of eye lesions in men and women before the treatment

In men, 212 eyes had a normal visual acuity (VA) before the treatment and remained normal during the treatment, while 946 eyes had an impaired VA and were selected for the calculations. The mean VA before the treatment was 3.6/10, the standard deviation (SD) was 3.6, and the

confidence interval (CI) at 95% was 0.2. In women, 128 eyes had a normal VA before the treatment and remained normal during the treatment, while 643 eyes had an impaired VA and were selected for the calculations. The mean VA was 4.2/10, SD was 3.6, and CI was 0.3. Comparison of the VA between men and women by the Student t test demonstrated a t value of 3.259 with a degree of freedom (DF) of 1587. The difference was statistically significant with a p value of 0.0012 (Table 1).

Table 1. Severity of eye lesions in men and women

	MEN	*WOMEN*	P
Visual Acuity	3.6	4.2	0.0012
Anterior Uveitis	2.5	2.4	0.51
Posterior Uveitis	2.1	2.0	0.20
Retinal Vasculitis	2.5	2.3	0.19

Anterior uveitis (AU) was absent in men in 575 eyes before the treatment and remained absent during the treatment, while 577 eyes had AU and were selected for the calculations. In women, AU was absent in 381 eyes before the treatment and remained absent during the treatment, while 391 eyes had AU and were selected for the calculations. In men the mean activity index for AU before the treatment was 2.5, SD was 2.4, and CI was 0.2. In women the mean AU was 2.4, SD was 2.2, and CI was 0.2. Comparison of AU between men and women by t test showed a t value of 0.657 with a DF of 966. The difference was not statistically significant, the p value was 0.51.

Posterior uveitis (PU) was absent in men in 245 eyes before the treatment and remained absent during the treatment, while 809 eyes had PU and were selected for the calculations. In women, PU was absent in 184 eyes before the treatment and remained absent during the treatment, while 526 eyes had PU and were selected for the calculations. In men the mean activity index for PU before the treatment was 2.1, SD was 1.5, and CI was 0.1. In women the mean PU was 2, SD was 1.2, and CI was 0.1. Comparison of PU between men and women by t test showed a t value of 1.284 with a DF of 1333. The difference was not statistically significant, the p value was 0.20.

Retinal vasculitis (RV) was absent in men in 299 eyes before the treatment and remained absent during the treatment, while 649 eyes had RV and were selected for the calculations. In women, RV was absent in 237 eyes before the treatment and remained absent during the treatment, while 410 eyes had RV and were selected for the calculations. In men the mean activity index for RV before the treatment was 2.5, SD was 2.4, and CI was 0.2. In women the mean RV was 2.3, SD was 2.5, and CI was 0.2. Comparison of RV between men and women by t test showed a t value of 1.298 with a DF of 1057. The difference was not statistically significant, the p value was 0.19.

3.3 Improvement of eye lesions in men and women after the treatment

The mean VA in men improved from 3.6 to 4.4. Comparison of the VA before and after the treatment by the Student paired t test demonstrated a t value of 7.332. The difference was statistically significant with a p value <0.000001 (Table 2). The mean VA in women improved from 4.2 to 5.2. Comparison of the VA before and after the treatment by the Student paired t test demonstrated a t value of 6.838. The difference was statistically significant with a p value <0.000001. The mean improvement in VA for men was 0.8 and for women 1.

Table 2. Mean improvement after the treatment in men and women

	MEN	*WOMEN*
Visual Acuity	0.8	1
Anterior Uveitis	1.7	1.7
Posterior Uveitis	1.1	1.2
Retinal Vasculitis	1	0.9

The mean AU in men improved from 2.5 to 0.8. Comparison of AU before and after the treatment by the Student paired t test demonstrated a t value of 14.027. The difference was statistically significant with a p value <0.000001. The mean AU in women improved from 2.4 to 0.7. Comparison of AU before and after the treatment by the Student paired t test demonstrated a t value of 12.31. The difference was statistically significant with a p value <0.000001. The mean improvement in AU for men was 1.7 and for women 1.7.

The mean PU in men improved from 2.1 to 1. Comparison of PU before and after the treatment by the Student paired t test demonstrated a t value of 19.47. The difference was statistically significant with a p value <0.000001. The mean PU in women improved from 2 to 0.8. Comparison of PU before and after the treatment by the Student paired t test demonstrated a t value of 19.612. The difference was statistically significant with p<0.00000l. The mean improvement in PU for men was 1.1 and for women 1.2.

The mean RV in men improved from 2.5 to 1.5. Comparison of RV before and after the treatment by the Student paired t test demonstrated a t value of 8.759. The difference was statistically significant with a p value <0.000001. The mean RV in women improved from 2.3 to 1.4. Comparison of RV before and after the treatment by the Student paired t test demonstrated a t value of 6.552. The difference was statistically significant with a p value <0.000001. The mean improvement in RV for men was 1 and for women 0.9.

4. DISCUSSION

Since a long time, it is widely accepted that men present a more severe involvement of BD than women[1-2]. Our analysis showed that men suffered from severer impairment of their visual acuity than women before treatment. The mean difference was 0.6 on a scale of 10/10. The difference was statistically significant. However, a mean difference of less than one line on the Snellen chart is of no clinical relevance. The difference in mean inflammatory activity indices (AU, PU, RV) were identical in men and women before treatment. The similarities of the activity indices demonstrate that there is no difference in the severity of eye lesions between men and women.

The mean improvement for VA, AU, PU, and RV was approximately the same in men and women. The similarity in the improvement of the above parameters in men and women demonstrate that men's response to the treatment is as good as women's response. Men are not more resistant to the treatment than women.

5. CONCLUSION

Men and women have the same severity (inflammatory indices) and the same outcome (treatment improvement) of eye lesions. The slight difference observed in visual acuity was of no clinical relevance.

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The Implications of Nonaphthous Beginning of Behçet's Disease

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1. INTRODUCTION

Although recurrent oral ulceration (oral aphthae, OA; Table 1) is the earliest and most common manifestation of Behçet's disease (BD) at onset (aphthous beginning, AB) and during the course, less commonly it is absent initially (non-aphthous beginning, NAB). As we reported previously, NAB was established in almost 30% of our 331 cases of BD[1]. This was confirmed by many authors from different countries with a prevalence rate of 15-50%[2].

The purpose of this study was to reevaluate the characteristics of patients with NAB and AB at onset and during the course of the disease, and establish its value in the diagnosis of BD.

2. PATIENTS AND METHODS

We analyzed 598 patients with BD diagnosed according to our new set of diagnostic criteria[2] (379 men, 219 women, M:F 1.73) regarding to NAB (28.6%) and AB (71.4%). We documented the demographic and clinical features at onset and during the course.

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Table 1. Abbreviations used

BD	Behçet's disease	PP	Populopustular lesions
NAB	Nonaphthous beginning	AG	Arthralgia
AB	Aphthous beginning	PA	Peripheral arthritis
PAP	Preaphthous phase	AS	Ankylosing spondylitis
OA	Oral aphthae	CNS	Central nervous system
GU	Genital ulceration	GIS	Gastrointestinal system
TP	Thrombophlebitis	AMY	Amyloidosis
EN	Erythema nodosum	VOI	Vital organ involvement

3. RESULTS

Male predominance was found to be much higher in patients with NAB (M:F was 2.1 vs 1.6). The duration of pre-aphthous phase (PAP) (the time lapsed between the onset of NAB and the onset of OA) was shorter in males (mean 3.5 ± 3.4 yrs, range 0.5-15 yrs vs mean 7.2 ± 7.2 yrs, range 0.5-22 yrs). Juvenile onset (<16 yrs) of BD was found to be more often in females with NAB than AB (30.9% vs 14.6%).

In 85% of patients with NAB, BD started with a single symptom, whereas with 67% of patients with AB the disease started with OA alone.

Table 2 gives the characteristics of the manifestations.

Table 2. Main manifestations in non-aphthous beginning (NAB) and aphthous beginning (AB) groups

	AT ONSET			DURING THE COURSE		
	NAB (n=171)	AB (n=427)	TOTAL (n=598)	NAB (n=171)	AB (n=427)	TOTAL (n=598)
ROU	-	100 (1)	71.4 (1)	98.3(1)	100(1)	99.5 (1)
GU	19.3 (3)	23.4 (2)	22.2 (2)	78.9(3)	78.7(2)	78.8 (3)
SKIN	29.2* (2)	12.4 (3)	17.2 (4)	86.6(2)	77.0(4)	79.7 (2)
EYE	11.7* (5)	5.9 (4)	7.5 (5)	56.7(5)	50.1(5)	52.0 (5)
JOINT	39.2** (1)	2.8 (6)	12.5 (3)	70.2(4)	70.7(3)	70.5 (4)
TP	16.4** (4)	3.5 (5)	7.2 (6)	47.9(6)	31.8(6)	36.4 (6)
LUNG	-	-	-	16.4	17.5	17.2
CNS	0.58	-	0.17	16.9	6.1	7.5
GIS	-	-	-	3.5	4.4	4.1
EO	-	-	-	2.6	3.4	3.1 (male)
AMY	-	-	-	3.5	0.7	1.5
VOI	25.2**	9.6	14.4	68.4***	53.3	57

*p < 0.05, ** p < 0.001, *** p < 0.01, ( ) : order of frequency

The order of frequency of the initial manifestations in cases of NAB was as follows: joint, skin, genital ulceration, eye, and thrombophlebitis. This was oral aphthae, genital ulceration, skin, eye, thrombophlebitis, and joint in AB group. The prevalence of these manifestations were much higher in NAB group. Patients in NAB group developed more vital organ involvement (VOI) at onset and during the course of the disease. The details of skin and joint involvement are given in Table 3.

Table 3. Details of skin and joint involvement (%)

	AT ONSET			DURING THE COURSE		
	NAB	AB	TOTAL	NAB	AB	TOTAL
SKIN	29.2*	12.4	17.2	86.6	77.0	79.7
a) EN	12.3**	3.7	6.2	28.0	18.0	20.9
b) PP	15.8**	6.3	9.0	43.8	49.4	47.8
c) a+b	1.2	2.3	2.0	14.0	9.6	10.8
JOINT	39.2**	2.8	12.5	70.2	70.7	70.5
AG	10.5**	0.9	3.7	21.0	22.5	22.1
PA	26.3**	1.9	8.8	47.4	46.8	46.9
AS	0	0	0	11.1	7.2	8.36

*: $p < 0.01$, **: $p < 0.001$
AS: ankylosing spondylitis (all but 9 associated with peripheral arthritis)

Family history for BD was obtained more often in AB group. HLA-B5 or B51 determined in 22 patients with NAB and 67 with AB did not show any significant association. Table 4 shows the statistical significance of the more prevalent initial manifestations in NAB and AB groups.

Table 4. More prevalent initial manifestations in NAB and AB groups

MORE IN NAB		MORE IN AB	
IN TOTAL	P	IN TOTAL	P
EN	0.001	GU	NS
PP	0.001		
TP	0.001		
PA	0.001		
EYE	0.05		
VOI	0.001		
AS GENDER		AS GENDER	
in NAB WOMEN > NAB MEN		in AB WOMEN > AB MEN	
EN	0.001	EN	NS
in NAB MEN > NAB WOMEN		in AB MEN > AB WOMEN	
GU	NS	EYE	NS
EYE	NS	PP	0.05
PP	0.05	TP	0.05
TP	0.05	PA	NS

4. DISCUSSION AND CONCLUSIONS

As a continuation of our previous study[1], this is the largest and only one in regard of non-aphthous beginning and its implications in BD.

We had objected to ISGBD diagnostic criteria[3] because of the acceptance of OA as a sine qua non and exclusion of TP, therefore we proposed a new diagnostic criterion in Seoul[2]. World literature and our studies confirm our above view.

Main items of our findings from this study are as follows:

NAB was found in 28.6% of our cases.

M:F was higher in NAB group. PAP is shorter in males. Beginning of BD with one symptom was more frequent in NAB group.

VOI was more common in NAB patients.

The prevalence of all initial manifestations except GU was more frequent in NAB.

We conclude that NAB in BD is an important phenomenon. This study may intrigue us to find a new hypothesis in explaining its etiopathogenesis, and its role on the clinical course and prognosis of BD.

Since OA is a sine qua non in diagnostic criteria of ISGBD one is destined to miss a correct diagnosis for almost 6 years in 20-30% of cases of NAB until the development of OA.

We believe that our new set of diagnostic criteria, not considering OA as an indispensible criterion and accepting TP as another one, is simpler and more reasonable than ISGBD criteria.

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Clinical Manifestations and Course of 200 Japanese Patients with Behçet's Disease

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1. INTRODUCTION

Behçet's disease (BD) is an inflammatory disease in which various symptoms appear. The time of onset and the combination of symptoms differ by individual patients[1-4]. We have conducted a study by questionnaire using 1400 members of the BD Society in order to clarify the natural history of the disease. Of approximately 900 responses that were obtained we examined the time of onset of each symptom, incidence, and the combination of symptoms of individual cases of 200 patients. The findings of 200 patients with BD are hereby reported as the first report.

2. PATIENTS AND METHODS

Data were collected from patients by questionnaire: clinical course and the time of onset of several manifestations which were ocular symptoms, oral aphthae, skin lesions, genital ulcers, arthritis, epididymitis, gastrointestinal lesions, vascular lesions, and neurological manifestations. We examined the time of onset of each symptom, incidence, and the combination of symptoms of 200 patients. Kaplan-Meier method was used for the analysis of incidence of each symptom.

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3. RESULTS

Of the 200 patients, 104 were male and 96 were female. According to the Japanese diagnosis criteria, 103 had the complete type, 82 had the incomplete type, and 15 were suspected of having Behçet's disease. Complications of gastrointestinal lesions were observed in 41 patients, vascular lesions were observed in 18 patients and neurological manifestations were observed in 29 patients. Classification of the types based on symptom combination shown by the incomplete type revealed that 34 patients had combinations of oral aphthae, skin lesions, and genital ulcers; and 28 patients had combinations of eye lesions, oral aphthae, and skin lesions, which accounted for 75% of the incomplete types.

The cumulative incidence for oral aphthae was 56%, and for skin lesions 43% in patients in their thirties. For ocular symptoms, genital ulcer was 30% in patients in their thirties, respectively. The cumulative final incidence of oral aphthae was 95%, skin lesion was 90%, and eye lesions and genital ulcers was 80%, respectively. In comparison to other symptoms, the time of onset of neural, gastrointestinal, and vascular symptoms was delayed, being less than 10% in patients in their thirties. The cumulative final incidence of arthritis was 72%, epididymitis was 28%, gastrointestinal lesions was 25%, vascular lesions was 20%, and neurological manifestations was 11%, respectively. Complications of neurological symptoms were seen in 29 patients, which comprised a case group with ocular symptoms in 25 patients, and a case group without ocular symptoms in 4 patients. The cumulative final incidence for neurological manifestations with eye lesions was 25%, and for neurological lesions without eye lesions 5% in patients, which demonstrated a link between ocular and neurological symptoms.

4. CONCLUSION

BD is a multisystemic inflammatory disorder with heterogeneous clinical features. The time of onset of oral aphthae was earlier than any other manifestation, and final incidence was 95% of our patients, and incidence was 56% in their thirties. The time of onset of several other clinical manifestations, including uveitis, skin lesions, genital ulcers and arthralgia was delayed, being 30 to 40% in patients in their thirties. Oral aphthae was a different characteristic symptom as opposed to another clinical manifestation in BD. The cumulative final incidence of neurological symptoms was 11%, comprising a case group with ocular symptoms (25%) and a case group without ocular symptoms (5%) which demonstrated a link between ocular and neurological symptoms.

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Clinical Presentation of Behçet's Disease in Internal Medicine

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1. INTRODUCTION

The clinical presentation of Behçet's disease varies from a full blown inflammatory disease with all classical manifestations to a condition with minimal symptoms. In the majority of Behçet cases, because of the predominance of the muco-cutaneous signs and symptoms, the first physician to be consulted is a dermatologist. However when an internist is consulted, it is usually for a more severe problem as arthritis, phlebitis, etc. At this moment the mucocutaneous diagnostic criteria might be absent or not sufficiently prominent among the other clinical manifestations. Therefore the internist must be aware of the different possible initial symptoms and signs in order to consider the diagnosis of the Behçet's disease early enough.

The major aim of our study was to evaluate these initial clinical presentations in 56 cases finally diagnosed as Behçet's disease at the time of their first medical visit by an internist or at first hospitalisation in an internal medicine department.

2. MATERIALS AND METHODS

In a retrospective study we determined the clinical presentation of 56 cases of Behçet's disease seen in two internal medicine departments in the

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south-west of France from 1992 to 2001. All cases had finally fulfilled the diagnostic criteria of the "International Study Group for Behçet's Disease", although this took a long time for many of them.

Frequencies of various symptoms and signs leading to a first medical visit at an internist or hospitalisation were evaluated.

3. RESULTS

The patients were 41 women and 15 men with 36.9 years mean age (15-56) at the time of disease onset. The frequency of the different symptoms is as follows: Oral ulcers 66%, arthritis (oligoarthritis or polyarthritis) 53%, central nervous system involvement (aseptic meningitis, hydrocephalus, cerebral vasculitis etc.) 25%, genital ulcers 19%, skin lesions (pseudo-folliculitis, erythema nodusum, etc.) 19%, fever (usually recurrent and periodical) 17%, eye lesions (anterior or posterior uveitis, retinal vasculitis, papillary oedema, optic neuritis, etc.) 16%, gastro-intestinal problems 16%, pericarditis 3%, myalgia 3%, and anaemia 3%. The HLA B51 was detected in 60% of the patients.

In 23 patients (41%) a diagnostic delay with an average of 6 months (2 months to 14 years) was observed. This was mainly because of the absence of oral ulcers and/or insufficient diagnostic criteria in the beginning of the disease. In one case with 13 years of recurrent uveitis and intermittent oral ulcers, a positive pathergy test completed the diagnostic criteria. If this test had been done earlier the diagnostic delay could have been decreased significantly.

4. CONCLUSION

Even in internal medicine oral ulcers are the most frequent presentation of Behçet's disease, however they are not necessarily present at the very early stages. To avoid the diagnostic delay that results from a late appearance of the complete diagnostic criteria, the internist must think of the diagnosis of Behçet's disease in cases of arthritis, and neurological and gastrointestinal problems. Using the associated elements as ethnicity, and the presence of HLA B51 might also be helpful. Although actually rarely used in the western world, the pathergy test can complete the diagnostic criteria when positive, and reduce the diagnostic delay in certain cases.

The Change of Clinical Manifestations of Patients with Behçet's Disease in Japan

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1. INTRODUCTION

Behçet's disease is widespread in a number of countries world-wide, especially in Asian, Eurasian, and Middle Eastern countries along the Silk Road. Japan is at the eastern edge of this area and the estimated number of patients in Japan is about 20,000. The purpose of this study is to examine the change of clinical manifestations of Behçet's disease at our clinic.

2. PATIENTS AND METHODS

We reviewed patients with Behçet's disease whose first visit to our clinic was between 1970 and 2000. We compared clinical manifestations of early patients with those of recent patients. The diagnosis of Behçet's disease is made according to criteria established by the Behçet's Disease Research Committee of Japan.

3. RESULTS AND DISCUSSION

Until the mid 1980's, 20 to 35 new patients visited our clinic each year. However, after 1987, only about 10 new patients visited our clinic each year.

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Thus, the number of new patients has obviously been declining recently. The etiological incidence of Behçet's disease in endogenous uveitis was 32.2% in 1981. Behçet's disease was the most frequently encountered form of endogenous uveitis at our clinic by 1999. However, it only accounted for 19.0% in 2000, whereas sarcoidosis accounted for 20.3%.

In the recent study, oral aphthae were seen in 99%, eye lesions in 73%, skin lesions in 80%, and genital ulcers in 59% of the patients. The patients with all 4 major symptoms were classified under the complete type. In the recent study, 29% of the patients were classified as having the complete type, and 71 % of the patients as having the incomplete type. The ratio of patients with the complete type has decreased in comparison with the previous study. This tendency was particularly marked in male patients. The ratio of male patients with the complete type was 56% in the previous study, while it was only 16% in the recent study.

We evaluated the visual prognosis of the patients who had been attending follow-up sessions for at least 5 years at our clinic. The visual prognosis of patients who first visited our clinic between 1985 and 1994 and that of patients who first visited our clinic between 1975 and 1979 were compared. Behçet's disease is a chronic disease. Therefore, a 5-year follow-up is the minimum necessary for evaluating visual prognosis. In 1984 cyclosporine was introduced to Japan for the treatment of patients with Behçet's disease. Therefore, therapy differed between the 2 groups. In the recent study, 30% of patients was given cyclosporine. The visual prognosis in recent cases looks better than that in previous cases after 5 years of observation. In any case, the visual acuity of 27% of patients' eyes fell to 0.1 or less after 5 years in the recent study. The visual acuity of female patients was much better than that of male patients. Only 16% of patients' eyes showed a visual acuity at 0.1 or less after 5 years in the recent study. This result is almost identical with that from the previous study.

4. CONCLUSION

The incidence of Behçet's disease has been decreasing recently at our clinic. Still, this disease remains to be a big threat to vision.

Chronology of Clinical Manifestations in Behçet's Disease

Analysis of 4024 cases

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1. INTRODUCTION

Behçet's disease (BD) is a multi-systemic disease with heterogeneous clinical features[1]. Its manifestations appear one after another during the course of the disease with attacks and remissions[2-4]. Some involvements such as mucous lesions seem to appear early, and others such as gastrointestinal (GI), vascular, and central nervous system (CNS) involvements many years later[5,6].

The aim of this study was to investigate the chronology of different manifestations of BD during its course.

2. MATERIALS AND METHODS

In a retrospective study, we reviewed the medical records of 4024 patients with BD registered at our BD Unit during the past 14 years (between October 1988 and June 2002) to determine the time of appearance of different manifestations in the course of their diseases. The symptoms of the disease had been recorded in the time order of manifestations for each patient.

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The data presented here differ and are higher in a population than those in the abstract. The mean delay for each item was determined. A confidence interval (CI) at 95 percent for each item, and a standard deviation (SD) for the means and the percentages were calculated. The comparisons were made by chi square test.

3. RESULTS

3.1 Time of appearance of different symptoms

The mean delay for development of oral aphthosis after onset of the disease was 0.4 year (CI:0.1, SD:1.7). It was 3.8 years (CI:0.2, SD:5.8) for genital aphthosis, 3.6 years (CI:0.2, SD:5.1) for skin lesions, and 4.3 years (CI:0.3, SD:6.5) for ocular lesion.

The mean delay time for appearance of minor symptoms was as follows: 3.8 years (CI:0.3, SD:5.1) for joint involvement, 4 years (CI:0.7, SD:5.5) for vascular lesions, 3.3 years (CI:0.7, SD:5.1) for CNS involvement, and 5 years (CI:1.1, SD:9.3) for GI involvement. For male patients the mean delay time for appearance of epididymitis was 3.2 years (CI:0.6, SD:4.7).

3.2 First manifestation

Oral aphthosis was the most frequent presenting sign seen in 80.2% (CI:1.1) of the cases. Genital aphthosis in 10.3% (CI:0.9), ocular lesions as uveitis in 9.7% (CI:0.8), and retinal vasculitis in 0.3% (CI:0.2), joint involvement in 5.3% (CI:0.6), and the other manifestations (mostly skin lesions) in 8.1% (CI:0.8) were the other initial manifestations of the disease.

3.3 First year incidence of different symptoms

In the first year of the disease, the incidence of oral aphthosis reached up to 85.4% (CI:1.1) in this study. The incidence was 17.8% (CI:1.2) for genital aphthosis, 18.9% (CI:1.2) for skin lesions, and 13.6% (CI:1.1) for ocular lesions.

The first year incidence for minor manifestations was as follow: 9.3% (CI:0.9) for joint involvement, 2.5% (CI:0.5) for vascular lesions, 2.3% (CI:0.4) for CNS involvement, 2.3% (CI:0.4) for GI involvement, and 5.3% (CI:0.9) for epididymo-orchitis in male patients.

3.4 Time of appearance of different manifestations after the first year of onset

Regarding the previous point, the mean delay for the appearance of various symptoms differs. For example, if oral aphthosis had not appeared in the first year of onset, the mean delay time for its appearance would be 3.8 years (CI:0.3, SD:3.5) instead of 0.4 year. For other symptoms it was longer: 5.3 years (CI:0.3, SD:6) for genital aphthosis, 5.2 years (CI:0.3, SD:5.4) for skin lesions, and 5.9 years (CI:0.3, SD:6.8) for ocular lesions.

This was even longer for minor manifestations of the disease: 5.8 years (CI:0.4, SD:5.3) for joint involvement, 6.5 years (CI:0.9, SD:5.6) for vascular lesions, 6.2 years (CI:1.1, SD:5.5) for CNS involvement, and 7.7 years (CI:1.6, SD:10.3) for GI involvement. In male patients with BD, the mean delay for the appearance of epididymo-orchitis was 5.5 years (CI:0.8, SD:5.4).

3.5 Prevalence of different symptoms during the course of disease

Prevalence of different symptoms of BD changed during consecutive years of its progression. Table 1 shows these changes in the prevalence of each symptom in the first year after the onset of the disease, and in different time intervals thereafter.

Table 1. Prevalence of various manifestations during progression of the disease

	0-1	1-3	3-5	5-10	10-15	15-20	>20
Oral aphthosis	85.4	90.9	93.4	95.8	96.4	96.6	96.6
Genital aphthosis	17.8	34.9	45.3	56.5	61.7	62.8	63.8
Skin lesions	18.9	35	43.5	53.5	57.6	58.9	60
Ocular lesions	13.6	26	34.5	44.5	47.9	49.7	51.2
Joint involvement	9.3	15.7	19.3	24.3	26.5	27.2	27.8
Thrombophlebitis	2.5	3.6	4.3	5.6	6.1	6.3	6.5
CNS involvement	2.3	3	3.5	4.4	4.7	4.8	4.9
GI involvement	2.3	3.1	3.9	5.3	5.9	6.1	6.4
Epididymo-orchitis	5.3	7.6	9.6	11.5	12	12.3	12.6

These changes are shown in Fig. 1 for major symptoms and in Fig. 2 for minor manifestations. Arrows mark the time when the prevalence of each symptom reached up to 95% of its actual frequency. It is interesting to note that except for oral aphthosis and gastrointestinal involvement, this time interval was around 15 years for most symptoms. For oral aphthosis it was much earlier (between 3-4 years), and for GI manifestations it was later (between 18-19 years). The period required to reach up to 90% of their

actual frequency was around 10 years for most symptoms (not shown in the figures).

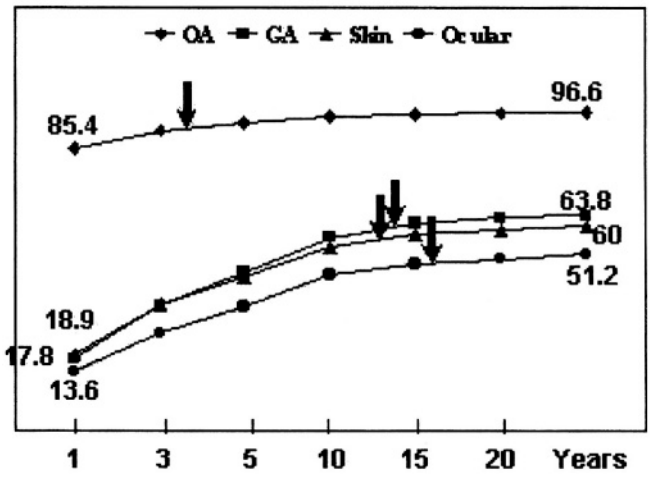

Figure 1. Prevalence during progression (Major manifestations)

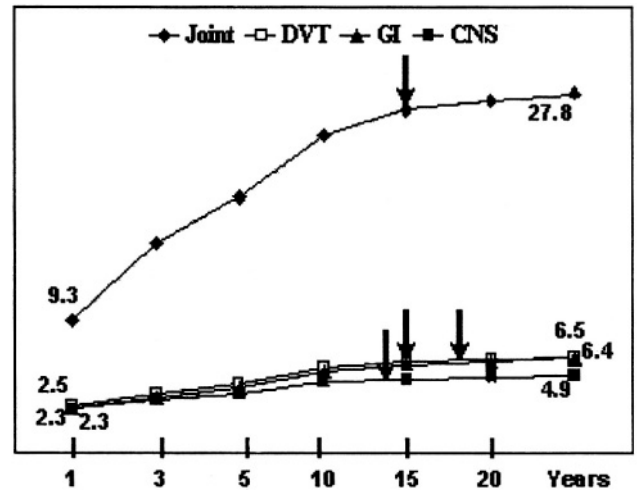

Figure 2. Prevalence during progression (Minor manifestations)

We can interpret this point in different way. If we study these patients for the frequency of various manifestations in different time intervals after the disease onset, another interesting result may be achieved. This is due to the nature of BD, and the delay in appearance of its various manifestations. It is

interesting that after 10 to 15 years, the results did not change significantly and a rather steady state was reached.

4. DISCUSSION

This study showed different time delay for the appearance of various manifestations of BD. It was short for oral aphthosis, and rather long for symptoms such as GI involvement.

A fully established disease, regarding the full appearance of symptoms in 90 to 95% of cases, was reached between 10 to 15 years after the appearance of the first manifestation of the disease.

These results emphasize the importance of follow-up time in clinical studies on BD, and may explain partly (if not to say mainly) the cause of differences in the prevalence of symptoms in different reports on BD patients[1,4,7]. Perhaps we may suggest a mean disease duration of at least 10 years after the disease onset in such studies for their better reliability.

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General Aspects and Clinical Manifestations of 100 Iraqi Patients with Behçet's Disease

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1. INTRODUCTION

Behçet's disease (BD) is a chronic multisystemic inflammatory disorder with mysterious aetiology. The prevalence varies widely from country to country, from race to race, and seems to be strongly dependent on the longitude or latitude of residence[1,2]. BD usually occurs around the third decade of life; concerning male / female ratio, studies denote different results[3,4,5]. The aim of this study was to shed light on the clinical patterns of BD in 100 Iraqi Arab patients with the general demographic data.

2. PATIENTS AND METHODS

One hundred Arab Iraqi patients with BD were involved in this study while attending the multi-discipline BD Clinic at Baghdad Teaching Hospital from Sept. 1999-June 2001. They all fulfilled the ISG criteria for diagnosis of BD.

Results were expressed as mean ± standard deviation, percentage and ratio, with chi square test for comparisons.

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3. RESULTS

Demographic features of these patients were as follows: 65% of patients were from Baghdad. Male/female ratio was 2.6, mean age was 26.38 years, mean age of onset was 27.5 years, mean duration of the disease was 9.3 years, and frequency of family history was 40% (Table 1).

Table 1. Demographic features of 100 Iraqi patients with Behçet's disease (BD)

Total number of BD patients	100
Number of BD patients from Baghdad	65
Number of BD patients from other provinces	35
Number of males	72
Number of females	28
Male/Female ratio	2.6
Age (mean ±SD) years	36.8±11.5
Age of onset (mean±SD) years	27.5±9.3
Duration of disease (mean ±SD) years	9.3±6.3
Family history of BD	40.0%

Distribution of cases by age, age of onset and sex is presented in Tables 2 and 3. The overall frequency of clinical manifestations is clearly shown in Fig. 1.

Table 2. Distribution of Behçet's disease (BD) patients by age and sex

Age Groups (years)	Sex		Total No. (%)
	Male No. (%)	Female No. (%)	
10-19	3 (4.2)	1 (3.6)	4 (4.0)
20-29	19 (24.6)	5 (17.9)	24 (24.0)
30-39	28 (38.9)	11 (39.3)	39 (39.0)
40-49	12 (16.7)	8 (28.6)	20 (20.0)
50-59	6 (8.3)	2 (17.1)	8 (8.0)
60-69	3 (4.2)	1 (3.6)	4 (4.0)
70-79	1 (1.4)	0 (0.0)	1 (1.0)
Total	72 (100.0)	28 (100.0)	100 (100.0)

4. DISCUSSION

In this work demographic data are similar in one way or another to those reported abroad and locally[2,3,4,5].

Clinically, manifestations were somehow comparable to those previously reported in Iraq, with exception the high occurrence of joint involvement, on

which should be reflected: Is it possibly a result of polyclonal B-cell activation? Whereas polyclonal autoantibodies is one of the predominant manifestations of BD[6], hyperproteinemia, which is clearly observed in these patients, has been associated with polyclonal B-cell activity[6,7]. Hypoalbuminemia is another finding in BD patients[6].

Meanwhile, increase of ALP activity and decrease in selenium concentration could be considered as additional factors to high occurrence of joint manifestation[6,8]. Furthermore, joint involvement occurs more frequently in females (Fig. 1), which may be due to many factors, among them hormonal factors and physiological specificity[9,10].

Table 3. Distribution of Behçet's disease (BD) patients by age of onset and sex

Age Groups of onset (years)	Sex		Total No. (%)
	Male No. (%)	Female No. (%)	
10-19	14 (9.4)	6 (21.4)	20 (20.0)
20-29	33 (45.8)	8 (28.6)	41 (41.0)
30-39	17 (23.6)	12 (42.9)	29 (29.0)
40-49	6 (8.3)	1 (3.6)	7 (7.0)
50-59	1 (1.4)	1 (13.6)	2 (2.0)
60-69	1 (1.4)	0 (0.0)	1 (1.0)
Total	72 (100.0)	28 (100.0)	100 (100.0)

5. CONCLUSIONS

Most patients were in the third and fourth decades of life, males were stronger affected than females, age of onset and gender did not significantly influence the disease expression in Iraqi patients; higher frequency of familial occurrence and of joint involvement were clearly observed

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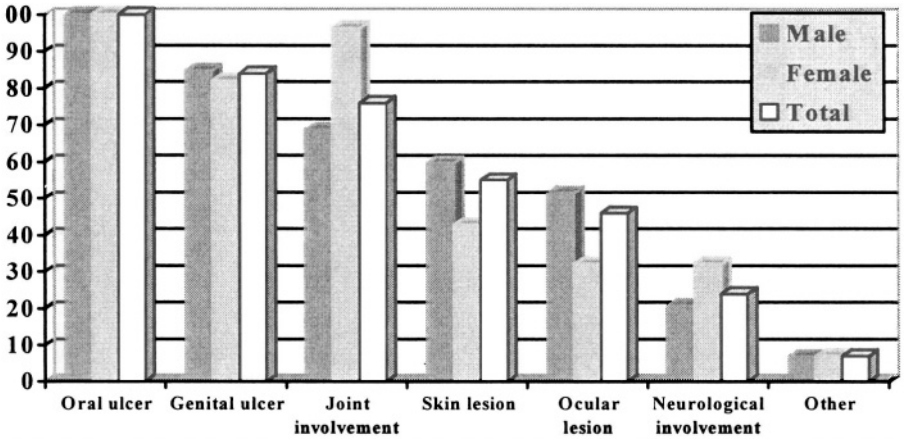

Figure 1. Frequencies of the main clinical features in 100 Iraqi patients with BD.

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The Prevalence of Behçet's Syndrome and Its Neurological Complications in Hertfordshire, U.K.

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1. INTRODUCTION

The prevalence of Behçet's syndrome in countries of high disease incidence, along the eastern shores of the Mediterranean and Middle East and eastern Asian countries, is of the order of 5–10 per 100,000 inhabitants[1,2]. New epidemiological data from these countries have been presented during the 10th International Conference on Behçet's Disease in Berlin, but there have only been regional formal studies of the prevalence of the syndrome in Western Europe or North America[3].

Additional prevalence data on the neurological complications which arise come from hospital based retrospective studies, and these papers suggest that neurological involvement occurs in some 3–16% of cases[4-6], although an autopsy series showed pathological evidence for neurological involvement in 20%[7].

Epidemiological data are crucial to the understanding of the natural history and prognosis of the syndrome in different populations and to the planning of treatment trials.

2. METHOD

Hertfordshire is county adjacent to London which is predominately rural but with towns of high density consisting mainly of London commuters. The population is of the order of one million people and the ethnic subgroups are

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85% white Europeans, 5% Indian subcontinent, 6% black African or Carribean, and 4% Oriental.

All general practitioner surgeries were invited to perform a database search for patients diagnosed as having Behçet's syndrome. Patients identified were sent a letter of introduction in which they were asked to consent to being approached by the investigator in order to identify the clinical syndrome, confirm Behçet's syndrome and to identify whether or not they had had neurological symptoms. Those who had were invited to attend for a neurological examination. All other communication with subjects was carried out by telephone or by letter. Additional data were acquired by hospital medical records department searches. All communications conformed to the data protection act of Great Britain. The study was approved by the North London MREC.

3. RESULTS

There are 141 GP surgeries in Hertforshire and data were made available by 56 (40%) of them. This translates to a study population of about 400,000. Twenty patients with Behçet's syndrome defined by the ISG criteria were identified. Eight patients had had neurological symptoms, of whom three were considered by the investigator to have neurological involvement by Behçet's syndrome. This gives a minimum prevalence of Behçet's syndrome in Hertfordshire of 5 per 100,000 inhabitants and a prevalence of neurological complications of 0.75 per 100,000 inhabitants.

4. CONCLUSION

Behçet's syndrome is uncommon in Hertfordshire and the prevalence is less than that in areas of the Silk Route but not as low as was previously thought. Since this county is typical of other UK counties, it is possible to surmise that the data reflect a national prevalence in the UK of some 1500 patients with the systemic manifestations of the disease and some 225 with neurological complications.

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Clinical and Genetic Characteristics of Late-Onset Behçet's Disease

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1. INTRODUCTION

In approximately 80% of patients with Behçet's disease (BD), the first symptom occurs between the age of 16 to 35 years, and in 97% of patients before the age of 40 (Shizumi, unpublished data). Onset of the disease after age 40 is relatively rare. We evaluated the clinical spectrum of patients with BD in whom the disease manifested after age 40 or more, as well as the genetic factors that may be associated with this late expression.

2. PATIENTS AND METHODS

BD was defined according to the ISG criteria. Data from medical files and from patient interviews were collected. The results were analyzed separately for adults and late-onset patients with BD. Adult BD was defined if the first disease manifestation appeared between the ages of 18 and 40 years. Late-onset disease was defined if onset of BD symptoms occurred after the age of 40. Severity score was calculated as the sum of 1 point for each of mild symptoms, 2 points for each of moderate symptoms and 3 points for each of severe disease manifestations, according to Krause et al[1]. The HLA-A, B and C tissue typing were performed as previously described[2]. Statistical analysis was performed employing Student's *t*-test for mean values and chi square (χ^2) for table analysis.

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3. RESULTS

Our study included 67 BD patients: 26 males (39%) and 41 females (61%). Thirteen patients (19.4% of all our BD patients) were identified as having late-onset BD.

3.1 Clinical manifestations of adult-onset BD

Oral aphthosis was the most common initial disease manifestation. All 13 patients presented first with recurrent aphthous stomatitis. Genital ulcers appeared in all 13 patients. Major eye involvement occurred in nine patients with adult-onset BD. Typical skin lesions occurred in three males and four females. A positive pathergy test was found at a similar rate in males and females, totaling five of 10 tested patients (50%). Nine patients (69.2%) had recurrent arthralgia, six patients (46.1%) had arthritis. Non-specific mild gastrointestinal symptoms occurred in seven patients (36.8%). None of the patients was suspected of having Familial Mediterranean Fever, which was also not reported in close family members. Pleuropulmonary manifestations appeared in one patient. Frequent headaches were reported by seven patients (36.8%). Other expressions of neuro-Behçet were diagnosed in another five patients. Vascular involvement, in the form of deep or superficial vein thrombosis of the lower limbs, was found in seven patients. A positive family history of oral aphthosis was reported in three patients (23%).

3.2 Adult-onset vs. adult BD

Major disease manifestations: The frequency of major manifestations in adult-onset BD and adult BD is presented in Fig. 1.

Minor disease manifestations: Similar prevalences were found in adult-onset and adult BD concerning overall joint disease, vascular involvement and recurrent headache.

The response to treatment was moderate in the two groups of patients. The curve of severity score increases from the age of 18 till the age of 35 to 40 at which time it starts to decline. Mean severity score of adult BD was 7.46 ± 3.4 and of the adult onset patients 7.77 ± 2.61 ($p = 0.7$).

Of the adult-onset patients, 85% were found to carry HLA-B5 whereas 66% of patients with adult BD carried HLA-B5.

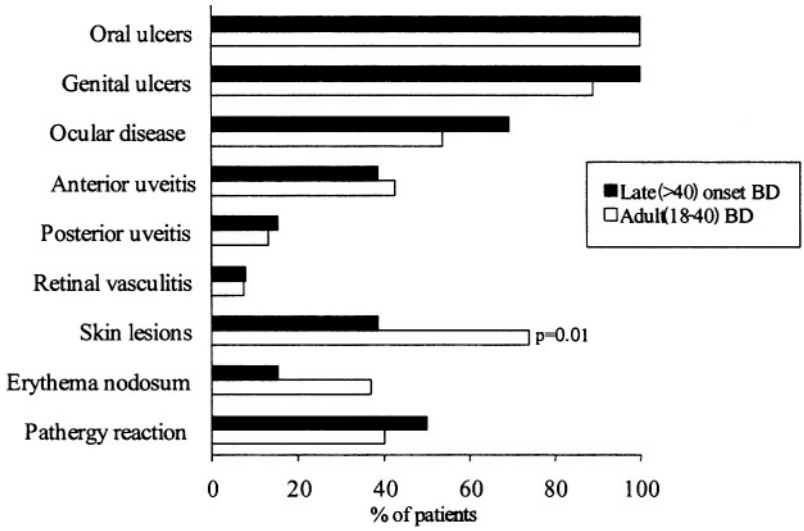

Figure 1. Major clinical manifestations comparison between adult and late-onset Behçet's disease

4. COMMENTS

Our findings of male: female ratio of 1:16 in adult-onset BD is a mirror image of previous reports on juvenile-onset BD, demonstrating a male:female ratio ranging from 1.1 to 1.4:1[3]. Overall we found the clinical spectrum of adult-onset BD to resemble that of adult disease. Similarly, no difference could be observed between the two groups of patients regarding the severity of the disease. HLA-B5 was found to be higher in the late-onset patients.

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DIAGNOSTIC CRITERIA, PROGNOSTIC PARAMETERS, ASSESSMENT OF DISEASE ACTIVITY, AND QUALITY OF LIFE

The Importance of the Manifestations Besides the Ones Included in International Criteria for Behçet's Disease

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1. INTRODUCTION

It is obvious that many manifestations and findings which are not included in ISGBD diagnostic criteria[1] carry a great importance for the diagnosis, prognosis and treatment of Behçet's disease (BD), whereas most of them were included in the previous ones. The purpose of this study is to analyse their prevalence and characteristics and reevaluate their importance.

2. PATIENTS AND METHODS

556 patients with BD diagnosed according to our new criteria[2] (354 men, 202 women, M:F 1.75) were evaluated (Table 1). The demographics and prevalence of the manifestations not included in ISGBD criteria were determined. The distribution of non-aphthous beginning (NAB) versus aphthous beginning (AB) was 28.9% and 71.1% respectively.

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Table 1. Our new set of criteria and the guide for the diagnosis of BD

Recurrent Oral Ulcerations
Recurrent Genital Ulcerations
Skin Lesions
Eye Lesions
Thrombophlebitis
+) Skin Pathergy Test
Other causative factors should be excluded
Diagnosis: any 3 of the features above are required

3. RESULTS

Manifestations of this group of patients are shown in Table 2.

Table 2. Manifestations during the course

	N	%	M:F	from onset	VOI
JOINT	390	70.0	1.7	√	-
AG	122	22.0	0.8	√	-
PA	260	46.8	2.1	√	-
AS	48	8.6	7.0	-	-
TP	210	37.7	5.0	√	√
S	142	25.5	4.1	√	-
D+Mj	49	8.8	11.2	√	√
Both	19	3.4	8.5	√	√
LUNG	83	14.9	6.5	-	√
CNS	42	7.5	3.6	?	√
GIS	25	4.5	5.2	-	√
EO	11/354	3.2	all M	-	-
ART	14	2.5	13	-	√
HIGH FEVER	14	2.5	6	√	?
CARDIAC	10	1.8	all M	-	√
AMY	9	1.6	all M	-	√

AG:arthralgia, PA:peripheral arthritis, AS:ankylosing spondylitis, TP:thrombophlebitis, S:superficial, D:deep, Mj:major, CNS:central nervous system, GIS:gastrointestinal system, EO:epididymoorchitis, ART:arterial, AMY:amyloidosis, VOI: vital organ involvement, M:male, F:female

Male predominance was much higher in most of the manifestations compared to the total group. Some of the manifestations started from the onset, e.g. arthralgia, peripheral arthritis, thrombophlebitis (every kind) and high fever. The order of frequency of manifestations during the course was as follows: peripheral arthritis, thrombophlebitis, lung involvement, ankylosing spondylitis, CNS involvement, epididymoorchitis, arterial involvement, high fever, GIS involvement, cardiac involvement, and amyloidosis.

Table 3 demonstrates the comparative prevalence of TP, PA and eye lesion in NAB and AB groups at onset and during the course.

The prevalence of peripheral arthritis and thrombophlebitis both at onset and during the course is almost equal to that of eye involvement.

Table 3. Comparative prevalences of TP, PA and eye lesions in NAB and AB groups at onset and during the course

At onset

	NAB		AB		TOTAL	
	n=160	%	N=396	%	n=556	%
TP	26a	16.3	14b	3.5	40	7.2
PA	41c	25.6	8d	2.0	49	8.8
EYE*	19e	11.9	23f	5.8	42	7.6

a vs b: $p<0.001$ c vs d: $p < 0.001$ e vs f: $p < 0.05$

During the course

	NAB		AB		TOTAL	
	n=160	%	n=396	%	n=556	%
TP	79a	49.3	131b	33.1	210	37.7
PA	75c	46.8	185d	46.7	260	46.8
EYE *	90e	56.2	199f	50.2	289	52.0

a vs b: $p<0.001$ c vs d: NS e vs f: NS

*: for comparison from ISGBD criteria

4. DISCUSSION AND CONCLUSION

So far we could not ascertain any study investigating the prevalence, characteristics and importance of the manifestations left out of ISGBD criteria.

Male to female ratio was found to be much higher in many manifestations except PA compared to the whole group. Many of them existed from the onset of the disease, e.g. joint manifestations, TP, and fever and carry a vital importance, e.g. TP, lung, CNS, GIS, arterial, cardiac involvement, EO, high fever, and amyloidosis. Whereas only eye involvement has this character in ISGBD criteria. We,among others,included many of these manifestations in the relavant diagnostic criteria.

Our study revealed that particularly PA and TP carry great importance since they have high prevalance at onset and during the course, and TP (deep, major) belongs VOI.

In conclusion, many manifestations which are not listed in ISGBD criteria have a great importance for early diagnosis, differential diagnosis, prognosis, and treatment of BD. TP should be included in the diagnostic criteria for BD as we do.

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Influence of Pathergy Test on the Accuracy of Different Diagnosis Criteria for Behçet's Disease

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1. INTRODUCTION

Pathergy test (PAT) is used in the majority of diagnosis criteria sets for Behçet's disease (BD). They are Dilsen criteria (D)[1], Japan revised criteria (J)[2-3], International criteria (I)[4], Iran traditional format criteria (IR)[5], and the Classification Tree (CT)[6].

Many factors influence the result of the PAT: Needle gauge (from 25 to 21), sharpness of the tip (sharp tip of disposable needles or manually blunted tip), direction of the needle penetration (vertical or diagonal, intra-dermal or subcutaneous), in situ injection (serum saline, sodium urate, streptococcal antigens) or just a skin puncture with no injection, the number of skin punctures (from one to several), the delay to read the result (24 to 48 hours), the description of a positive reaction (papule or pustule, reactive erythema surrounding the papule or pustule), and finally where to draw the line between a positive and a negative result. All these factors may influence the result of the PAT, and none of them is standardized. Therefore the results differ from one center to another, introducing a great bias to the accuracy of different diagnosis criteria.

The aim of this study was to analyze the sensitivity, the specificity, and the accuracy of these criteria with the PAT as a criterion being included, and excluded.

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2. MATERIALS AND METHODS

2.1 Inclusion criteria

The diagnosis was clinical (it was not based on a particular diagnosis criterion).

2.2 Method

All Behçet's patients of the Behçet's Disease Registry (4717 on January 20, 2002) were included in the study. Consecutive control patients (2407) were those who were referred to the BD Unit with a possible diagnosis of BD, but proving the contrary after evaluation.

2.3 Statistical analysis

The sensitivity, the specificity, and the accuracy of the diagnosis criteria were calculated first by taking into account the result of the PAT, and second by excluding the result of the PAT.

A confidence interval (CI) at 95% was calculated for each percentage.

3. RESULTS

3.1 General data

There was a significant drop in the sensitivity and the accuracy of all diagnosis criteria, while the specificity improved slightly.

3.2 Dilsen criteria

The sensitivity dropped from 86% (CI: 1) to 68% (CI: 1.3). The specificity improved from 91% (CI: 1.1) to 99% (CI: 0.3) whereas the accuracy dropped from 88% (CI: 0.7) to 79% (CI: 0.9). The differences in all the parameters were statistically significant.

3.3 Japan revised criteria

The sensitivity dropped from 88% (CI: 0.9) to 80% (CI: 1.1). The specificity did not change. It was 91% (CI: 0.7) with and without the PAT.

The accuracy dropped from 91% (CI: 0.7) to 86% (CI: 0.8). The difference in the sensitivity and the accuracy was statistically significant.

3.4 International criteria

The sensitivity dropped from 82% (CI: 1.1) to 66% (CI: 1.4). The specificity improved from 98% (CI: 0.5) to 99% (CI: 0.9) whereas the accuracy dropped from 88% (CI: 0.7) to 77% (CI: 0.9). The difference in the sensitivity and the accuracy was statistically significant.

3.5 Iran criteria (traditional format)

The sensitivity dropped from 93% (CI: 0.7) to 79% (CI: 1.2). The specificity improved from 96% (CI: 0.8) to 97% (CI: 0.6) whereas the accuracy dropped from 97% (CI: 0.4) to 85% (CI: 0.8). The difference in the sensitivity and the accuracy was statistically significant.

3.6 Classification tree criteria

The sensitivity dropped from 97% (CI: 0.5) to 89% (CI: 0.9). The specificity improved from 97% (CI: 0.7) to 98% (CI: 0.5) whereas the accuracy dropped from 97% (CI: 0.4) to 92% (CI: 0.6). The difference in the sensitivity and the accuracy was statistically significant.

Table 1. Sensitivity, specificity, and accuracy of diagnosis criteria

	PATHERGY INCLUDED						PATHERGY EXCLUDED					
	Sensitivity		Specificity		Accuracy		Sensitivity		Specificity		Accuracy	
CRITERIA	*%*	*CI*	*%*	*CI*	*%*	*CI*	*%*	*CI*	*%*	*CI*	*%*	*CI*
Dilsen	86	1.0	91	1.1	88	0.7	68	1.3	99	0.3	79	0.9
Japan	88	0.9	97	0.7	91	0.7	80	1.1	97	0.7	86	0.8
International	82	1.1	98	0.5	88	0.8	66	1.4	99	0.9	77	1.0
Iran	93	0.7	96	0.8	97	0.4	79	1.2	97	0.6	85	0.8
Classification Tree	91	0.5	97	0.7	97	0.4	89	0.9	98	0.5	92	0.6

4. DISCUSSION

The pathergy test is an important criterion for the modern diagnosis and classification criteria of BD. As stated in the introduction part, many factors influence the result of the pathergy test. As a result, many scientists hesitate to employ this tool, especially those not being very familiar with BD.

The sensitivity of all the criteria using the pathergy test dropped when its results were disregarded. The Dilsen criteria was the big looser, it lost 18% of its sensitivity. It was followed by the International criteria (16%) while the least affected were the Classification Tree, and the Japan revised criteria with 8% each.

The absence of the pathergy test promotes greatly the specificity of the Dilsen criteria by improving it by 8%. For the other criteria the improvement was just 1%, or absent (Japan revised criteria).

The accuracy also dropped in the absence of the pathergy test. Most affected were the Iran (traditional format) and the International criteria, while the least affected was the Classification Tree criteria.

The highest accuracy remained for the Classification Tree criteria at 92%.

5. CONCLUSION

The Classification Tree criteria are the most sensitive and the most accurate criteria with or without the result of the pathergy test. They are also least affected by the absence of the pathergy test.

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Measuring Health Related Quality of Life

An international perspective

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1. INTRODUCTION

During the past 30 years a paradigm shift in the evaluation of medical treatments has occurred. Not only the change in clinical symptomatology or survival span but the patient's own view of her or his health state is now considered an evaluation criterion for medical treatments[1] . The term "health-related quality of life" reflects this conceptual change. Although maintaining or even improving quality of life has been a long-standing moral imperative in any patient-physician encounter, assessing quality of life of patients not only implicitly in the patient-physician relationship but also explicitly measuring it in evaluating medical treatments is new. The acceptance of the term "quality of life" does reflect a critique of classical clinical endpoints and is related to recent developments in population health. The increase in the population of elderly, potentially chronically ill populations in need of long-term treatment as well as financial cutbacks in almost all health systems world-wide demand a critical review of the health care delivery and criteria for evaluating its quality[2]. The development of quality of life research extends basically over the last twenty years, in which according to a recent literature search over 30.000 publications have appeared concerning conceptual as well as methodological approaches to assessing quality of life and its application in different studies[3].

The term "quality of life" has its origins in politics, as can be seen in the concept of "pursuit of happiness" as an essential component of a governance[4]. In the social sciences literature including sociology, politology, anthropology, and psychology, the term "quality of life" has already been

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used since 1940. Large studies have been conducted to investigate the quality of life assessed primarily via socioeconomic indicators in various countries. A more individually focussed approach to studying quality of life involved the concept of life satisfaction[5,6].

From 1967 on, the term "health-related quality of life" has been coined differentiating the medical from the more sociological oriented quality of life concepts, since in medicine specifically health aspects of well-being and function were to be represented[2].

The development of the comparatively young "quality of life" research field consists of four phases so far. The first phase in the middle of the 1970's was characterized by wealth of philosophical work relating to the question what quality is and how to measure it. In the 1980's, the second phase with a more explicit assessment of quality of life and a corresponding development of measurement instruments began. Since the early 90's a third phase of application of quality of life instruments in terms of including quality of life measures in several types of clinical studies evolved, and with the new millenium the impact of quality of life research on medical practice is now being emphasized. Over the time, quality of life research has been confronted with three essential questions which mainly stem from external perception of the field (e.g. medicine). The first question relates to a basic scepticism toward the possibility of defining and operationalising the quality of life. The second problem relates to construction and quality of methods to assess quality of life, suspecting these would be too subjectively biased and therefore without value[7]. The third aspect relates to the benefit of assessing quality of life, that is to the question to which extent research results are relevant for health-political action or individual medical activities[8].

The aims of quality of life research deal with the description of quality of life for specific populations in a given country or nation in order to obtain information for health-political planning. Users of such data come from health systems research, health-policy and epidemiology. A second level pertains to the evaluation of treatments using cross- sectional and longitudinal studies as well as randomized, controlled clinical trials. Users are health insurance companies but also health care providers. The third aspect of health economical use of quality of life research involves the evaluation of complex treatment strategies related to prevention or rehabilitation on the health-political and economic level. Quality of life is one criterion in so-called cost benefit analyses in which benefit pertains also to the quality of life of the health care consumers.

2. CONCEPTUAL BASIS

In contrast to the vast body of literature relating to "happiness", "well-being" and "satisfaction", the term "quality of life" in medicine has been associated with an astonishing lack of theory. Principally three types of quality of life models can be distinguished[9].

The first type of model is individually centered and implies that quality of life is principally not measurable in persons because it varies form person to person in its dimensionality. Proponents of this approach maintain that quality of life can only be intraindividually described. An abstraction including different persons is only possible on the basis of a difference between goals and their attainment in which the difference between goals and expectation is a critical value in quality of life research.

A second type of model views quality of life as evaluable using a defined number of different dimensions which are relevant for all persons. These dimensions are physical, psychological and social well-being in accordance with earlier definitions of the World Health Organization. Some authors explicitly include functional capacity, others extend the dimensions by further aspects such as spirituality. According to this approach, measurements try to capture the dimensionality of quality of life concepts and to assess persons in the amount in which the quality of life dimension is represented.

A third group of definitions maintains that quality of life cannot be measured directly, neither intraindividually nor interindividually. Quality of life is viewed as an implicit construct in which implicit measurements of patient preference rather than direct questions towards the well-being are used. This is the model of so-called health economical or cost utility approaches to quality of life assessment in which either specific scenarios or gambling theoretical approaches have been constructed. Each of these conceptual approaches has been associated with the development of a specific assessment methodology which is reflected in the development of measurement instruments.

There is a growing international consensus that quality of life is definable in operational rather than in nominal terms[10]. Operational definitions have been preferred by researchers assuming an interindividual universality of the quality of life concept. In qualitative studies about patient's definitions of quality of life, dimensions of quality of life seem to be intersubjectively comparable[11].

3. ASSESSMENT METHODS

A major effort in quality of life research concerns instrument development which is relatively advanced[12]. The development of instruments refers first to the development of generic instruments, and second to the development of disease-specific questionnaires. Both assessment types depart from the notion of a multi-dimensionality of the quality of life concept and the involvement of the patient's perspective, which does not preclude external ratings but necessitates caution in equating proxy-assessments of quality of life with patient's self-report.

Internationally, a wealth of generic instruments to assess quality of life is available which have been translated into different languages, are psychometrically tested in the respective cultures, and are useful for clinical research. There are also guide-lines with regard to translating and testing as well as norming of instruments[13]. The care with which translations and psychometric approaches have been conducted shows how much importance is attached to linguistic and cultural influences on quality of life instruments by the researchers. A common feature of this cross-cultural work is the attempt to provide culturally adequate psychometrically robust measurement instruments. There are several international working groups which relate to the EORTC Questionnaire[14], the Nottingham Health Profile[15], to the SF-36 Health Survey[16], and to the WHO QOL[17] with several other international groups forming now[13].

Of special interest is the research project of the World Health Organization (WHO) which includes the culture-centered definition of quality of life from various cultures. The resulting discussion led to a matrix of relevant quality of life dimensions which, using focus groups in various cultures, were to be formulated as items. Persons from fifteen nations participating in the study developed over 3.000 questions which were finally reduced to 300. Psychometric analysis of data of 4.500 respondents showed that using structural analytical equation modelling, there is reason to suspect cross-culturally relevant dimensions of quality of life which concern psychological, social, physical, spiritual, functional and economic aspects as well as the environmental levels. The research showed that people world-wide differ less than expected in their basic understanding of quality of life. Independent of age, gender, and culture it seems to be relevant to feel physically fit, to be socially integrated, to feel psychologically stable, to be able to fulfil daily roles, and to experience social support in a materially and economically safe environment. Homogeneity in quality of life definitions do not imply that countries would not differ in the state of quality of life of the interviewees, they rather suggest that quality of life could be an intercultural, universal experience.

According to psychological test theory, reliability, validity, and especially sensitivity or responsiveness of measurement instruments is of utmost importance[18]. Classical test theory as well as new approaches (e.g. Rasch scaling) are used to test the structure of the scales and their psychometric properties. A minimal prerequisite for a psychometrically tested instrument is that there is evidence for its performance in a group of healthy persons as well as patients. Acceptable test-theoretical coefficients include reliability (Cronbach's Alpha) as well as test retest reliability (usually repeating measurements in clinically unchanged populations after one week). As concerns validity, construct and criterion validity are assessed by factor analysis, known groups comparisons, and correlations with other instruments.

More recently, these so-called conformitory analyses as well as test statistics from the multi-trait multi-methods models are used to test a priorily identified scale structure. Statistical programs, such as the multi-trait analysis program (MAP) are economic and precise tools for identifying relevant scale and item characteristics.

Especially important is the sensitivity of quality of life instruments, usually viewed as special case of criterion validity. In evaluation research, sensitivity, that is the differential change over time with regard to specific intervention, is important because only then intervention effects can be estimated[7].

Disease-specific instruments have been developed in an increased number throughout the last ten years, especially in oncology but also in allergology, neurology, and psychiatry. Widely used instruments in oncology are e.g. the EORTC-questionnaire[14] or the Functional Assessment of Cancer Treatment Questionnaire (FACT)[19]. In general, the phase of developing and testing of instruments has reached a climax. A broad and internationally available methodologically adequate pool of instruments has been developed to assess quality of life in various studies. Of course, specific diseases or treatments necessitate further development and an increased effort to develop further instruments for health-economical studies is still needed as well.

4. APPLICATION OF LIFE INSTRUMENTS

A literature search regarding clinical studies showed an increased number of 154 listings of clinical studies including quality of life parameters[20]. These papers can be classified according to the patient population or the therapeutic approaches chosen. In addition, they can also be differentiated according to the study objectives and designs ranging from cross sectional

over longitudinal studies evaluating the quality of life from before to after a treatment or to randomised clinical trials in which two or more therapeutic arms are compared with each other. Most of the research in the adult clinical trial area has been performed in oncology and cardiology and more recently in psychiatry.

Oncology was one of the first disciplines to approach the topic of quality of life. One reason for this was the question whether the extension of life time for a few months with chemotherapeutic interventions in solid tumours was appropriate if prognosis and expected therapeutic benefit were poor[10]. From this point, quality of life studies soon evolved around how to improve care for cancer patients. Most research was committed to developing and applying research instruments rather than descriptive clinical studies. Randomized clinical studies were primarily conducted by the large oncological clinical trials groups in North America as well as Europe. In these study groups quality of life has gained an increased consideration as an outcome parameter, e.g. in testing chemotherapy regimens in patients with breast cancer[21].

Cardiology was also early to include the topic of quality of life as a study objective[22]. In contrast to oncology however, specific measurement instruments were not developed but rather existing instruments from public health research were used. Widely discussed was the classical study by Croog et al.[23] in which in a cohort of over 500 hypertensive men the superiority of one medication in several of the documented quality of life areas was shown.

Another main area of research in quality of life in clinical trials concerns coronary heart disease[22]. Most of the clinical studies work with standardized generic measurement supplemented by a specific questionnaire[24] In general the quality of life indicators for cardiovascular populations show that coronary heart disease is associated with a marked deterioration in quality of life which seems to be dependent on the NYHA classification, and gender[25].

In psychiatry, the topic of quality of life research has appeared rather lately, possibly due to a long-standing view that classical psychiatric instruments do already reflect patients' quality of life. The focus, however, was mainly on expert or external ratings of symptomatology; self-reported perception of patients has not been systematically included in psychiatric literature. New approaches to assess quality of life from the patient's point of view are being developed, e.g. in schizophrenia[26]. In a study with 357 patients suffering from psychiatric disorders, a dramatic decrease in quality of life after disease onset in several relevant dimensions was reported. In neurology, epilepsy as well as stroke have received much attention in terms of adult quality of life research. In epilepsy, development of specific measurement instruments has been performed[27]. Clinical studies in epilepsy,

however, are rare and suggest that anti-epileptic medications with the better side effect profile are associated with better quality of life ratings[28]. Clinical studies have been reported in other areas as well, e.g. in nephrology, as concerns the use of erythropoetin in dialysis patients[29]. In pain syndromes, especially headaches, the SF-36 Health Survey was frequently used[30]. As regards HIV-infections and AIDS, epidemiological studies show that in addition to the severity of disease unfavourable living conditions and financial problems contribute to a decreased quality of life in HIV-infected and AIDS-patients[31], and treatment with erythropoetin showed positive effects on quality of life in 251 anaemic patients with HIV-infection[32]. In Surgery, most studies relating to quality of life are available in gastrointestinal surgery, e.g. pouch reconstruction or in limp surgery hip replacement[33].

In clinical application, the quality of life studies have followed a certain pattern. As a first step, theoretical and conceptual articles about quality of life assessment have been published followed by methodological work on the development of new assessment instruments or the use of available ones. Cross-sectional and longitudinal observational studies have followed to assess the quality of life impairments in specific patient populations which is dramatic according to a recent literature review and also demonstrates marked difference from the medical criteria. Only in few disease areas, clinical trials have been conducted. Most successful were approaches in which multinational clinical study groups have joined efforts to produce state of the art study protocols to include quality of life assessment in clinical trials. New developments in quality of life research pertain to the health sciences in which quality of life is a new health status indicator in public health studies (e.g. for evaluating the effect of preventive programs). Also, in health economy the use of quality of life instruments has to be more critically discussed. Lately, publications have compared the psychometric quality of different measurement instruments for a specific disease.

What does this mean concerning Adamantiades-Behçet's disease? It is basically the necessity to develop appropriate instruments. Also, implementing quality of life outcomes in a epidemiological, clinical and health-economic study is recommended.

5. DISCUSSION

During the past 30 years the quality of life field has changed from a seemingly esoterical topic to a well-founded component of evaluation research[4]. This is indicated by an increase in publications as well as the availability of psychometrically robust assessment instruments for quality of

life. In addition, the quality of life research field has given a fresh impetus to an exchange between the social-behavioural sciences and medicine. The criticism of classical end-points in medical studies has allowed to focus the psychological situation of the patient and has again stressed the psychological expertise in evaluating the effects of medical care.

Internationally, quality of life research has led to cooperation structures in research as well as application. The strength of quality of life research is not so much the conceptual but clearly the methodological, and also practical field. Open questions in quality of life research pertain to individual representation of quality of life concepts[34], to the question of correspondence between self- and external reports, to the confusion relating to differences between well-being, depression, health status and quality of life, to the weighing of quality of life indicators, and to the clinical relevance of quality of life research in practical terms[34].

In addition, psycho-social factors are associated with quality of life: coping strategies as well as health locus of control and social support are important psycho-social determinants which should be addressed in interventions[3].

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Univariate and Multivariate Analyses Comparing Demographic, Genetic, Clinical, and Serological Risk Factors for Severe Adamantiades-Behçet's Disease

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1. INTRODUCTION

Although a severe course can develop in a considerable rate of patients with Adamantiades-Behçet's disease (ABD)[1], only a few trials have assessed prognostic criteria so far[2-5]. Therefore, we conducted a retrospective study to analyse associations of demographic, genetic, clinical, and serological risk factors with severe ABD.

2. PATIENTS AND METHODS

We examined a sample of 372 patients from the German Registry of Adamantiades-Behçet's disease[6]. All patients suffered from manifest ABD and fulfilled the criteria of the Behçet's disease classification tree[7]. We analysed the association structure of gender, nationality, family history, HLA-B_{51} and anti-cardiolipin antibodies positivity, onset sign, duration of the development of the disease, and severe disease in the patients sample. "Severe disease" included death, blindness, and documented severe vascular, CNS, lung, gastrointestinal, and articular involvement. "Vascular sign" included ocular changes, erythema nodosum, and thrombophlebitis. For

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statistical analysis we used chi-square tests, Mann-Whitney tests, and multiple logistic regression analysis.

3. RESULTS

A rate of 60.5% of the sample was male. 55.5% were of Turkish and 44.5% of German descent, 12.3% comprised family history, 60.6% were HLA-B_{51} positive, and 44.9% presented positive anti-cardiolipin antibodies. In 79.7% of the patients the disease occurred with aphthous oral ulcers, and in 10.4% with a vascular sign. 16.6% of the patients developed a documented severe disease.

In a univariate analysisavascular onset sign (severe course: 54.3% in patients with vascular onset sign vs. 11.8% in patients with oral aphthous ulcers, genital ulcers or articular involvement as onset sign , $p<0.00l$) was related to the severity of the disease, also, trends in male patients (19.4% vs. 12.3% in female, $p=0.1$) and patients with positive anti-cardiolipin antibodies (32.4% vs. 16.3%, $p=0.11$) could be detected. In the subsample with mucocutaneous and articular onset signs (n=300), HLA-B_{51} positive (72.0% vs. 58.3%, $p=0.04$) and male (69.9% vs. 59.2% in female, $p=0.06$) patients developed more often ocular lesions.

In the multivariate analysis, 62.1% of the male patients with a vascular onset sign developed severe course of the disease in comparison to 13.6% (11.7-16.7%) of all other groups [male with non-vascular onset sign and female with or without a vascular onset sign, $p<0.001$, odds ratio 10.2 (95% CI 1.5-66.6)]. In the subsample with mucocutaneous and articular onset signs, 72.0% of the HLA-B_{51} positive patients developed ocular lesions in comparison to 58.3% of the HLA-B_{51} negative ones ($p=0.04$, odds ratio 1.8), whereas a trend was detected according to the gender since 69.9% of male patients developed ocular lesions in comparison to 59.2% of the female ones ($p=0.06$).

4. DISCUSSION

Our results provide further evidence of the genetic components of ABD and the significance of the specific onset sign concerning the prognosis of the disease. The combination of vascular onset sign (ocular changes, erythema nodosum, and thrombophlebitis) with male gender is significantly associated with the occurrence of a severe course. In patients with a non-vascular onset sign (oral aphthous ulcers, genital ulcers or articular involvement), HLA-B_{51} positivity is a negative prognostic marker (Fig. 1).

Multinational multicentre studies should be established to investigate the impact of these components

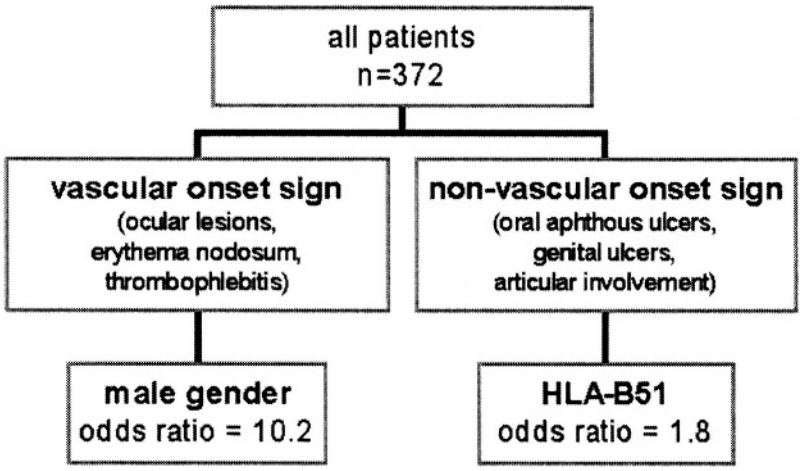

Figure 1. Risk factors for severe course of Adamantiades-Behçet's disease

Male gender is a widely accepted risk factor for severe course of ABD[2-5]. On the other hand, non-aphthous ulcers onset has also been reported to be a risk factor by Dilsen[4]. $HLAB_{51}$ is not associated with the disease itself[1] but has been disputed as a prognostic factor[1,5,8]. This study has found $HLAB_{51}$ to be, indeed, a risk factor for the majority of patients who develop the disease with a non-vascular onset sign, thus confirming our previous data[1,6]. Other demographic and clinical data, such as age of onset[4], or skin lesions, arthritis, posterior attacks, and other complications[2] could not be identified as risk factors for developing a severe course of ABD in our patients.

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Is Long Term Observation of Patients with Recurrent Aphthous Stomatitis Necessary?

Clinical follow-up of 1238 cases

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1. INTRODUCTION

At multidisciplinary Behçet's Disease Unit of Ankara University Faculty of Medicine, we have 2755 Behçet's disease patients being regularly followed-up. In addition, we are following-up the patients with recurrent aphthous stomatitis for the development of Behçet's disease. The aim of our study was to evaluate whether a long term observation of patients with recurrent aphthous stomatitis is necessary or not and to determine the possible risk factors.

2. PATIENTS AND METHODS

The records of 1238 patients with recurrent aphthous stomatitis followed-up at the Multidisciplinary Behçet's Disease Unit at Ankara University Faculty of Medicine during the years 1988-2002 were retrospectively analysed. The patients with systemic diseases known to be associated with oral aphthous lesions such as systemic lupus erythematosus and inflammatory bowel diseases were excluded. At the first visit every patient was evaluated with complete dermatologic examination and ophthalmologic examination. Laboratory tests including complete blood count, serum

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biochemistry, vitamin B_{12}, folic acid, ferritin, ANA and anti-ds DNA levels and three-step pathergy test were performed. During the follow-up period the patients were evaluated every six months and whenever needed. Any additional symptom related with Behçet's disease was recorded.

The data extracted from the record of each patient included the demographic features (age, sex), the age at onset of oral aphthous lesions, localisation and morphology of aphthous lesions (minor, major, herpetiform), the result of the three-step pathergy test, ophthalmologic examination, and the time of onset of any other manifestation of Behcet's disease. The diagnosis of Behçet's disease in patients with additional symptoms was made according to the criteria of International Study Group for Behçet's disease.

We compared the demographic features, morphology and the age at onset of oral aphthous lesions in patients with recurrent aphthous stomatitis with those patients who developed Behçet's disease during the follow-up period to determine the risk factors for the development of Behçet's disease. The results were analysed statistically by using student's t-tests and chi square (χ^2) tests.

3. RESULTS

Of the 1238 patients with recurrent oral aphthous lesions, 542 were (43.8%) male and 696 were (56.2%) female. The male/female ratio was 0.7. The age of the patients ranged from 2 to 70 with a mean age of 33.25±11.64 years. The mean age at onset of aphthous lesions was 27.35±11.75 years. The follow-up period of patients varied from 1 to 11 years with a mean of 4.5 years.

Minor aphthous ulcerations were present in all patients. One thousand and seven (81.3%) patients had only minor ulcerations. Two hundred and seven (16.7%) patients had both minor and major ulcerations and 24 (1.9%) patients had minor, major and herpetiform ulcerations. The lesions were most frequently located on tongue and lips.

Ophthalmologic examination was normal in all patients and pathergy test was found to be positive in 55 patients (4.4%).

Out of the 1238 patients with recurrent oral aphthous lesions, 47 patients (3%) developed an additional manifestation of Behçet's disease within a mean period of 5.61 years.

Thirty six (2.9%) of these patients met the diagnostic criteria for Behçet's disease. Sixteen (44.4%) were male and 20 (55.6%) were female (male/female ratio was 0.8) with a mean age of 29.13±9.30 years.

The mean age at onset of the oral lesions was 22.44±7.80 years. Twenty patients (55.6%) had only minor ulcerations and 16 (44.4%) had both minor and major ulcerations. The mean period for the development of an additional manifestation of Behçet's disease in these patients was 5.61 years (min: 2 months, max: 22 years). The three step pathergy test was positive in 7 (19.4%) patients at the initial evaluation for recurrent aphthous stomatitis.

The second symptom of Behçet's disease was genital ulcer in 29 (80.5%), skin lesions in 4 (11.1%) patients, ocular involvement in one (2.7%), neurological involvement in one (2.7%) and thrombophlebitis in one (2.7%) patient.

In order to evaluate the possible risk factors for the development of Behçet's disease in patients with RAS, we compared the demographic features and clinical findings of patients who developed Behçet's Disease with the others. Statistical evaluation revealed that the mean age and the age at onset of aphthous lesions were significantly lower ($p<0,05$) in patients who developed Behçet's disease. Major aphthous ulcerations were also found to be more frequent in these patients ($p<0.001$) (Table 1).

Table 1. Comparison of clinical and demographic features of patients with Behçet's disease and RAS

	Behçet's disease	Recurrent aphthous stomatitis	P value
Total number of patient	36	1202	
Male	16 (44.4%)	526 (43.8%)	
Female	20 (55.6%)	676 (56.2%)	
Male/Female	0.8	0.7	p>0.05
Mean age	29.13±9.30 years	33.37±11.68 years	p<0.05
Mean age at onset of aphtous lesions	22.44±7.80 years	27.49±11.82 years	p<0.01
Types of oral ulcerations			
Minor	20 (55,6 %)	987 (82.1%)	
Major	16 (44,4 %)	191 (15.9%)	p<0.001
Herpetiform	0	24 (2%)	

4. CONCLUSION

Behçet's disease is a chronic inflammatory disorder effecting many organ systems[1,2]. Clinical manifestations of Behçet's disease may occur concurrently or subsequently. Generally oral aphthous lesions are the initial symptoms of Behçet's disease and other manifestations occur subsequently within varying periods during the course of the disease[3,4]. However, it is a great dilemma to predict if patients presenting only with recurrent aphthous stomatitis will indeed develop Behçet's disease in the future or not[5-6].

The retrospective analysis of the clinical data of 1238 RAS patients who were regularly followed up at multidisciplinary Behçet's Disease Unit showed that 36 (2.9%) of them developed Behçet's disease within a period ranging between 2 months and 15 years after the onset of aphthous lesions. Our results indicate that even though recurrent aphthous stomatitis is a rather common condition in general population, especially those patients who have major ulcerations and earlier onset of aphthous lesions should be regularly followed-up. We also suggest to perform the three-step pathergy test in every patient with recurrent aphthous stomatitis and closely follow-up patients with positive test results particularly in countries where Behçet's disease is prevalent.

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Acetylator Phenotype in Behçet's Disease

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1. INTRODUCTION

Acetylation is considered a major metabolic pathway in the biotransformation of a number of drugs, such as: procainamide, hydralazine, sulphonamide, isoniazide, sulphadimidine, dapsone, phenelzine, clonazepam, acebutolol, and a caffeine metabolite[1]. Acetylation principally occurs in the liver, extrahepatic sites of acetylation have also been identified[2].

Acetylation exhibits a genetically controlled bimodal distribution within any given population. Individuals can be phenotyped either slow or rapid acetylator using a test drug. Slow acetylation is inherited in an autosomal recessive fashion[1]. Polymorphic N-acetylation has been linked to variation in drug response, susceptibility to adverse reactions, and increased incidence of certain spontaneous disorders including cancer.

Behçet's disease (BD) is a chronic relapsing disease characterized by multiple signs and symptoms such as recurrent orogenital ulcerations, eye involvement, skin manifestations and other system affections[3]. In 1937, it was first described by the Turkish dermatologist Hulûsi Behçet as "recurrent oral aphthous ulcers, genital ulcers and hypopyon-uveitis"[4].

Dapsone, which is used for treatment of BD[5,6], is metabolized by N-acetylation. Reviewing the literature, we found no reports for determination of the acetylator status in BD.

Therefore, the aim of this work was to determine the acetylator status in BD in order to know the degree of metabolism of dapsone in the liver by N-acetylation, and to identify the frequency of acetylator phenotypes either

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slow or rapid in BD patients and compare it to healthy individuals. In addition, the study aimed to explore if there is any association between the acetylator status and HLA-B_{51} typing and the severity of the disease.

2. PATIENTS AND METHODS

Forty-one BD patients, 25 male and 16 female, aged from 19-46 years, registered at the BD Clinic at Saddam Medical City and fulfilling the International Study Group Criteria (ISGC) for the diagnosis of BD[7] and 37 healthy individuals, 19 males and 18 females, with an age ranging from 25-38 years, participated in the study. Control individuals had no history of serious illness, were normal on physical examinations and had no history of sulphonamide allergy or G6PD deficiency. Smokers, drinkers and those taking medications in the last week were excluded. All subjects gave their informed consent. The study was approved by the ethics committee.

Detailed history was taken from the patients including age, sex, occupation, age at onset of the disease, the first presenting feature as well as duration and frequency of occurrence of symptoms. The Clinical Manifestation Index (CMI) was calculated for each patient. The index for the individual patient is the numerical sum of clinical features, the total grading of CMI equal to 31[6]. In addition, HLA-B_{51} positivity (human leukocyte antigen or major histocompatibility complex MHC) was done for each patient by microlymphocyte cytotoxicity test in Al-Karama Teaching Hospital, and hemoglobin (Hb) and erythrocyte sedimentation rate (ESR), white blood corpuscle (WBC) and differential count, c-reactive protein (CRP) and liver function tests were examined in the Teaching Laboratories of Saddam Medical City.

After an overnight fast, each subject received a single oral 100 mg of dapsone (Al-Nile, Cairo, Egypt). Drinking of caffeine-containing beverages was not allowed throughout the study period. A blood sample (5 ml) was obtained 3 hours after drug intake by venepuncture which was added to a 10 ml polyethylene tube containing 50 µl heparin (Heparin Leo 5000 iu/ml; Leo, Denmark). Plasma was seperated within one hour after collection by centrifugation at 3000 rpm for 10 min. The samples were subsequently stored frozen at -20°C pending analysis.

Standards and chemicals used in this method were analytical reagents. Dapsone (DDS) and monoacetyldapsone (MADDS) powders were obtained from Al-Nile. The reagents used were perchloric acid (60%), methanol, and 0.067 M phosphate buffer pH 5.9. Chemicals were purchased from British Drug House (Pool, England) and were of analar grade.

A rapid, simple, one-stage protein precipitation method for the estimation of plasma DDS and MADDS concentrations by HPLC (Shimadzu, Kyoto, Japan) was applied[8].

3. RESULTS

The frequency of slow acetylators in healthy individuals was 70.2% [95% confidence interval (CI) 55.5 - 84.9%]; n = 26 control subjects of the total 37 (12 male and 14 female) with mean age 30.7 years (Table 1). The MADDS / DDS ratio ranged from 0.03 to 0.29. On the other hand, the frequency of rapid acetylators among healthy individuals was 29.8%, n = 11 subjects from 37 (7 male and 4 female) with mean age 30.6 years. The MADDS / DDS ratio ranged from 0.55 - 1.12 (Fig. 1).

The frequency of slow acetylators in BD patients was 53.7% [95% CI 38.5-68.9%), n = 22 patients from 41 (13 male and 9 female) with mean age 32.5 years Table 1. The frequency of non-acetylators with a zero acetylation (MADDS/DDS) ratio was 46.3%, n = 19 patient from 41 (12 male and 7 female), with mean age 32.8 years, (Fig. 2).

There was significant difference between the mean acetylation ratio in patients and control group (P = 0.001), (Table 2), on the other hand no significant difference was detected between the mean age in patients and control group (P = 0.179) (Table 2).

Comparing the acetylator status of BD patients to that of the control group we found that there is a significant difference in the frequency of non-acetylators between patients and control as well as the frequency of rapid acetylators between patients and controls. But there is no significant difference in the frequency of slow acetylators between patients and controls. Slow acetylators BD patients (n= 22) can be subdivided into 2 groups according to the MADDS/DDS ratio.

Table 1. Descriptive statistics of the difference between age and acetylation ratio in patients and control group

Age	N	Mean	Std. Deviation	Std. Error mean	C.S	
					t-Test	Significance
Patient	41	32.65	7.82	1.2226	1.355	P = 0.179
Control	37	30.72	3.88	0.638		
Acetylation Ratio						
Patient	41	0.07	0.085	0.013	-5.557	P < 0.001
Control	37	0.34	0.304	0.049		

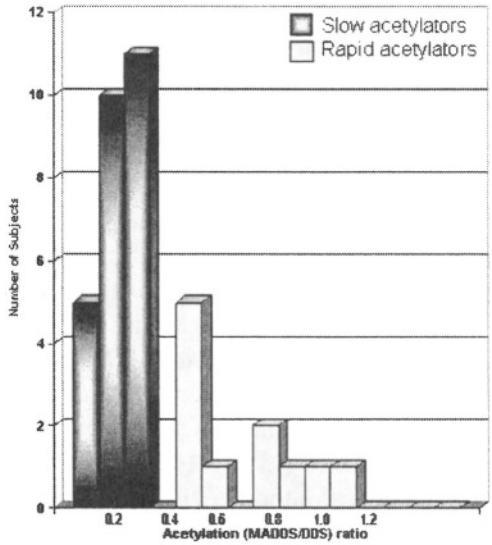

Figure 1. Frequency distribution histogram of the plasma MADDS/DDS ratio in 37 unrelated healthy Iraqi subjects

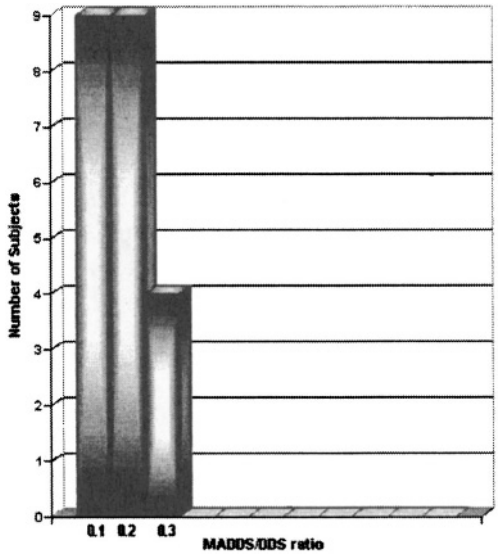

Figure 2. Frequency distribution histogram of the plasma MADDS/DDS ratio in 41 BD patients show 22 patients of slow acetylation phenotyping

Moderately slow acetylators with MADDS/DDS ratio ranging from 0.1-0.3, were 59.1% (13 patients from 22), 8 male and 5 female. Very slow

acetylators with MADDS/DDS ratio ranging from 0.01-0.09, were 40.9% (9 patients from 22), 5 male and 4 female. There were no rapid acetylators in BD patients.

Table 2. Distribution of acetylator phenotype (none , slow and rapid acetylators) in patients and control with their statistical significance

Acetylator phenotype	Patients	Control	CS	
			t-Test	Significance
None	19 (46.3%)	0	4.761	S
Slow	22 (53.7%)	26 (70.2%)	1.506	NS
Rapid	0	11 (29.8%)	3.767	S
Total	41	37		

CS = comparison statistics; S = significant; NS = not significant

The Clinical Manifestation Index (CMI) can determine the severity of BD. A CMI > 15 of the total grading (31) represents a severe disease , which includes most of the manifestations of BD, such as mucocutaneous lesions, eye and joint involvement in addition to positive pathergy test[6]. Our results revealed that 63.4% of BD patients had a CMI > 15, and 36.6% < 15.When the CMI was correlated to the acetylator status the results revealed that there was strong negative association between the acetylator status and the severity of BD. Thus, the more severe the disease, the slower the N-acetylation of dapsone including slow and non-acetylators (with zero MADDS/ DDS ratio), and these results were statistically significant ($r = -0.854$, $P < 0.001$).

The frequency of patients with positive HLA-B_{51} was 68.2%, n = 28 from 41 patients, 17 male and 11 female. Furthermore, moderately slow acetylators were all negative for HLA-B_{51}, while all very slow acetylators and non acetylators were positive for HLA-B_{51}. These results indicate that there is a statistically significant correlation between acetylator status and HLA-B_{51} in BD patients ($r = 0.850$, $P < 0.001$).

4. DISCUSSION

In this study acetylator status was determined in normal subjects and patients with BD. The results revealed that the frequency of slow acetylators among a sample of healthy Iraqi volunteers (n=37), using DDS as a test drug, was 70.2% while the frequency of rapid acetylators was 29.8%. A previous Iraqi study from Mosul was done for determination of acetylator phenotype in Iraqi population taking 57 healthy volunteers. Each subject received 50 mg of DDS orally, and the concentrations of DDS and MADDS

were estimated by thin layer chromatography (TLC). The results revealed that 58% were slow acetylators, 7% were intermediate acetylators, and 35% were rapid acetylators[11]. The difference in the frequency of acetylator phenotypes between this previous study and our present study may be due to many factors such as geographic location and the use of HPLC rather than TLC.

The findings of our study are similar to those reported from other Arab countries. The frequency of slow acetylators in healthy Jordanian individuals using DDS as a test drug was 67.5%[12]. In addition, the frequency of slow acetylators in Saudi Arabia was found to be 63.4%[13], in Libya 65%[14], and 82% among Egyptians[15]. Our calculated frequency of the recessive allele controlling slow acetylation (q) was 0.84±0.003 in healthy individuals, which is similar to that reported from other Arab countries. The results reported from Jordan were 0.82, from Libya 0.81, from Saudi Arabia 0.80, and were 0.91 from Egypt [12-15]. In our results, the expected genotype frequencies for homozygous slow acetylators (q^2), heterozygous (2Pq), and homozygous (P^2) rapid acetylators were 0.70, 0.28, and 0.02 respectively These results are similar to that reported from a Jordanian study which revealed that the expected genotype frequencies were 0.67, 0.30, and 0.03 for q^2, $2Pq$, and P^2, respectively[12].

The apparent trimodality noticed in the frequency distribution histogram in our study might suggest that the plasma acetylation (MADDS/DDS) ratio which equals to 0.75 is likely to discriminate between homozygous and heterozygous rapid acetylators (n=5, n=6), respectively. This does not agree with those calculated according to the Hardy-Weinberg law of population genetics (n=1, n=10) for homozygous and heterozygous rapid acetylators, respectively. Trimodality of the frequency distribution histogram and disagreement between observed and calculated values for both homozygous and heterozygous rapid acetylators have been reported by others[16-18].

Acetylator status has not been studied in BD. DDS, which is one of the drugs metabolized by acetylation, has been used since 1984 in treatment of BD[5]. A recent double blind, placebo-controlled, crossover study has confirmed that dapsone has a therapeutic role in BD[6]. In addition, this study revealed that DDS as a drug has few adverse reactions in BD patients for a long period of daily treatment with 100mg of DDS. Therefore we studied the acetylator status in BD for the first time. The results revealed that BD patients are slow metabolisers for DDS. Acetylator status of the slow phenotype or the non-acetylator status can serve as a marker for BD. The association between acetylator status and the severity as measured by the CMI of BD further supports this finding. These results might point to a possible role of acetylation in the pathogenesis of BD.

Another finding was that $HLA\text{-}B_{51}$ subtype was strongly associated with acetylator status in BD patients. $HLA\text{-}B_{51}$ was reported to be significantly

associated with BD[19,20]. Our results revealed that 68.2% of BD patient were positive $HLA\text{-}B_{51}$, which is in line with previous reports. The HLA system is a genetically-related system located on the short arm of chromosome number 6 and consisting of 3 classes: class I, class II and class III. Class I is furthermore subdivided into HLA-A, HLA-B, and HLA-C. N-acetylation is also genetically determined; slow acetylators are homozygous for an autosomal recessive gene (rr), while rapid acetylators may be either homozygous (RR), or heterozygous (Rr) for autosomal dominant allele[21]. We could not find other reports in literature relating the acetylator status to $HLA\text{-}B_{51}$ typing. Further research is needed to explore any such possible association.

Dapsone is a drug used in treatment of BD[6,7]. In normal people, approximately 20% of each dose of DDS is excreted in urine as unchanged drug, 70-85% is excreted in urine as water soluble metabolites, and a small amount is excreted in feces[22]. Two metabolic pathways are responsible for metabolism including N-acetylation and N-hydroxylation. The latter producing the metabolite amino-hydroxylamino diphenyl sulfone DDS-NOH which is responsible for the hematological adverse effect (DDS-hydroxylamine induced hemolytic anemia)[22]. The findings in our study have several implications on the kinetics of DDS. The first is that DDS is less metabolised in the liver by a conjugation reaction catalysed by N-acetyltransferase enzyme to MADDS. This will enhance high concentration of DDS in the circulation, giving good therapeutic effect. On the other hand, since most of our patients acetyletate DDS very slowly or not at all, we expect the second pathway to be responsible for metabolism. However, BD patients were treated with DDS for a long duration with few adverse reactions[5,6]. No serious adverse reactions appeared in BD patients while taking DDS for different durations with a standard dose[5,6], inspite of their slow acetylator or non-acetylator status (poor metaboliser). This may possibly indicate different metabolic pathways for metabolism of DDS in BD patients. Further studies are suggested to determine the metabolic pathway of DDS in BD patients. Further studies are also needed using molecular genetics in order to identify the genetic constitution in BD patients and the acetylator genotypes as well as to identify the gene frequency controlling the acetylator status in these patients.

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HLA Antigen, Severity and Acetylator Phenotype in Behçet's Disease Patients with Non-Relative Parents

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1. INTRODUCTION

Behçet's disease (BD) has a strong genetic background with a complex inheritance model[1]. On the other hand, consanguinity is very common in Iraq[2] and many Middle Eastern countries[3]. In one series from Iraq the incidence of consanguineous matings was 57.87%[2]. Several investigators have stressed the relationship between consanguinity and health[3].

Therefore the present paper explores the effect of consanguinity on BD. The parameters studied included the Clinical Manifestation Index (CMI) previously described, which was found to be a useful tool in assessing the severity of the disease[4]. The other factor was positivity for $HLA\text{-}B_{51}$. Several large series in Britain, Japan, Korea and Middle East have shown a significant association between BD and $HLA\text{-}B_{51}$[5].

Acetylation is considered a major metabolic pathway in the biotransformation of a number of drugs[6]. Acetylation exhibits a genetically controlled bimodal distribution within any given population[6]. In a previous report we have shown that patients with BD tend to have a slow acetylation[7]. Therefore the effect of consanguinity on acetylation in BD was also explored.

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2. PATIENTS AND METHODS

Forty-one BD patients, 25 male and 16 female, their age ranging from 19-46 years. The patients registered at BD Clinic at Saddam Medical City, and fulfilled the International Study Group Criteria (ISGC) for the diagnosis of BD[8]. The informed consent of BD patients was obtained. The study was approved by the ethical committee.

Detailed history was taken from the patients including age, sex, occupation, age at onset of the disease, the first presenting feature as well as duration and frequency of occurrence of symptoms. A detailed family history was taken including consanguinity between parents. Patients were considered consanguineous when the parents had at least one ancestor in common and, in practice, this common ancestor was no more remote than a great-great grandfather[9]. Family history of the disease was also taken.

HLA-B_{51} positivity (human leukocyte antigen or major histocompatibility complex MHC) was done for each patient by microlymphocyte cytotoxicity test in Al-Karama Teaching Hospital.

The Clinical Manifestation Index (CMI) was calculated for each patient. The index for the individual patient is the numerical sum of clinical features, the total grading of CMI equal to 31[4]. The pathergy test was done for each patient. In addition, other investigations were done for each patient in the Teaching Laboratories of Saddam Medical City, including hemoglobin (Hb) and erythrocyte sedimentation rate (ESR), white blood corpuscle (WBC) and differential count, c-reactive protein, and liver function test.

The acetylator status was determined by a rapid, simple, one-stage protein precipitation method for determination of acetylator phenotype. The method depends on the estimation of plasma dapsone and its metabolite monacetyle dapsone concentrations by a high performance liquid chromatography (HPLC)[7].

Statistical analyses were done using SPSS version 7.5 computer software (Statistical Package for Social Sciences). Results were presented as mean ± SEM. Differences between groups were assessed by chi square test. An estimate was considered statistically significant if the P value was <0.05. Linear correlation was tested by simple regression analysis[10].

3. RESULTS

There were 41 patients included in the study. Twenty-five patients had non-consanguineous parents while the other 16 had consanguineous parents. Nine patients had a positive family history for BD, which gave a percentage of 21.9%. Seven of these patients had non-consanguineous parents.

Results show that 23 patients out of 25 (92%) with non-consanguineous parents were positive for HLA-B_{51} whereas the frequency of HLA-B_{51} in patients with consanguineous parents was 31.2% (5 patients from a total of 16). The association of positive HLA-B_{51} histocompatibility typing in BD patients with non-consanguineous parents was significant (r=-0.637, P<0.001). Patients with positive HLA-B_{51} had a severer disease represented by a higher CMI. This association was significant (r=0.778, P<0.001).

BD patients with non-consanguineous parents had a severer disease represented by a higher CMI (i.e. a total score of 15 or more out of 31). The total number of patients with non-consanguineous parents with sever disease was 21 out of 25 (84%). All of these patients had positive HLA-B_{51} typing. This correlation was statistically significant (r=0.504, P<0.001). BD patients with non-consanguineous parents also had a significantly higher percentage of slow or non-acetylators (r=-0.675, P=0.001).

Table 2 represents the mean acetylator ratio in BD patients with consanguineous and non-consanguineous parents. Patients with consanguineous parents had a significantly higher mean acetylation ratio.

4. DISCUSSION

BD patients with non-relative parents had the more severe disease. This may be related to HLA system and genetic susceptibility to BD. We expect that these patients may have HLA-B_{51} xenoantigens while patients of relative parents may possibly have HLA-DR_5 alloantigens[11], which may be associated with less severe manifestations of this disease. In contrary, HLA-B_{51} positivity is associated with a severe course of BD.

Furthermore, there is an association with HLA-B_{51} typing as follows: 92% of patients with non-relative parents were HLA-B_{51} positive, while the percentage in patients with relative parents was 31%. This may indicate that BD patients of non-relative parents are more likely to be HLA-B_{51} positive and this correlation also reaches statistical significance (r= -0.637, P<0.001).

Familial clustering of BD is not common but has been reported[12]. In our results, the frequency of patients with positive family history for BD was 21.9% (n=9 from 41), of which 7 patients were HLA-B_{51} positive with non-relative parents. This explains the association between the family history for BD and the absence of relationship between the parents of BD patients. Pathergy test was positive in 73% of our patients and this is similar to other reports of Iraq, where pathergy test was positive in 71% of patients with BD[13].

The frequency of BD patients with non-relative parents in our study was 94.7% in non-acetylators, 55.5% in very slow acetylators, and 15.3% in

moderately slow acetylators. This indicates that the absence of relationship between the parents of patients is negatively correlated with the degree of N-acetylation. This correlation was statistically significant (r=-0.675, P<0.001).

Concerning the genetic predisposition of BD, several studies have shown a significant association between BD and HLA-B_{51}[5]. This was also reported in a study from our country which demonstrated a significant association between HLA-B_{51} and BD[13]. Furthermore, molecular genetic studies have revealed a novel susceptibility gene for BD at a locus about 17 cM to HLA-B locus on the short arm of chromosome 6 (6P22-23). This locus for BD might be in linkage disequilibrium with HLA-B_{51}[1]. These data are indicative for a genetic background of BD with an association between HLA-B_{51}, a novel susceptibility gene, the severity of BD and acetylator status.

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Risk Factors of Neuro-Behçet

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1. INTRODUCTION

Central nervous system (CNS) involvement in Behçet's disease (BD) has a higher variability of prevalence (2-44%) depending on diagnostic criteria and ethnic populations[1-3]. It is related either to direct meningoparenchymal (MP) lesions or to cerebral vascular involvement dominated by cerebral venous thrombosis. CNS involvement in BD is a remarkable cause of morbidity, and approximately 50% of the CNS-BD patients still suffer from moderate to severe disability after 10 years of disease. The aim of this study was to evaluate clinical aspects and course of CNS involvement in BD in view to determine a subgroup of patients at high risk for this complication.

2. PATIENTS AND METHODS

105 patients fulfilling criteria of the International Study Group for BD[4] were retrospectively included. Patients were categorized into two groups according to the presence (group 1) or absence (group 2) of neurological and/or psychiatric involvement. Patients with isolated headaches and those taking drugs and/or affected by disease known to induce CNS involvement were excluded from group 1. Group 1 was also divided into two subgroups according to neurological lesions type: patients with MP involvement, and those with cerebral vascular involvement (CV). Cerebrospinal fluid (CSF) analysis was performed in 19 patients. A pathergy test was performed in 27 patients (19 in group 1 and 8 in group 2) by intradermal needle pick and read

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after 24-48 hours. A cranial tomodensitometry scan was carried out for 19 patients (17 in group 1 and 2 in group 2). Cerebral MRI was performed in 24 patients (18 in group 1 and 6 in group 2). Genetic factors (HLA B51 and MICA6) were determined in 41 patients and 43 controls. HLA B51 allele was determined using a complement-dependent microlymphocyte toxicity assay, and triplet repeat polymorphism of MICA was analysed on a denaturating polyacrynamide gel, and alleles were visualised by autoradiography. Demographic, clinical and genetic features in all groups and subgroups were analysed and compared by Kruskall wallis and chi-square test. P value below 0.05 was considered statistically significant.

3. RESULTS

Twenty-seven patients (27/105 = 25.7%) (20 males and 7 females) had clinical evidence of neurological and or psychiatric involvement (group 1, Table 1). The mean age at onset of BD and at neurological onset was respectively 28.5 and 34.3 years. The mean disease duration before neurological involvement occurrence was 6.4 years (2 months–23 years). Nineteen patients (70.3%) had MP involvement (brainstem: 9, hemisphere: 6, spinal cord: 4, psychiatric involvement: 2, isolated pyramidal signs: 1, isolated aseptic meningitis: 1). Eleven patients (40.7%) had isolated parenchymal involvement. Seven patients (25.9%) had CV involvement (cerebral venous thrombosis: 4, idiopathic intracranial hypertension: 1, cerebral haemorrhage: 1, cerebral arterial thrombosis: 1). A single patient had both MP and CV involvement (venous sinus thrombosis). Headache was noted in 63% of cases, pyramidal syndrome in 52%, cranial nerves in 37% of cases and cerebral stroke like in 26% of cases. Headache was significantly more frequent in CV patients, while motor symptoms and psychiatric involvement were more frequent in the MP subgroup and epilepsy in the CV subgroup. All these differences were not significant. CSF was abnormal in 11/19 cases (57.8%) (7 cases in MP group, 3 cases in CV group and one in both CV and MP subgroups). Inflammatory findings in CSF were pleocytosis observed in ten patients. The CSF opening pressure was increased in only two patients with CV involvement. CT performed in 17 patients, was abnormal in 12 cases (70.5%), and MRI was abnormal in 16 patients (88.9%). Brainstem involvement was observed in 9 patients. It was associated to other locations in six cases (spinal cord in 2 cases and hemispheric white matter in 4 cases). A hemispheric lesion was seen in 6 patients, isolated in 3 cases and associated to brainstem in the others. A spinal involvement was observed in 4 patients, associated to a sus medullar involvement in 3 cases, as psychiatric involvement in 2 cases, isolated

pyramidal signs in one case, and an aseptic meningitis in one case. In CV involvement, 5 patients had intracranial hypertension due to a cerebral vein thrombosis confirmed by IRM in 3 cases, a cerebral haemorrhage in one case, and a cerebral arterial thrombosis in one case. In the patient with both MP and CV involvement, a hemispheric lesion associated to left lateral sinus thrombosis was seen. All patients were treated with colchicine (1 mg/day). Oral steroid (0.5 to 1 mg/kg/day) was used in twenty-three (85%) cases. Intravenous prednisolone was administered in 8 cases. Monthly intravenous cyclophosphamide bolus was used in 15 cases. Anticoagulant agents were administered in cases of confirmed cerebral vein thrombosis. Twenty one patients were followed-up for a mean duration of 3.5 years. In group 1, compete recovery or improvement with mild neurological impairment was seen in 13 cases, improvement with severe disability in 3 cases, worsening in one case, and the course was stationary in one case. Three patients died (11.2%) (cerebral haemorrhage, epilepsy and embolism pulmonary).

Table 1. Clinical and genetic features in group 1 and group 2

	Patients with neurological involvement Group 1 (n = 78)	Patients without neurological involvement Group 2 (n = 27)	P
Male/female	57/21	20/7	NS
Mean age at onset	28.30 ± 8.655	28.462 ± 9.18	NS
Oral aphtosis	78 (100%)	27 (100%)	NS
Genital aphtosis	63 (80.8%)	24 (88.9%)	NS
Pseudofolliculitis	52 (66.7%)	15 (55.6%)	NS
Erythema nodosum	18 (23.1%)	6 (22.2%)	NS
Cutaneous aphtosis	12 (15.4%)	3 (11.1%)	NS
Uveitis	36 (46,2%)	11 (40.7%)	NS
Retinal Vasculitis	22 (28.2%)	11 (40.7%)	NS
Articular involvement	42 (53.8%)	13 (48.1%)	NS
Venous thrombosis	31 (39.7%)	13 (48.1%)	NS
Arterial thrombosis	1 (1.3%)	1 (3.7%)	NS
Arterial Aneurysm	3 (3.8%)	4 (14.8%)	0.048
HLA B51	18/42 (42.8%)	2/7 (28.7%)	NS
MICA 6	17/21 (80.9%)	6/7 (85.7%)	NS

4. DISCUSSION

In our study the frequency of neurological involvements in BD was rather high 25.7%. To determine risk factors of this complication, we compared demographic and clinical features between patients with (Gl) and without neurological involvement (G 2); vascular involvement was more frequent in group 1 than group 2 (48.1% vs 39.7%) but without significant

difference. Only arterial aneurysms were significantly more frequent in neuro-Behçet's patients (Table 1).

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Association of HLA-B51 with Clinical Expression of Behçet's Disease

Analysis of 201 Iranian patients

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1. INTRODUCTION

Despite the strong association between HLA-B51 and Behçet's disease (BD), there is little evidence for its pathogenecity. And the clinical significance of this genetic marker has not been fully elucidated[1].

The aims of this study were to investigate the clinical significance of HLA-B51 and its association with various manifestations of the disease, and to compare it with those of HLA-B5.

2. MATERIALS AND METHODS

In a prospective study, all consecutive new patients with BD according to Iran or Japan criteria, seen in our Behçet's clinic from April 2001 to January 2002, were evaluated. They were classified into two groups according to the presence or absence of HLA-B51. A computerized form, including 100 clinical and paraclinical data, was used to collect different manifestations of the disease. These data were compared with the two groups by chi square test, and corrected by Fisher exact test. A confidence interval at 95% (CI) was calculated for each item.

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3. RESULTS

Of 201 new BD patients studied, HLA-B51 was positive in 78 cases (39%, CI:6.7). Despite the lower disease duration in B51-positive group (5.3±4.9 vs. 7.3±7.1, $p<0.05$), their mean age of onset was higher (29±13.7 vs. 24.2±10.2, $p<0.009$). Disease onset before the age of 16 (9% vs. 25%) and the juvenile form of the disease (1.3% vs. 12%) were both significantly lower in B51 positive patients ($p<0.003$). There was no significant difference between the two groups in any major or minor disease manifestation, presenting signs, or any paraclinical findings.

The same comparison was done in 4531 registered BD patients of our database, between B5 positive and negative group. B5 positive group had a higher frequency of male patients (male/female ratio 1.26 vs. 1.06, $p<0.005$) and a slightly lower rate of childhood onset of the disease (13% vs. 16%, $p<0.002$). The prevalence of ocular (57% vs. 54%, $p<0.009$), articular (36% vs. 32%, $p<0.007$), gastro-intestinal (9% vs. 7%, $p<0.02$), and CNS involvements (3.7% vs. 2.3%, $p<0.008$), as well as epididymitis (12% vs. 9%, $p<0.04$) was higher in B5 positive group. No significant difference was detected for presenting signs, while positive pathergy test was higher in B5 positive patients (61% vs. 55%, $p<0.00006$).

4. DISCUSSION

In conclusion, we have not found any association between B51 and various disease expressions in new BD patients. The delaying effect of B51 on the disease onset has never been mentioned before. This was in contrast to the effect of B5 on various disease expressions[2,3], while the same but rather milder delaying effect was seen with B5[2].

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The Behçet's Disease Activity Index

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1. INTRODUCTION

Currently, there are few, if any, reliable laboratory markers that reflect the fluctuating overall disease activity in Behçet's disease. Judgment of disease activity by clinicians is therefore subjective. Because of this, there is a great need for the development of a standardized scheme to assess current disease activity for use in monitoring disease progression, and for evaluating the effects of therapeutic interventions. In addition to this, any form would need to be investigated before it is used internationally for cross-cultural comparison.

Keeping this in mind, the Behçet's Disease Current Activity Form (BDCAF) was developed as a piece of work for the International Scientific Committee on Behçet's Disease. The aim of this project was to produce a subset of clinical features according to the BDCAF which could sum up to an index describing the grade of disease activity in a patient at a certain time.

2. METHOD

The BDCAF was collected from a number of clinicians in 5 countries (China, Korea, Iraq, Turkey, and the UK). Responses to 12 items (from a previous work[1]) were extracted out of the completed forms to create the index, and to be entered into analysis. These were: Headache, mouth ulcers, genital ulcers, erythema nodosum or superficial thrombophlebitis and pustules, arthritis and arthralgia, nausea and vomiting, diarrhoea/ bleeding

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p.r., any eye involvement, any new CNS involvement, and any new MV involvement.

Data from these 12 items were fitted to the Rasch model which assumes that the probability of a particular patient affirming a given item is a logistic function of the difficulty of the item and the ability of the person[2]. The overall fit of the scale to the Rasch model is given by the Item-Trait Interaction chi square statistic. This should demonstrate a non-significant deviation from the Rasch model, and gives an indication of how well the items fit together to form a hierarchy. The analysis also allows us to consider the consistency of the fit of data from different countries. This is by analysis of Differential Item Functioning. The Software package RUMM 2010[3] was used to complete the Rasch analysis of the data.

3. RESULTS

Five hundred and twenty three forms were collected. The patients had a mean age of 37.5 years, and 57.4% were male. There was a problem with the three category response options required for the clinical features questions. This indicates that this response function is not working as intended. Therefore, in each country all clinical features items were re-scored into either "no symptom or symptoms for less than 2 weeks", or "symptoms for more than 2 weeks".

Following the re-scoring, the scale appeared to fit the Rasch model well in China, Iraq and the UK. In Korea and Turkey the scale fitted the Rasch model to an acceptable level, with one misfitting item in each country (Korea – "any eye problems", Turkey – "any new CNS involvement").

When the data were pooled, the Item-Trait Interaction Statistic, which identifies the degree of the overall fit of the index to the Rasch Model, was significant (chi square=51.252, DF=12, p=0.000001). This indicates that the data are deviating significantly from the model and do not represent a unidimensional and hierarchical scale.

One possible cause of item misfit is Differential Item Functioning (DIF), therefore DIF was examined in relation to the specific country. The items "genital ulcers", "skin pustules", "any eye problem", and "any MV involvement" displayed Cross-Cultural DIF. Post-hoc analysis (Tukey's test) revealed that China, Korea, and Turkey showed the largest deviation from the model. When this occurs, the question can still be included in the index but when analysed it is treated as a separate question for each country. In this way items showed good fit to the Rasch model both at the individual level and overall (chi square=40.894, DF=23, p=0.012176), which

confirmed that the problem of item misfit in the pooled data was driven by differences at the country level.

4. DISCUSSION

The results of the analysis show that the three category scoring function for clinical feature items does not work as intended, i.e. a higher score does not indicate "more" disease activity. Therefore, a two category scoring function is necessary to satisfy the requirements of the Rasch model.

Based on the new scoring function, the 12 summary items from the Behçet's Disease Current Activity Form produce a unidimensional index of disease activity within the countries involved in the study. Though the data from all countries cannot be pooled for comparison (with the exception of Iraq and the UK) by splitting the items and fitting the data to the Rasch model it is possible to use the index to make comparisons between countries. This means that the BDAI will be a useful tool for international research.

ACKNOWLEDGEMENTS

We are very grateful to all the clinicians who provided us with data.

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Development of Clinical Activity Form for Korean Patients with Behçet's Disease

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1. INTRODUCTION

The Behçet's Disease Current Activity Form (BDCAF) has been extensively piloted and studied with data collected from several countries. However, the marked geographic differences in disease expression of Behçet's disease as well as possible ethnic and intercultural differences may hinder precise evaluation. Physicians specializing in various organs involved in Behçet's disease gathered together to compose a new activity grading system, which determines the activity by the symptoms and signs reflecting the inflammation of each organ. It does not include professional terms previously used in activity grading systems, enabling nonprofessionals to grade more correctly. And it also excludes any terms which could be biased, such as fatigue or headache.

This study was to assess the The Behcet's Disease Activity Form for Korean patients as an objective measurement of clinical activity, so that any physician regardless of experience with Behcet's disease can evaluate correctly.

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2. SUBJECTS

Sixty three patients were recruited who fulfilled the criteria of the International Study Group for Behçet's Disease or corresponding to complete or incomplete type of the revised criteria of Behçet's Disease Research Committee of Japan. All patients have been seen regularly with one-month interval, and treated with various drugs, such as colchicine, minocycline, pentoxiphyllin, or steroids.

3. INSTRUMENTS

The Behçet's Disease Activity Form for Korean patients as described above has been made nearly 8 months after the first meeting of physicians. This activity form was completed by patients before physician's examination and assessed by physicians.

3.1 Clinical features to be assessed

1. Self rating scale of well being
2. Oral ulcerations
3. Genital ulcerations
4. Skin lesions
5. Ocular symptoms
6. Vascular symptoms
7. Gastrointestinal symptoms
8. Arthritic symptoms
9. Neurologic symptoms
10. Epididymitis
11. Others

3.2 Scoring system

Symptoms	Score
Oral ulcerations	20
Genital ulcerations	20
Skin lesions	20
Ocular symptoms	20
Vascular symptoms	2
Intestinal symptoms	5
Arthritic symptoms	7

Neurologic symptoms	2
Epididymitis	2
Other symptoms	2

4. RESULTS

Among 63 patients, male to female ratio was 1:3.85 (13:50). The mean age was 39 ± 8.7 years. The rates of recurrent symptoms and the degree of agreement between patients and assessor are shown in Tables 1 and 2, respectively.

Table 1. Presence of recurrent symptoms

Symptoms	Positive rate, n=63
Oral ulcerations	55 (87%)
Genital ulcerations	8 (13%)
Erythema nodosum	26 (41%)
Eye injection	14 (22%), but non-specific
Arthralgia	33 (55%)
Other symptoms	Less than 10%

Table 2. Degree of agreement between patients and assessor

Symptoms	Degree of agreement
Oral ulcerations	Very good
Genital ulcerations	Good
Skin lesions	Good
Ocular symptoms	Moderate
Vascular symptoms	Poor
Gastrointestinal symptoms	Poor
Arthritic symptoms	Fair
Neurologic symptoms	Poor
Epididymitis	Very good

5. SUMMARY

The Behcet's Disease Activity Form for Korean patients was easy to complete by patients without assistance.

Since all patients have been treated long-time, the spectrum of recurrent symptoms was limited. Most common recurrent symptom was oral ulcerations, followed by arthralgia, and erythema nodosum.

Sometimes only a single symptom was enough to make patient feel very ill.

Despite avoiding professional term to describe the symptom, there was poor agreement between patients and assessors in some symptoms, such as vascular, gastrointestinal and neurological symptoms. It may come from poor understanding of symptoms specific to Behcet's disease.

6. CONCLUSION

This study was exploratory and applied to a small number of patients. Therefore, The Behcet's Disease Activity Form for Korean patients needs to be tested on a large number of patients, and assessed by many physicians.

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Living with Behçet's Disease

An insight into the impact of the illness on people's lives

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1. INTRODUCTION

Behçet's Disease (BD) is a chronic multisystemic disorder with a complex pattern of signs and symptoms. Studies are planned to look at predictors of the outcome, and to evaluate new therapies for BD. However, current outcome measures are focused on impairment but do not take into account the wider impact of the condition on the individual's lifestyle. The measurement of the quality of life has a broader focus than simply impairment and activity by considering what the patients' state of health prevents them from doing, and their emotional response to these restrictions. The work presented here is the first step into the development of a Quality of Life Index for BD.

2. METHOD

Twenty five patients were interviewed, all subjects had consultant confirmed diagnosis of BD and were older than 18 years. A theoretical sampling frame was used to ensure a representative sample of subjects relating to age, gender, and presentation of the disease. Subjects could choose the gender of the interviewer; they were encouraged to talk freely about all aspects of their illness and the impact it had on their lives. All

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interviews were taped in full extension and typed interview transcripts were produced.

3. RESULTS

Content analysis was started as soon as the first 5 interviews were transcribed to identify the main themes that emerged. These included "Relationships", for example with partner, children and parents; "Limitations" and "Moods and Emotions". Limitations of the disease included daily activities and social life but subjects also spoke about the impact of their illness on "the big picture" or large life events including career planning and even where they chose to live. A wide range of emotions was expressed from fear and depression to anger and embarrassment. These quotes are typical of the feeling expressed by the participants:

"It's an absolutely totally crazy mixed-up illness, you are never the same from one day to the next"

"She knows I'm not well but I don't think she really understands, she still thinks I can run around and do everything"

4. CONCLUSION

The unpredictable course of BD has far reaching effects on individuals' lives influencing relationships, day to day activities as well as more significant life events. The emotional impact of living with this illness should not be underestimated.

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PATHOGENESIS

Pathogenesis of Adamantiades-Behçet's disease

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1. INTRODUCTION

Adamantiades-Behçet's disease (ABD) is a multisystemic, inflammatory disorder with chronic recurrent course, whereas mucocutaneous lesions exhibit histological changes of vascular reaction or vasculitis[1]. It is characterised by the classical clinical trias of recurrent oral aphthous ulcers, genital ulcers and iritis/uveitis. The aetiology of the disease remains unknown; whereas genetic factors, infectious agents and environmental pollution, immunological mechanisms, and endothelial and clothing factors have been implicated and studied intensively. The major involvement of certain ethnic groups and the wide variation of the prevalence of the disease in the same ethnic group in association to the geographic area of residence indicate environmental triggering of a genetically determined disorder[2,3] (Fig. 1).

2. GENETIC FACTORS

There is no specific mode of Mendelian transmission in ABD[4]. Familial occurrence is one of the mostly reported epidemiological features[2,3], however there are regional differences with familial occurrence being more frequent in Korea (15.4%) than in Japan or China (2.2-2.6%) and in Arab countries, Israel and Turkey (2.0-18.2%) than in Europe (0.0-4.5%; $p<0.001$)[2]. Genetic anticipation in the form of earlier disease onset in the children compared with their parents has been identified corroborating the

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higher frequency of familial cases in juveniles than in adults and the possibility of a genetic predisposition in ABD[5].

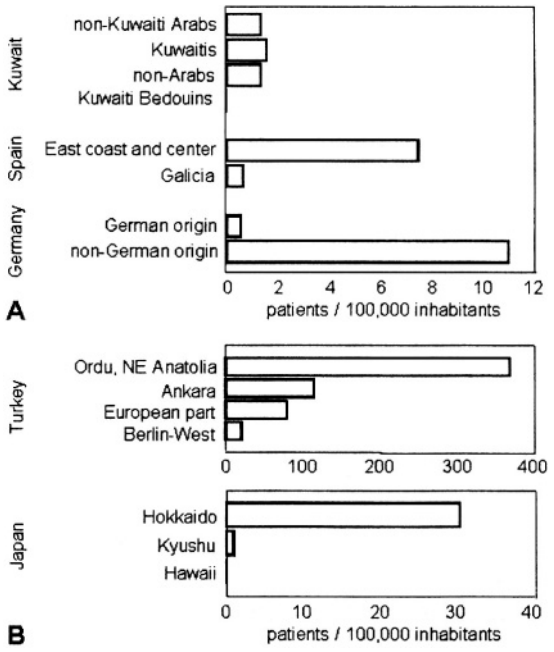

Figure 1. Indications for Adamantiades-Behçet's disease being an environmentally triggered, genetically determined disorder. (A) Different rates of disease prevalence in a common residential area according to the ethnic origin of the patients group. (B) Different rates of disease prevalence in a single ethnic group according to the area of residence.

Several studies have demonstrated a significant association and an increased incidence of HLA B_{51} in ABD world wide, whereas there are regional differences in HLA-B_{51}-associated risk. HLA-B5-positive individuals of German origin as well as from other northern European countries present a lower risk to develop the disease compared to southern Europeans[3] (Fig. 2). However, none of the functional correlates of the disease appear to be restricted by HLA-B_{51}. The role of HLA-B_{51} has been studied extensively and current evidence is shifting towards the view that HLA-B_{51} is not involved directly in the aetiology of the disease but might be closely linked to disease-related gene(s)[6,7]. On the other hand, HLA-B_{51} was found to be a marker for unfavourable prognosis[2,3]. The alleles encoding the HLA-B_{51} antigen include HLA-$B_{5101\text{-}5106}$. HLA-B_{5101} is the allele that is most frequently observed in the normal population and in patients with ABD[8].

Genes possibly associated with ABD have been localised on chromosome 6, in the region between TNF and HLA-B or HLA-C genes[9,10] (Fig. 3), including the MICA, PERB, and NOB genes[11]. MICA allele is a polymorphic major histocompatibility complex (MHC) class 1-related gene. MICA6 allele has recently been shown to be significantly associated with ABD[12]. Moreover, the Tap (transporter) genes, which encode proteins that regulate the intracellular transport of antigens to the MHC molecules, were investigated for polymorphisms and it was determined that the Tap 1C allele was absent in all 58 patients with ABD recruited[6]. In another experiment, the Tau-A microsatellite sequence localised between the HLA-B and TNF regions seemed to be a candidate gene sequence for the disease.

Figure 2. HLA-B51-dependent odds ratios for the occurrence of Adamantiades-Behçet's disease.

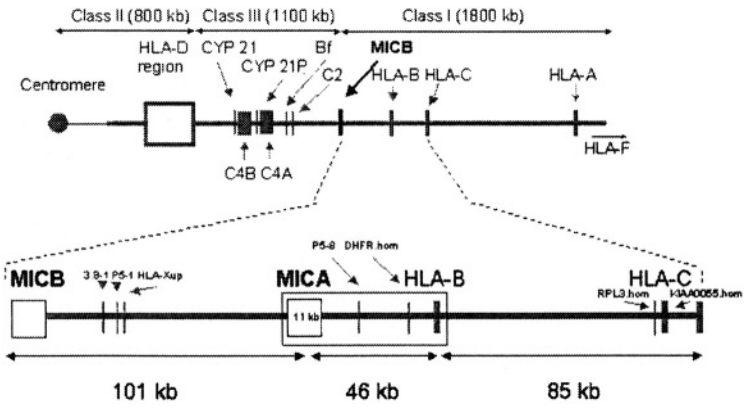

Figure 3. The gene locus for Adamantiades-Behçet's disease (MIM 109650) is located in the area of the MHC, i.e. on the short arm of chromosome 6 (6p21.3).

3. INFECTIOUS AGENTS AND ENVIRONMENTAL POLLUTION

The disease is not considered to be contagious since no horizontal transmission has ever been reported in contrast to the solely vertical one as reported above. However, in addition to the genetic background, offending agent(s) may trigger an immune defence reaction. Bacterial as well as viral infections environmental agents have been implicated to initiate immunopathological pathways leading to the onset of the disease.

3.1 Bacterial agents

A bacterial aetiology has been suggested by Adamantiades in his first publication[13]. The Behçet's Disease Research Committee of Japan reported systemic ABD signs in patients within 1-2 weeks after controlled contact with *Streptococcus sanguis*, *pyogenes*, *faecalis* and *salivarius*, and with *Escherichia coli* and *Klebsiella pneumoniae*[14]. The *Streptococcus* strains *sanguis* and *oralis* dominate the flora of the oral mucosa in patients with the disease and appear to be the most relevant bacteria among those suggested as provoking factor for the initiation of ABD[15,16]. Streptococcal antigens and antistreptococcal antibodies are frequently found in the oral mucosa and serum of patients with the disease[17-19]. The involvement of IgA protease-producing Streptococcus *sanguis* species is proposed as explanation for a chronification of the infection[20]. The BeS-1 gene encoding the immunogenic antigen of *Streptococcus sanguis* KTH-1, a 95 kDa-large antigen, isolated from the patients with Behçet's disease has been cloned and sequenced[21]. In addition, exposure of the patients to streptococcal antigens may be a major provoking factor for the activity of ABD. The proposed mechanism is through activation of neutrophils, which are elevated in patients with ABD[18].

Currently, we have detected MALP-404, a *Mycoplasma fermentas* lipoprotein, in serum of 32% of ABD patients compared to none of the healthy controls[22]. Interestingly, MALP-404 contains the peptide motif -G-------F, which can be presented by HLA-B51[6]. Mycoplasmas cause infections of mucosal tissue, colonizing epithelia of the respiratory or genital tract and especially *Mycoplasma fermentans* is associated with rheumatoid disease. Most mycoplasmas contain macrophage activating components[23], and macrophages have been shown to be strongly stimulated by factor(s) circulating in the serum of ABD patients[24].

3.2 Viral agents

A viral aetiology was suggested by Behçet in his original publication[25], based on observations of inclusion bodies in oral and genital ulcerations. A 211-bp Herpes simplex virus type 1 (HSV-1) DNA fragment was identified and partial transcription of the HSV-1 genome has been detected in patients' peripheral blood mononuclear cells[26,27]. HSV-1 DNA was detected in 43% of 91 saliva patients' samples compared to 14% of 87 samples from healthy controls ($p<0.01$)[28] and antibodies against the virus have been found in serum of patients with ABD[29]. Increased T cells and high levels of anti-HSV antibodies have been observed in patients with CNS involvement. In contrast, no cytomegalovirus has been isolated from biopsy specimens or serum of patients with ABD[30]. Antibodies against hepatitis viruses, known to play a role in vasculitis, have been detected in serum of patients with ABD[31,32] but no causative correlation between them and ABD has been established[33].

3.3 Environmental pollution

The experimental administration of chemical environmental compounds to swines was followed - 4-10 months later - by oral, genital, and intestinal ulcers and cutaneous lesions[34]. On thed other hand, increased levels of environmental pollutants were found in several cell types and in serum of patients[35].

4. IMMUNOLOGICAL MECHANISMS

Immunological mechanisms are considered to play a major role in the pathogenesis of ABD[36] (Fig. 4). They include the involvement of heat shock proteins, alterations of the neutrophils and macrophage activity, expression of numerous cytokines and chemokines, and autoimmune mechanisms.

4.1 Heat shock proteins

Heat shock proteins (HSP) are molecules that are synthesised in response to various kinds of stress in all eukaryotic cells[37]. They also share antigenic epitopes with several bacterial micro-organisms which have been implicated in the pathogenesis of ABD, such as HSV, Streptococcus species and Mycobacteria. T cell epitope mapping has identified four peptides derived from the sequence of a 65 kDa bacterial HSP which stimulate proliferation

of $\gamma\delta^{+}$TCR lymphocytes of patients with ABD[38-40]. These peptides show significant homology with the corresponding peptides derived from the human 60 kDa mitochondrial HSP[41]. In the cerebrospinal fluid of patients who had parenchymal neurological involvement an increased level of anti-HSP antibodies could be found[42]. On the other hand, immunoglobulin A isotype of antibodies specific for mycobacterial tuberculosis HSP-65 could cross react with certain serotypes of *Streptococcus sanguis*[43]. In general, cross reactions between microbial and human 65-kDa HSP possibly link infection with autoimmunity.

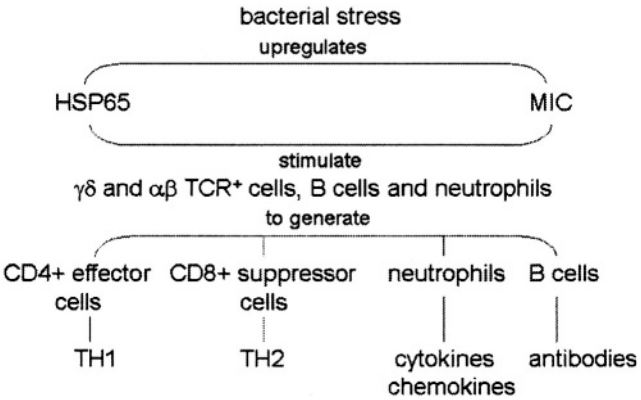

Figure 4. The suggested immunopathogenesis of Adamantiades-Behçet's disease (from Lehner, T., 1999, *Ann. Med. Interne (Paris)* **150**: 483-487[36])

The hypothesis that a 65-kDa HSP antigen determinant is involved in the aetiology and pathogenesis of BD is supported by (a) raised serum IgA antibodies to mycobacterial 65-kDa HSP[40], (b) autoantibodies to oral epithelial antigens[43], (c) cross reactivity between a number of monoclonal antibodies to mycobacterial HSP and *Streptococcus sanguis*[42], (d) cross-reactivity between polyclonal antibodies to mycobacterial HSP and oral epithelial antigens, and (e) significant increase of both circulating and inflammatory site infiltrating T cells during the active phase of the disease. The up-regulation of T cells observed in ABD patients suggests that the aetiological agent(s) may include 65-kDa HSP peptides shared between common bacteria and human tissues, superantigens such as bacterial toxins, or viruses[39,44]. Interestingly, the MICA protein of the MHC region is recognised by T-cells with a variable Vd1$\gamma\delta$ region in their T-cell receptor and antigens presented to $\gamma\delta$+CD8+ T-cells are assisted by the MICA molecule[45].

4.2 Neutrophil function

Neutrophil chemotaxis and phagocytosis are increased in cutaneous lesions of ABD patients[46-48]. Leukocyte adhesion molecules (L-Selectin, MAC-1 and CD44) are expressed on peripheral leucocytes and may participate in the sequential cascades of leucocyte chemotaxis and adhesion. Endothelial adhesion properties are enhanced due to the increased expression of CD11a/CD18 in neutrophil surfaces and ICAM-1 in the endothelium. A higher level of superoxide production in the neutrophils of patients with ABD seems to be related to the presence of HLA-B51[48]. In conclusion, the enhanced superoxide and excessive production of lysosomal enzymes and enhanced chemotaxis of neutrophils from patients with ABD indicate that the neutrophils are overactive, which leads to tissue injury[49,50].

4.3 Monocyte function

The activation of monocytes may explain the production of pro-inflammatory cytokines responsible for the chronicity of inflammation[51]. Spontaneous overproduction of TNF-α, IL-6, and IL-8 by patients' monocytes is apparently related to the disease activity. Patients' serum was shown to be able to inhibit the anti-inflammatory molecule AMAC-1 expression on healthy monocytes induced IL-4 and dexamethasone[24].

4.4 Cytokine and chemokine mediators

Various pro-inflammatory cytokines such as IL-1, IL-8 and TNF-α are elevated in the sera of ABD patients. Especially, IL-8 seems to play an important role, can be also released by endothelial cells, and is a sensitive marker of disease activity[52,53]. Cytokine release may be dependent on the involved organ. Elevated levels of IL-6 in the cerebrospinal fluid was found in patients with neurological involvement[54], whereas patients with CNS involvement and oral aphthous ulcers exhibit elevated serum levels of IL-8[52]. Patients with ocular involvement show increased IL-2-producing $CD4^+$ cells[55]. In addition the correlation of TNF receptor-75 levels with disease activity indicates that TNF receptor-75 may serve as a biological marker of disease activity[56]. The elevation of plasma IL-12 was also shown and the correlation of IL-12 plasma levels with disease activity may suggest a pathogenetic role of a Th1-type immune response in active disease[57].

4.5 Autoimmune mechanisms

Autoimmune mechanisms are involved in the pathogenesis of ABD. A number of immunological abnormalities have been identified and circulating immune complexes are compatible with the development of clinical features. The major microscopic finding at most sites of active ABD is an immune-mediated occlusive vasculitis[50,58]. At the cellular level, CD4-T cells were found in the perivascular inflammatory exudate and Thl-cells respond to various stimuli to produce IL-2, IFN-γ, and TNF-α[46]. In addition, patients' lymphocytes express CD29 molecules and bind to endothelial cells in active disease[46,59]. Cytokines induce B cell proliferation. On the other hand, IL-12 is generated by stimulation of CD4-T cells with the HSP peptide 336-351, and can also be secreted by neutrophils in ABD[41]. In addition, IL-8 levels are higher in patients with active disease; this cytokine has a potent effect on the inflammatory response[52]. Natural killer (NK) cells are increased in peripheral blood in patients with active disease, but their activity was found to be relatively low at the intervals[60,61]. Decreased NK cell activity may be correlated with increased levels of prostaglandin E_2, whereas prostaglandin E_2 depresses NK cells activity[61,62]. Circulating immune complexes together with enhanced neutrophil migration may be involved in the pathogenesis of systemic and mucocutaneous effects of ABD[46,52,63.]

5. ENDOTHELIUM AND CLOTHING FACTORS

5.1 Endothelial cells

Recurrent vasculitis and thrombosis are key findings in ABD. The decreased levels of prostacyclin observed in serum of ABD patients and auto-antibodies against oxidatively modified low density lipoprotein implicated a role of endothelial dysfunction in the disease[64,65]. Lee et al. have detected circulating IgM complexes directed against a disease-specific 44-kDa cell membrane-bound receptor on microvascular endothelial cells[63,66]. Binding of the circulating IgM complexes to their endothelial receptor does not lead to endothelial cell death but induces a type III immunologic reaction, such as the synthesis and release of cytokines[52,59,66] (Fig. 5). Enhancement of endothelial cell E-selection expression by patients' serum and increased inflammmatory cell binding to endothelial cells in the presence of infectious agents have also been detected[67,68].

5.2 Clothing factors

Vascular changes leading to vasculitis and thrombosis are additional important pathological features of ABD. Auto-antibodies against cardiolipin have been identified[69]. Endothelial cell damage causes auto-oxidative damage and an increase of oxygen radicals[70]. There is increased risk of thrombosis during the course of ABD[50,71]. Plasma endothelin-1 concentrations were found significantly increased, indicating vaso-contraction[72] and being direct result of elevated synthesis from injured vascular endothelial cells. Thrombomodulin, a cell surface glycoprotein of vascular endothelium, which was also detected to be increased in plasma of patients with active ABD, potentially damages the endothelial cells[71,73].

6. CONCLUSION

Current research on the pathogenesis of Adamantiades-Behçet's disease points on a genetically determined disorder with certain MHC genes on chromosome 6 being the most obvious candidates. Environmental factors, especially a chronic infection, are probably responsible for triggering an immunological reaction on genetically determined individuals. Extrinsically induced tissue stress or heat shock proteins act as a common factor linking some of the possible pathogenetic agents to cross react with host tissues and elicit a significant Th1 cell response. Additional activation of endothelial cells leads to tissue damage and self-maintenance of inflammation. The chronic local inflammation process together with clothing factors lead to enhanced coagulation and thrombosis.

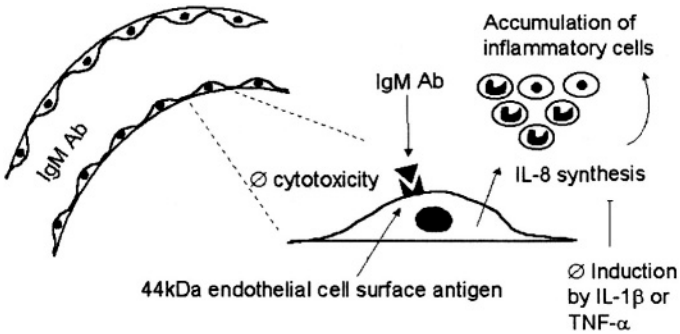

Figure 5. The involvement of endothelial cells in Adamantiades-Behçet's disease. Circulating antibodies against a specific endothelial cell surface antigen activate vascular endothelial cells to produce cytokines which induce accumulation of inflammatory cells.

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Immunopathogenesis and Prevention of Uveitis with the Behçet's Disease-Specific Peptide Linked to Cholera Toxin B

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1. AETIOLOGY OF BD

1.1 Viral aetiology

Herpes simplex virus 1 (HSV1) has been studied extensively and it appears that Behçet's disease (BD) is not a result of direct infection by HSV1 but possibly a dysfunction of the immune system to the viral antigens[1,2]. DNA hybridization and PCR studies have shown increased mRNA and DNA of HSV1 (but not those of HSV-2) in BD[3-6]. HSV1-specific immune complexes, raised antibodies and cytotoxic T cells were found in the circulation of patients with BD[7,8]. A model of HSV1 induced BD has been developed in ICR mice[9].

1.2 Streptococcal aetiology

Streptococcus sanguis or its cross-reactivity with oral epithelial antigens has been implicated in the aetiology of recurrent oral ulcers[10-12]. Later 4 species of streptococci (*S. sanguis, S. pyogenes, S. faecalis and S.*

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salivarius)– were suggested as aetiological agents in BD[13,14]. The *S. sanguis* found in lesions of patients with BD were uncommon serotypes[15]. Significant levels of IgA and to a lesser extent IgG antibodies to these streptococci (KTH-1 to 3) were found in patients with BD[16]. *S. sanguis* stimulated T cell proliferation, IL-6 production and upregulation of mRNA or IL-2 and IFNγ[17,18]. Furthermore, *S. sanguis* found in BD cross-reacted with oral mucosal antigens and this was accounted for by shared homology between the microbial and human 65kD heat shock protein[16]. This may account for the early autoimmune findings in BD and recurrent oral ulceration[19,20].

2. HEAT SHOCK PROTEINS

The large number of strains of streptococci and the cross-reactivity between *Streptococcus sanguis* and oral mucosal antigens raised the hypothesis[16] that a common antigen, such as stress or heat shock protein (HSP) found in most Gram-positive bacteria[21] might account for these diverse observations. Indeed, Western blot analysis showed cross-reactivity between these streptococci and the HSP65, as well as significant increases in IgA and IgG antibodies to HSP65 in sera from patients with BD[16]. Although stimulation of PBMC with HSP65 yielded high stimulation indices in BD (10.9 ± 1.8), this was not significantly different from healthy controls, disease controls or patients with recurrent oral ulcers[22]. HSP from one bacterial species shows considerable homology with that of other bacterial species, so enhanced immunity may be boosted naturally by intercurrent infections that have been implicated in BD.

T cell epitope mapping identified 4 peptides derived from the sequence of the 65kD HSP[22] which stimulate specifically $\gamma\delta^+$ T cells from patients with BD[23]. These peptides (111-125, 154-172, 219-233 and 311-325) showed significant homology with the corresponding peptides derived from the human 60kD HSP[22]. The B cell epitopes within mycobacterial HSP65 or human HSP60 overlapped with the T cell epitopes, and both IgG and IgA antibodies were identified[24]. Among the 4 T and B cell epitopes, peptide 336-351 is significantly associated with BD in Britain[16,23], Japan[25] and Turkey[26]. HSP65 was also significantly increased in the epidermal cells of skin lesions in BD[27], and antibodies to HSP65 were raised in the cerebrospinal fluid from patients with neurological manifestations of BD[28]. Thus, there is an increasing body of evidence to suggest the involvement of HSP65 and specific epitopes within this molecule in the pathogenesis of BD.

It is noteworthy that peptide 91-105 is specific in stimulating T cell proliferation in patients with recurrent oral ulcers[29]. This epitope is separated

only by 5 residues from the BD-specific peptide 111-125. Although ROU is the only consistent feature of BD, the T cell proliferative response to peptide 91-105 is lost in BD. Thus, T cells from patients with ROU respond only to peptide 91-105, whereas patients with BD, still manifesting ROU, loose their reactivity to this peptide and develop a response especially to peptide 336-351. The intramolecular switch in HSP65 from aa 91-105 to 336-351 and the other 3 peptides is therefore of great significance and deserves further attention. Peptide 91-105 has been subjected to further studies[30] using alanine substitution of each amino acid, and this revealed that the critical residues are found in the carboxy terminal part of peptide 91-105 (residues Leu 98, Arg 100, Arg 104 and Asn 105).

3. HSP-PEPTIDE INDUCED EXPERIMENTAL UVEITIS

The pathogenicity of these peptides in inducing some of the manifestations of BD was then tested in Lewis rats. Subcutaneous immunization with any of the 4 peptides but especially with peptide 336-351 and adjuvants elicited uveitis in about 80% of Lewis rats[31,32]. A mucosal model of induction of uveitis was then developed in rats by oral or nasal administration of p336-351 without an adjuvant[33], and this is consistent with the onset of oral mucosal ulceration in more than 90% of patients with BD. Mucosal induction of experimental uveitis appears to be mediated by $CD4^+$ T cells, whereas suppression is induced by $CD8^+$ T cells or IL-4[33]. This finding is consistent with peptide 336-351 eliciting IL-12, a TH1 cytokine from $CD4^+$ T cells,whereas $CD8^+$ cells secrete cytokines which suppress TH1 cell function[34].

In addition, microbial HSP65 and HSP70 function as potent stimulators of the 3 CC chemokines, RANTES, MIP-1α, and MIP-1β but not MCP-1[35]. The chemokines are stimulated by HSP to a comparable extent, irrespective of whether the HSP is empty (i.e. ATP-treated) or loaded with a peptide. The function of these chemokines is to attract immature dendritic cells, macrophages, T and B cells[36-40]. It is therefore not surprising that HSP65 and HSP70 act as systemic[41-43], as well as mucosal adjuvants[35]. HSP65 and HSP70 are the only known reagents to modulate both systemic and mucosal immunity, and this property might be important in BD which is one of a few multisystem diseases with major mucosal and systemic manifestations. An important issue, however, needs to be resolved, that is whether the microbial HSP cross-reacting with human HSP is adequate to account for the immunopathogenesis of BD or if there is a peptide within the pocket of HSP that is specific for BD. It is noteworthy that HSP70 acts as molecular

chaperone in folding, unfolding of proteins and peptides[44]. It is also significant that HSP70 is one of the few external reagents that can translocate antigens into the class I pathway, thereby inducing THl-type immune responses[45]. HSP70 utilises the CD40 receptor[46,47] which is a costimulatory molecule on dendritic cells, macrophages and B cells and is critical in the interactions with the CD40 ligand, especially in $CD8^+$ T cell responses[48,49]. Interaction between HSP70 and CD40 costimulatory molecules stimulates CC chemokines and cytokines and may function as a bridge between innate and adaptive immunity[46,50].

HSP65 and HSP70 stimulate $\gamma\delta^+$ T cells to proliferate[51-53] and to generate the 3 CC chemokines[35]. $\gamma\delta^+$ T cells are present in circulating blood and mucosal tissues of healthy individuals and are usually either $CD3^+/CD4^-/CD8^-$ or $CD8^+$ T cells. The proportion of $\gamma\delta$ T cells is significantly increased in BD[54-57] and this is consistent with HSP65 and/or HSP70 stimulating an increase in number of $\gamma\delta$ T cells[51-53,35.] This subset differs from $\alpha\beta$ T cells in that it is not HLA restricted, and MICA molecules can present peptides to $\gamma\delta$ T cells[58]. HSP peptides specific for BD stimulate $\gamma\delta$ T cells to proliferate[23]. *S. sanguis* also stimulates $\gamma\delta$ T cells to generate IL-2 and IFNγ mRNA. $\gamma\delta$ T cells may play an essential role in the immunopathogenesis of BD, as they have been consistently found to be increased in BD[54-57].Stimulation of $\gamma\delta$ T cells with microbial HSP or their constitutive peptides, coupled with the capacity of MICA gene product to present peptides to $\gamma\delta$ T cells[58], enhances the significance of this subset of T cells in BD. The function of $\gamma\delta$ T cells in BD needs to be determined but they are involved in mucosal ulceration through their capacity to generate TH1 cytokines[59], to function as killer cells[60], by mediating expression of a keratinocyte growth factor[61], and by generating CC chemokines[35].

4. ACTIVATION OF HSP AND MICA

The multi-system pathology in BD might be initiated by microbial stress, activating HSP and the MICA cell stress response gene in epithelial cells. Indeed, MICA contains heat shock elements defined in HSP70 genes and shares nucleotide sequences homologous with the human HSP70 promoter[62]. Thus, microbial stress may activate HSP and MICA, and induce in genetically predisposed individuals a cascade of cytokines and chemokines to stimulate innate immune responses. CD 14 has been identified as a receptor for human HSP60[63], whereas the microbial and human HSP70 utilise the CD40 receptor[46,47,50]. CD 14 receptors are found on macrophages and neutrophils, whereas CD40 receptors have a wider distribution, on macrophages, dendritic cells, B cells and endothelial cells. These cells are

stimulated to generate an array of cytokines and chemokines that will elicit increased vascular permeability, acute phase proteins (including CRP, factor B and C9), and chemoattraction of mononuclear cells and neutrophils. Innate immunity may drive adaptive immunity with specific CD4, CD8, γδ T cell and B cell responses and polarization towards the TH1-type of cytokines. Overall, the recognition of interaction between HSP, MICA and $\gamma\delta^+$ T cells in the immunopathogenesis of BD raises the provocative hypothesis that BD might be a disease generated by over-reaction to microbial stress proteins.

5. PREVENTION OF MUCOSALLY INDUCED UVEITIS WITH A HSP60 DERIVED PEPTIDE, LINKED TO CHOLERA TOXIN B SUBUNIT (CTB)

Oral or nasal tolerization using p336-351 failed to prevent experimental uveitis in Lewis rats and indeed induced uveitis even without an adjuvant. However, when p336-351 was covalently linked to CTB and given orally, a significant decrease in uveitis was found[64]. The mechanism of tolerance preventing the development of uveitis may involve a TH2 subset of memory cells, an increase in TH2 and/or TH3 and decrease in TH1 cytokines in the mesenteric lymph nodes and the uveal tract of the eye[64]. In view of the successful prevention of the HSP-peptide induced uveitis by the peptide-CTB conjugate an open clinical trial was initiated in humans. Preliminary results suggest that oral administration of p336-351-rCTB conjugate enabled gradual withdrawal of existing treatment with immunosuppressive drugs in some patients with BD, without a relapse of uveitis (Stanford, M., Whittall, T., Lehner A, in preparation). This phase I/II clinical trial is due to be completed shortly when the full results will be reported and may pave the way to a double blind phase III clinical trial.

6. CONCLUSION

Behçet's disease (BD) is a multisystemic disease affecting muco-cutaneous, ocular, central nervous system, joint and vascular tissues. The aetiology of BD has been associated with a variety of microorganisms, especially Herpes simplex virus and some strains of *Streptococcus sanguis.* A common microbial agent may be involved, such as heat shock protein (HSP) which has significant homology with human cellular HSP. The evidence in favour of HSP as an aetiological factor in BD is based on T cell

proliferative responses and B cell antibodies to the defined HSP60 epitope p336-351 in patients with BD in Britain, Japan and Turkey. The proportion of γδ T cells is significantly increased in BD and HSP65 or HSP70 upregulates γδ T cells. The peptide determinant defined in patients with BD elicits uveitis in Lewis rats when administered by the oral mucosal or systemic route. It is noteworthy that the MICA gene is a cell stress response gene and shares nucleotide sequences with the human HSP70 promoter. The cytokine and chemokine networks stimulated by HSP induce polarisation towards the TH1 cytokines during active disease. The evidence from these studies has converged towards the concept that the multi-system immunopathology of BD is generated by an over-reaction to microbial stress proteins. We now demonstrate that p336-351 induced uveitis in rats can be prevented by oral tolerization with the peptide linked to recombinant cholera toxin B subunit. Preliminary evidence in an open clinical trial suggests that this novel tolerizing strategy may be effective in preventing relapse of uveitis in BD.

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The Role of Infectious Agents in the Pathogenesis of Behçet's Disease

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1. INTRODUCTION

Patients with Behçet's disease (BD) have generally chronic infectious foci in their oral cavity such as chronic tonsilitis, decayed teeth with apical odontitis, pyorrhea, etc. Their oral hygiene standard seems to be lower compared to healthy individuals of similar age. Non-pathogenic streptococci including S. sanguis can be detected in these infectious foci, and the patients have hypersensitivity against such streptococci[1,2]. S. sanguis taken from such patients is of the uncommon serotype KTH-1 (strain 113-20)[3] which is also detected in patients with Kawasaki disease.

Using streptococcal antigens in cell walls of S. sanguis, we can induce oral aphthous ulceration artificially, cutaneous hyperreaction by the prick test in vivo, and the over-production of inflammatory cytokines from peripheral blood mononuclear cells (PBMC) of BD patients in in-vitro tests[2,4]. It has been demonstrated that some immunological abnormalities, such as low activity of NK cells, increase of $\gamma\delta$ T cells, hypercomplementemia, etc. are present[5] and that heat shock protein (HSP) 60/65 is detectable in patients' sera and/or lesions[6,7].

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2. METHODS AND RESULTS

To examine the relationship between focal infections in the oral cavites and the muco-cutaneous lesions in BD patients, we prepared and sequenced the DNA (Bes-1) encoding the antigen of S. sanguis (KTH-1) from BD patients[8]. Using the primers of Bes-1 which are homologues to the human intraocular peptide, Brn-3b, we performed PCR analyses and PCR in situ hybridization with the lesional samples from BD patients and positive controls consisting of cellulitis infected by streptococci. In the lesions, aphthous and genital ulceration, and erythema nodosum (EN)-like eruption of BD patients, the PCR analysis revealed amplified bands, and PCR in situ hybridization was positive on cells adhering the vascular walls and infiltrated macrophages in EN-lesions[9].

3. DISCUSSION

The results suggest that the debris of S. sanguis which may be released from the infectious foci in the oral cavity reacted immunologically as antigens in the lesions of the patients.

In further experiments, we should try to find the relationship between the bacterial debris and HSP 60/65 in the various lesions of BD patients with an HLA-B51 genetic background.

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Detection of Microbial DNA in Skin Lesions from Patients with Behçet's Disease

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1. INTRODUCTION

The etiology and mechanism of pathogenesis of Behçet's disease (BD) is not known although there are several hypotheses about causative factors, such as viral or bacterial infection, or autoimmunity with a genetic basis. Patients with BD generally have a high incidence of chronic streptococcal infections such as tonsillitis and dental caries, and hyperreactivity to streptococcal antigens might be related to these foci of chronic infection[1]. BD patients have an intense delayed-type hypersensitivity to streptococci which is shown by their cutaneous reactions[2] as already demonstrated by us, among others, using streptococcus antigens and overproduction of inflammatory cytokines by their peripheral blood mononuclear cells (PBMCs)[3,4]. In this previous study, we found that *Streptococcus (S.) sanguis,* which is serologically different from the standard strains, was dominant in the oral flora of patients with BD[5,6]. These patients showed significantly higher titers of antibody against several 80-150 kDa membrane proteins from the isolated strain as well as greater hypersensitivity to streptococcal antigens than did normal controls[7,8]. Furthermore, the strain adhered avidly to the epithelial cells of the lesions from BD patients[6,9]. Recently, we cloned and sequenced the Bes-1 gene encoding the immunogenic antigen of *S. sanguis,* KTH-1 (uncommon serotype 1, strain 113-20), isolated from BD

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patients[10]. A portion of the amino acid sequence has 60% homology with the human intraocular peptide Brn-3b, which is a POU domain expressed in a subset of retinal ganglion cells (Fig. 1)[11]. Western blot analysis of the gene product of an immunopositive clone showed that samples from BD patients, but not from healthy controls, had a reactive band, demonstrating a cross-reactivity between the *S. sanguis* peptide and human intraocular peptide. Although attention had been focused mainly on streptococci[2,3], viral infection is also thought to be an etiologic factor. A number of different viruses have been implicated in BD and particular attention has been paid to evaluating the role of herpes viruses such as herpes simplex virus (HSV)[12]. In this study, we performed PCR and PCR in situ hybridization (PCR-ISH) analyses to detect Bes-1 DNA in BD muco-cutaneous lesional samples including oral aphthae, genital ulcers, folliculitis, and erythema nodosum (EN)-like eruptions, and compared the results obtained to those of samples of lesions from other related inflammatory diseases including non-BD-EN, Sweet's disease, and phlegmone due to streptococcal infection (Table 1). Another aim of this study was to screen BD patients for the presence of HSV-1, HSV-2, HHV-6, and HHV-7 genomes in their skin lesions, and to compare the results obtained with those from other related inflammatory disorders.

```
Identities=9/15(60%), Positives=10/15(66%)
     BES-1    229  AFIVPHGGHFHYIPK  243
                   ** +****  *  **
    Brn-3b    11   AFSMPHGGSLHVEPK  25
Identities=6/13(46%), Positives=7/13(53%)
     BES-1     373  HGDHHHFIPYDKL  385
                    *  ***  *+  *
    Brn-3b     177  HHHHHHHQPHQAL  189
```

Figure 1. Amino acid sequence similarities between BES-1 and Brn-3b

2. BES-1 DNA IN BD SKIN LESIONS

The 999 bp fragment of Bes-1 DNA was detected by PCR in muco-cutaneous lesions from patients with BD (Fig. 2). The product yielded the expected fragments when digested with Pst-1 restriction endonuclease (Fig. 3). Among the BD patients, 37% were positive for Bes-1 DNA (three of 11 cases) and this was confirmed using skin samples without epidermis (Fig. 3, Table 2). We also demonstrated by PCR the presence of Bes-1 DNA in

samples of a genital ulcer (No. 4, incomplete type) and an EN-like eruption (No. 22, complete type) from patients with BD, and Bes-1 DNA was found in the skin sample of an EN-like eruption (No. 7) and an oral aphtha (No. 8) in one patient with BD (suspected type). Bes-1 DNA was also detected by PCR-ISH in the muco-cutaneous lesions, which were positive by PCR (Table 2). The reaction product was deposited on the nuclei of cells adhering to the dermal vessel walls and some of the infiltrates around the vessels in the main inflammatory lesions (Fig. 4). The PCR-ISH-positive cells correlated with the presence of Bes-1 DNA in the lesions from patients with BD.

Table 1. Details of patients with Behçet's disease (BD) and other inflammatory disorders

No./Sex/Age	Diagnosis	Location	Duration and symptoms
1/F/44y	Sweet's disease	lower leg	2mo: myelodysplastic syndrome
2/F/30y	BD	lower leg	2y: EN-like symptom
3/M/29y*	BD	scrotum	5y: genital ulcer
4/M/29y*	BD	scrotum	5y: genital ulcer
5/F/48y	EN	lower leg	2w:
6/F/23y	EN	lower leg	1w:
7/F/47y**	BD	lower leg	1mo: EN-like symptom
8/F/47y**	BD	oral mucosa	1mo: oral aphthae
9/F/23y	Phlegmone	lower leg	2w:
10/M/56y	Sweet's disease	chest	3w:
11/F/70y	EN	lower leg	1mo: drug-induced
12/F/29y	BD	arm	7y: EN-like symptom
13/M/49y	BD	scrotum	4y: genital ulcer
14/M/50y***	BD	trunk	3y: folliculitis
15/M/50y***	BD	finger	3y: folliculitis
16/M/50y***	BD	finger	3y: folliculitis
17/F/41y	Sweet's disease	lower leg	2.5mo: hyperthyroidism
18/F/23y	BD	lower leg	2.5y: EN-like symptom
19/F/28y	BD	upper leg	1.5y: EN-like symptom
20/F/25y	BD	Lip	7y: oral aphthae
21/F/39y	BD	lower leg	4y: EN-like symptom
22/F/49y	BD	arm	24y: EN-like symptom

*,**,***; three patients have several lesions, EN: erythema nodosum

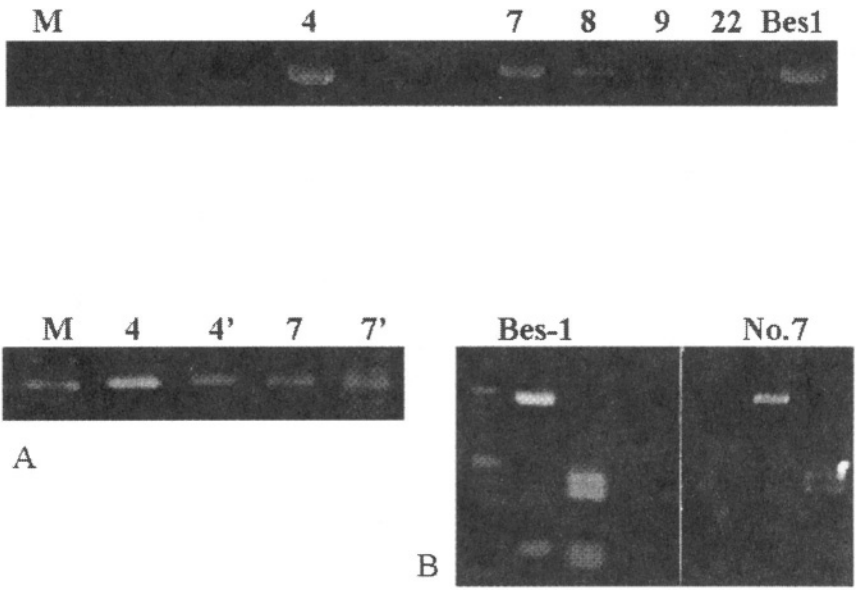

Figure 3. Bes-1 DNA in muco-cutaneous lesions. A) The epidermis was removed from Bes-1 positive samples by micro-dissection. Representative data are shown (No.4 and 7). Lanes 4 and 7 include epidermis and lanes 4' and 7' are without epidermis. Positive bands were detected in all lanes. B) Electrophoresis of restriction endonuclease digested DNA. Representative data are shown (No.7). Positive control, Bes-1 and No.7 showed the expected fragment pattern; uncut 999-bp sample and sample cut with Pst-1 showing 160- 389- and 450-bp fragments.

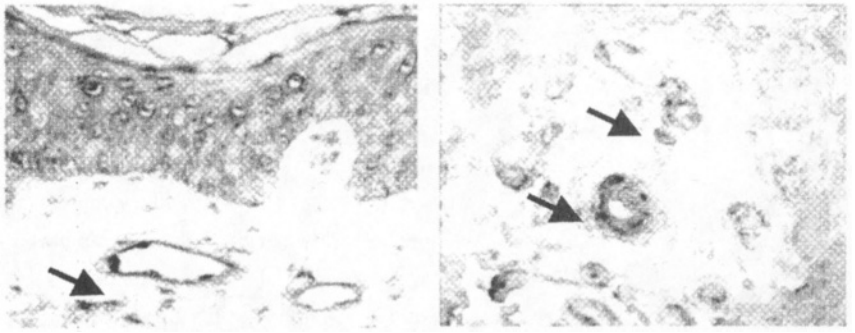

Figure 4. Bes-1 DNA in muco-cutaneous lesion. PCR-ISH analysis of EN-like eruption in one BD case (No. 7) shows the positive signals (arrows) for Bes-1 DNA in cells adhering to the superficial dermal vessel wall (left), and deep dermal vessel walls (right).

3. HERPES VIRUS DNA IN BD SKIN LESIONS

We also found HSV-1 and/or HSV-2 DNA in one (incomplete type) of 11 BD cases, two of three cases of Sweet's disease, and one of three non-BD-EN cases (Table-2). HSV-1/2 DNA was found in a lower percentage of BD cases (one of 11 cases) than Bes-1 DNA. Moreover, high titers of HSV-1/2 antibodies were closely correlated with positivity for HSV-1/2 DNA in the skin lesions, even in patients with both BD and non-BD syndromes. All cases were negative for HHV-6 and HHV-7 (Table 2).

Table 2. Results in patients positive for PCR and PCR-ISH

No. of Samples	Bes-1 PCR	Bes-1 PCR-ISH	HSV-1	HSV-2	HHV-6	HHV-7
1	-	-	Yes	-	-	-
2	-	-	-	-	-	-
3	-	Yes	-	-	-	-
4	Yes	Yes	-	-	-	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	Yes	Yes	Yes	Yes	-	-
8	Yes	Yes	-	-	-	-
9	Yes	Yes	-	-	-	-
10	-	-	-	-	-	-
11	-	-	Yes	Yes	-	-
12	-	-	-	-	-	-
13	-	-	-	-	-	-
14	-	N.D.	-	-	-	-
15	-	N.D.	-	-	-	-
16	-	N.D.	-	-	-	-
17	-	N.D.	Yes	-	-	-
18	-	N.D.	-	-	-	-
19	-	N.D.	-	-	-	-
20	-	N.D.	-	-	-	-
21	-	N.D.	-	-	-	-
22	Yes	N.D.	-	-	-	-

N.D.: not done

4. CONCLUSION

The presence of Bes-1 DNA seems to be more closely related to the pathogenesis of BD than the presence of HSV1/2. Our results suggest a causative role for Bes-1 in the pathogenesis of BD and a correlation with chronic focal infection in the oral cavity. Consistent PCR-ISH findings are crucial for demonstrating a causal association between an infectious agent, Bes-1, and skin lesions in patients with BD. Therefore, further studies,

probably utilizing double staining for characterizing of PCR-ISH-positive cells, may elucidate the role of the streptococcal antigen, Bes-1, in the pathogenesis of BD lesions.

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Association of *Mycoplasma fermentans* with Adamantiades-Behçet's disease

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1. INTRODUCTION

Mycoplasmas are wall-less bacteria causing infections of mucosal tissue. Many mycoplasmas are commensals, others, such as *Mycoplasma (M.) fermentans,* are associated with rheumatoid arthritis and other joint disorders[1]. Most, if not all, mycoplasmas contain macrophage activating components. Studies on a macrophage activator from mycoplasmas, comparable in activity to the lipopolysaccharide of Gram-negative bacteria, led to the identification of a macrophage-activating lipopeptide (MALP)-2 from *M. fermentans.* MALP-2 is a small lipopeptide originating from MALP-404, a 40 kDa-large lipoprotein[2]. MALP-2, among other mycoplasmal lipoproteins, has an unusual N-terminus. It consists of a cystein bearing a dihydroxypropyl group in thioether linkage. Both hydroxygroups are esterified with long chain fatty acids whereas the aminogroup is free. This distinguishes mycoplasmal lipoproteins from those in walled bacteria. Mycoplasmal lipoproteins are exceptionally good antigens because they have a built-in adjuvant site in the form of the lipid-substituted N-terminus. Thus, MALP-404 is an immunodominant determinant in *M. fermentans.* MALP-404 is expressed by the most *M. fermentans* strains including those isolated from the joints of patients with reactive arthritis (Mühlradt, unpublished).

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Like other bacterial proteins, MALP-404 contains the peptide motif -G-------F which can be presented by HLA-B51[3]. This transplantation antigen is associated with Adamantiades-Behçet's disease (ABD)[4]. ABD is a chronic, multisystemic inflammatory disorder, which is clinically characterized by relapsing oral aphthous and genital ulcers, ocular, and vascular lesions. The disease may affect small and large vessels in almost all organs. ABD is a universally rare disorder with varying prevalence, occurring endemically in the Eastern Mediterranean area and in Central and East Asia, with a peak onset in the 3rd decade of life[5]. A microbial infection has been implicated in the development of the disease to explain the strong inflammatory reactions observed, the activation of monocytes and macrophages, and the induction of proinflammatory cytokines and chemokines detected[6,7]. Recently, several reports demonstrated that crude fractions of lipoproteins derived from different mycoplasma strains showed macrophage-stimulatory activities by inducing the production of proinflammatory cytokines. For all these reasons, we investigated the presence of antibodies against MALP-404 in the sera of patients with ABD and examined a possible correlation of *M. fermentans* infection with the disease in an ethic committee-approved case-control study.

2. PATIENTS AND METHODS

The 22 patients [10 female, 12 male; median age 37 years; originating from Germany (n=3), Turkey (n=12), and other countries (n=7)] and 14 gender-, age-, and origin-matched healthy controls [7 female, 7 male; median age 36 years; originating from Germany (n=4), Turkey (n=7), and other countries (n=3)] were recruited after providing written consent. Patients fulfilled the criteria of the International Study Group for Behçet's disease[8]. The presence of the MALP-404 antigen was detected in the serum of the subjects examined by the Western blot method. Demographic and background characteristics were displayed using summary statistics. The primary and secondary variables were evaluated using chi square tests. For all comparisons, a significance level of 0.05 was applied.

3. RESULTS

From the 22 ABD patients, 7 (32%) were MALP-404-positive; among them 3 patients (14%) with strong antibody signals indicating a previous or currently active infection with *M. fermentans* (Fig. 1). In contrast, MALP-404 antibodies were not detected in any of the 14 matched controls

examined (odds ratios > 6.07 and 2.05, respectively; $p<0.05$). Four of the 7 MALP-404-positive patients (57%) but also 9 of the 15 MALP-404-negative patients (60%) were HLA-B51-positive (odds ratio = 0.89, ns). The presence of the MALP-404 antigen did not correlate with any demographic feature, the clinical signs or the prognostic factors of the disease (ns).

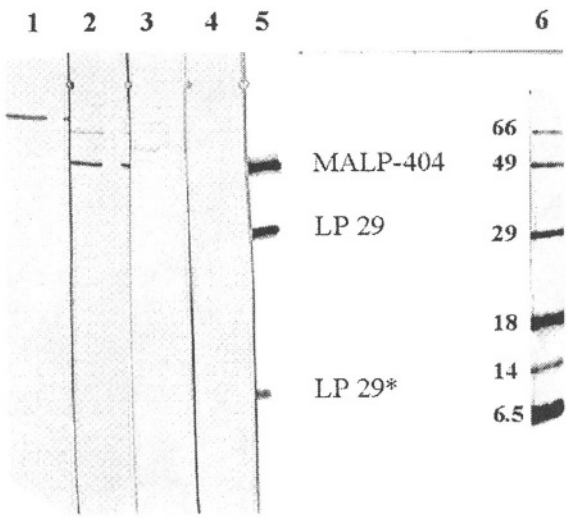

Figure 1. Western blot of selected sera from patients with Adamantiades-Behçet's disease (ABD). Mycoplasma lipoprotein antigens were enriched by cold octyl glucoside extraction of a *Mycoplasma fermentans* strain isolated from the knee joint of a rheumatoid arthritis patient[1]. The antigen mixture was separated on SDS-PAGE and electroblotted to a PVDF membrane. Lanes 1–4: sera from ABD patients; lane 5: monoclonal antibodies against the indicated lipoproteins (positive control); lane 6: molecular weight markers. Note typical positive reaction to MALP-404 in lane 2, and strong reaction with an unidentified *M. fermentans* antigen in lane 1.

4. DISCUSSION

These data suggest that a high percentage of patients with ABD presents with antibodies against *M. fermentans*. MALP-404 has been shown to be subject to site-specific proteolysis between residues 14 and 15 of the mature lipoprotein, resulting in the cell-bound MALP-2 and soluble released fragment products[9]. On the other hand, MALP-404 is responsible for macrophage activation; and macrophages of healthy donors have been strongly activated by sera of ABD patients[6]. Therefore, it is possible that a

M. fermentans infection in ABD patients predisposes these patients to a cross-reactive autoimmune response and precipitates the disease[4].

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Antimicrobial Activity of Synthetic Human CAP18 Peptides to *Streptococcus sanguis* Isolated from Patients with Behçet's Disease

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1. INTRODUCTION

Despite continuous challenges by a wide variety of microbes, mammals remain remarkably free of infectious diseases. Innate immunity, a system encompassing a complex array of defense elements mediated by both local and circulating effector cells, provides this crucial fist-line host defense. A number of antimicrobial peptides involved in host defense have been isolated from various animal species[1,2]. Cathelicidins are a family of antimicrobial peptides found in many mammalian species[3]. The cathelicidins contain a conserved cathelin domain and a C-terminal domain found in various species yielding multiple different antimicrobial peptides[2]. CAP 18, 18 kD, a cationic antimicrobial protein derived from human leukocytes, is a 140-amino acid protein recently demonstrated to have lipopolysaccharide (LPS)-binding and antimicrobial activity[4]. LL-37 is synthesized as a precursor, termed human cationic antimicrobial protein 18 (hCAP18). The precursor is cleaved to the mature peptide LL-37 by a serin protease, protease-3, in neutrophils after exocytosis[5]. LL-37 can bind to and neutralize bacterial LPS[6,7] and is chemotactic for human peripheral monocytes, neutrophils, and CD4 T lymphocytes[8,9]. The gene encoding LL-37/hCAP18,

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peptides had a stronger killing activity than $hCAP18_{109-135}$. Thus the araino acid sequence which influences charge distribution is essential for the bactericidal effect. Sequential permeabilization of outer and inner membranes of the target bacterium can cause its death.

In this study, eight *S. sanguis*(-like) strains, three isolated from patients with BD (BD113-20, BD114-23, BD118-1), two from patients with Kawasaki disease (SSH-83, KKH-T), and three type and reference strains of ATCC (ATCC10556: *S. sanguis,* ATCC10557: *S. oralis,* and ATCC10558: *S. gordonii)* were used. *S. sanguis*(-like) has been suggested as a causative agent of the disease. The proportion of the bacteria in the oral flora of patients with BD was significantly increased compared with controls[12]. Patients with BD showed hypersensitivity in skin tests with the streptococcal antigens, and symptoms typical of BD were sometimes provoked by an injection of the antigen[13].

Minimal inhibitory concentration (MIC) was defined as the lowest concentration of peptide causing at least 99.9% reduction of the number of microorganisms presented at the beginning of the MIC determination. Fifity percent inhibitory concentrations (IC50s) were determined by least-squares linear regression.

The antimicrobial activity of CAP 18 was tested by the MIC assay. The MIC of $hCAP18_{109-135}$ is more than 20 μg/ml. Two analog peptides LL/hCAP18 and FF/hCAP18 showed strong activities ranging from 0.3 to 20 μg/ml (MIC). In the IC50 assay, hCAP18 was active against all strains at concentrations ranging from 0.5 to 8.4 μg/ml (Table 1). The IC50 of CAP 18 analog peptides was at most 10-fold lower than that of $hCAP18_{109-135}$. The rate of bactericidal effect may be an important factor for assessing the activity of antimicrobial peptides in vivo, and determining their potential use as pharmaceuticals. Therefore, we examined the time course of bacterial killing. When the peptides were used at 10 μg/ml concentration, the time required for half killing ranged from <5.0 to 16.2 min. Two strains, ATCC10558 and ST-IV, were killed within 5 min, whereas the others required 9.8 min or more. The analog peptides LL/hCAP18 and FF/hCAP18 induced rapid killing.

2.2 Binding effect

The binding activity was examined by the erythrocyte agglutination assay. Briefly, one milliliter of 1% erythrocytes (human O type) was sensitized by incubation with 0.2 ml of various LPS solution (100 μg/ml in HBSS). Fifity microliters of a 1.0% suspension of sensitized erythrocytes was mixed with an equal volume of a twofold serial dilution of CAP 18 peptides in a U-bottom microtiter plate and incubated at 37 C for 1 h.

Activity of CAP 18 was expressed as the minimum agglutinating concentration (MAC).

Table 1. Anti-microbial activity of synthetic human CAP18 peptides IC50

Bacterial strain	IC50(μg/ml)		
	$hCAP18_{109-135}$	LL/hCAP18	FF/hCAP18
S.sanguis BD113-20	1.0	0.15	<0.15
S.sanguis BD114-23	3.3	2.5	<0.15
S.sanguis BD118-1	4.8	2.1	0.24
S.sanguis SSH-83	4.2	0.65	0.95
S.sanguis KTH-T	8.4	2.2	<0.15
S.sanguis ATCC10556	7.0	0.18	0.46
S.oralis ATCC10557	4.8	2.5	1.12
S.gordonii ATCC10558	0.53	0.21	0.52
S.gordonii ST-7	3.7	0.11	<0.15

CAP 18 peptides tested were agglutinated erythrocytes sensitized with purified cell wall, Ranz-Randall extract (RRE), glycerolipid, or lipoteichonic acid (LTA) at the concentration of 0.6 to 5μg/ml. (Table 2). Thus, CAP 18 is not only LPS-binding protein to Gram-negative bacteria but also a LTA/glycerolipid-binding protein to Gram-positive bacteria such as *S. sanguis.*

Table 2. Agglutination of component-sensitized erythrocytes by synthetic CAP18 peptide

Bacterial component	Hemagglutination: MAC(μg/ml)		
	$hCAP18_{109-135}$	LL/hCAP18	FF/hCAP18
Cell wall from *S. sanguis*	5.0	1.2	2.5
RRE from *S.sanguis*	5.0	1.2	2.5
Glycerolipid from *S. sanguis*	2.5	0.3	5.0
LTA from *S.sanguis*	1.2	0.3	2.5
MDP from *S.sanguis*	>20	>20	>20
LPS from *S. minnesota* R595	1.2	0.15	0.07
LPS from *E.coli* O111:B4	5.0	0.6	0.15
LPS from *S.flexneri* serotype 1A	20.0	2.5	0.6

3. ANTIMICROBIAL PEPTIDES IN INNATE IMMUNITY

In this study, we have found that CAP 18 peptides bind to cell wall components such as LTA. After binding, pore formation can occur and change the membrane permeability. In the future, it will be interesting to learn how the altered expression of antimicrobial peptides is related to genetic background or progression of BD. Fig. 1 shows a model of mucosal host defense in BD.

1. The initial encounter between the host and pathogens usually occurs at surface boundaries, especially wet mucosal surfaces. Not only is infection relatively uncommon, but signs of inflammation are remarkably rare, too. These observations imply that highly effective host defense mechanisms, capable of dealing with the vast majority of microbial encounters, exist at these sites.
2. Defense of mucosal surfaces includes inducible and constitutive expression of antimicrobial peptides. The peptides are also derived from circulating cells such as neutrophils.
3. Possible consequences of long-term deficits of these innate defenses would be recurrent and/or chronic infections or chronic mucosal inflammation.

CAP 18 may be related to the host innate immunity in patients with BD.

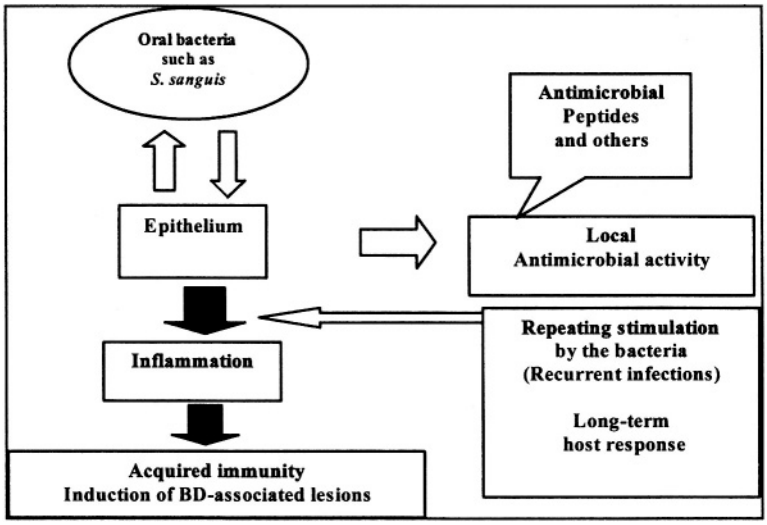

Figure 1. A model of mucosal host defense and BD

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Anti-*Saccharomyces cerevisiae* Antibodies

A novel serologic marker for Behçet's disease

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1. INTRODUCTION

Over the past few years, antibodies against *Saccharomyces cerevisiae* (ASCA), a yeast commonly used in the food industry, were reported in patients with Crohn's disease (CD) with a prevalence varying from 39% to 76%[1-3]. ASCA are considered specific for CD, and their major clinical use has been to differentiate CD from ulcerative colitis (UC) in both adult and pediatric populations[1,4]. ASCA are also found in healthy first-degree relatives of patients with CD, suggesting a genetic origin of the antibodies[5,6]. Behçet's disease (BD) is a multi-system disorder, the clinical expression of which may be dominated by mucocutaneous, articular, neurologic, urogenital, vascular, intestinal or pulmonary manifestations[7]. BD and CD share various clinical similarities, including mucocutaneous manifestations (recurrent oral ulcers, erythema nodosum), gastrointestinal disease favoring the terminal ileum, and recurrent arthritis as well as uveitis, thus raising the possibility of certain etiologic and pathogenic factors common with both diseases. Hence, we evaluated the prevalence of ASCA in patients with BD, and looked for possible associations between positive ASCA and various BD-related manifestations as well as disease severity.

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2. PATIENTS AND METHODS

Twenty-seven BD patients were studied, all of them fulfilled the International Study Group (ISG) criteria for BD[8]. None of the BD patients had inflammatory bowel disease (IBD), nor a first-degree family relative with IBD. Furthermore, none of the patients had gastrointestinal manifestation of BD.

The severity score was calculated as previously described[9-11].

ASCA levels were determined by ELISA employing commercial kits for IgG- or IgA-ASCA (QUANTA Lite™, INOVA Diagnostics, Inc. San Diego, CA, USA), and following manufacturer's instructions. Values of 25 or more units were regarded positive. IgG- and IgA-ASCA levels were measured in the group of BD patients and in two control groups: ten patients with idiopathic recurrent aphthous stomatitis (RAS) with no other clinical feature of BD, and ten healthy volunteers.

3. RESULTS

Twenty-seven BD patients were studied. There were 7 males (25.9%) and 20 females (74.1%), mean disease duration was 13.1±9.1 years. Thirteen BD patients were either IgG- or IgA-ASCA positive (48.1%), of whom two patients were positive for both IgA- and IgG-ASCA. In contrast, only one patient in each control group was positive for ASCA (10%, $p=0.01$). Fig. 1 shows the results of the IgG- and IgA-ASCA assays. The mean value of IgG-ASCA in the BD patients was 20.7±12.3 (range 6-45) units which was significantly higher than in the patients with RAS (10.0±5.5, $p<0.001$), or healthy volunteers (10.8±9.8, $p<0.02$). In the BD patients mean IgA-ASCA levels were 16.8±8.8 units being significantly higher than in the group of healthy volunteers (11.0±5.0, $p=0.02$) but similar to the patients with RAS (17.0±5.3). Regarding the BD patients, no correlation was found between positive ASCA values and the presence of genital ulcers, ocular disease, skin lesions, positive pathergy reaction, deep or superficial vein thrombosis, arterial disease, joint manifestations, neurological involvement or the presence of HLA-B5. There was also no difference in the rate of major oral ulcers between positive- and negative-ASCA patients (27.3% vs. 30.8%, NS), nor in the BD-severity score (7.31±1.80 vs. 7.28±2.27, NS).

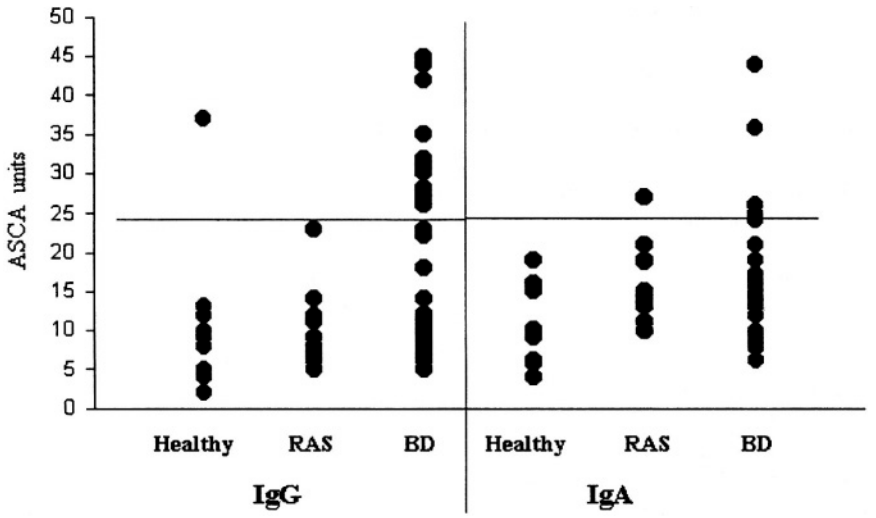

Figure 1. ASCA levels (IgG and IgA) in patients with Behçet's disease (BD), recurrent aphthous stomatitis (RAS) and healthy controls. Levels equal to or greater than 25 units are considered positive.

4. COMMENTS

The results of our study associate, for the first time, the presence of a distinct antibody with BD. We show that ASCA, until now considered rather specific for CD, appear in BD patients with a prevalence comparable to those reported for CD. In our study, in order to avoid possible overlap with CD, none of the patients nor any family relative had IBD. Furthermore, none of the patients had chronic gastrointestinal manifestations. It appears, therefore, that the presence of ASCA in BD is an integral part of the disease, not associated with intestinal or other specific target-organ involvement. Furthermore, the systemic severity score of BD was also similar in the two groups of patients. Thus, it is conceivable that the presence of ASCA does not pose an increased risk for a more severe disease course in BD. Further prospective studies are needed to evaluate whether ASCA titers are correlated with clinical relapses of the disease. Concerning the diverse clinical expression of BD in various geographical areas, it will also be of interest to evaluate the prevalence of ASCA among BD patients worldwide.

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GENETICS

The Human Genome Project: What We have Learnt about the MHC Region on Chromosome 6 and Its Potential to Behçet's Disease

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1. INTRODUCTION

Although research at the Sanger Institute is not focused on Behçet's Disease, many of the resources generated as part of the Human Genome Project (HGP) will be useful to advance the research into this comparatively rare disease with autoimmune aetiology. Like most autoimmune syndromes, Behçet's disease is thought to be caused (at least in part) by one or multiple genetic components within the Major Histocompatibility Complex (MHC) on the short arm of chromosome 6. In the following, the main resources already available at the Sanger Institute will briefly be described.

2. THE HUMAN GENOME PROJECT

A draft sequence of the human genome has been available since June 2000 and is already making a big impact on biomedical research[1]. At the time of writing, about 90% of the genome sequence was finished to an accuracy of less than 1 error in 10,000 bases and the remainder is expected to be completed by Spring 2003. The main findings of the initial analysis were:

- The genome was found to encode 30,000-40,000 protein-coding genes. The exact number is still difficult to determine but current estimates are closer to 30,000 genes.

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- The proteome was estimated to be considerably larger (greater than 100,000 proteins) owing largely to alternative splicing.
- Some genes may originate from horizontal transfer although this finding remains controversial.
- Close to half (45%) of the sequence was determined to consist of repeat elements providing a detailed "fossil" record of our genome.
- The rate of mutation was found to be twice as high in males as in females.
- The rate of recombination was found to be uneven across chromosomes (higher towards the telomeres).
- In a separate study, over 1.4 million single nucleotide polymorphisms (SNPs) were identified and mapped across the human genome[2]. These SNPs provide a valuable resource for linkage disequilibrium (LD) mapping of genes in complex diseases.

A lot of these data are freely available via the ENSEMBL Human Genome Browser[3] shown in Fig. 1.

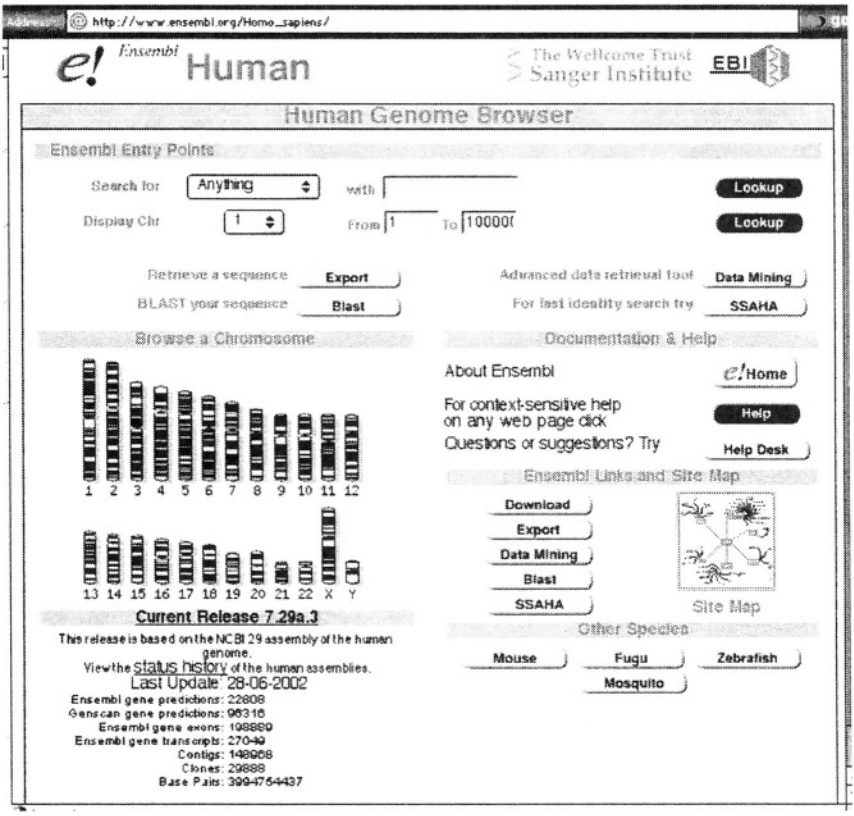

Figure 1. Homepage of the ENSEMBL Human Genome Browser

3. CHROMOSOME 6 AND MHC RESOURCES

The MHC is the most important genetic region in relation to common human diseases such as autoimmunity and infection. Because of this biomedical importance, the MHC was completely sequenced by 1999[4], well ahead of the human genome draft sequence. Driven by pathogen variability, the MHC is under enormous pressure to evolve and adapt quickly. Over time, it has become the most polymorphic region in the human genome with some genes such as HLA-B (which has been associated with Behcet's disease), having over 400 alleles. However, even subtle changes in the self/non-self recognition pathways can lead to genetic miscommunication and result in autoimmune diseases such as Diabetes, Multiple Sclerosis and Behcet's disease. Figure 2 shows a summary of chromosome 6 including the extended MHC which is located on the short arm at 6p21.31-22.1. The high gene and SNP densities of the MHC are clearly visible.

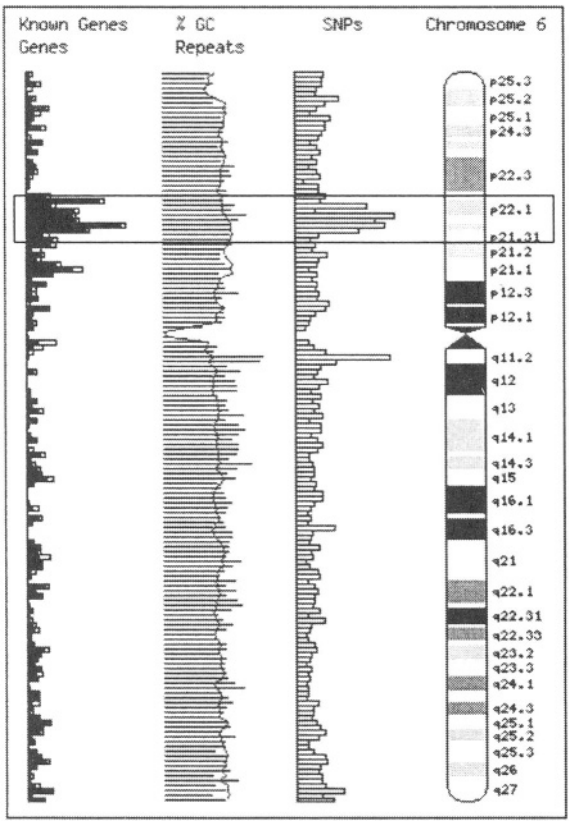

Figure 2. July 2002 status of chromosome 6 showing the distribution of over 1,000 genes and over 125,000 SNPs. The approximate position of the extended MHC is boxed.

termed CRAMP, contains binding sites for transcription factors activated by interleukin 6 (IL-6). Consistent with this, LL-37/hCAP18 expression appears to be regulated by IL-6 in some epithelial tissues[10]. LL37/CAP18 is upregulated in keratinocytes in response to inflammatory stimuli. Recently, we reported that the C-terminal 27 aminoacids in LL-37 comprise the LPS-binding and anti-microbial domain[11].

We speculate that LL-37/hCAP18 is related to oral mucosal defense and that the regulated expression and production of this peptide can be important for inhibition of BD. In the first step, we show that a synthetic peptide exerts a potent bactericidal activity against *Streptococcus sanguis* isolated from patients with BD. The activity of two synthetic analogues of hCAP18 is also reported. Target molecules against the bacteria are determined as well.

1.1 What is CAP18?

CAP 18 has a linear amphipathic α-helical structure that is important for its antimicrobial activity, and it differs markedly from human α– and β-defensins, which have three disulfide bonds and a β–sheet structure. CAP 18 is expressed in specific neutrophil granules and various epithelial cells. CAP 18 contains a conserved cathelin domain and a C-terminal domain. Similar conserved domains has been recognized in the other antimicrobial peptides of the cathelicidin family.

The human CAP 18 cDNA encodes a protein composed of a 30-amino-acid signal peptide, a 103-amino-acid N-terminal domain of unknown function, and a 27-amino-acid C-terminal domain. We used the C-terminal 27-amino-acid fragment (FRK SKEKIGKEFK RIVQRIKDFL RNLV) which was identified as the LPS-neutralizing and antimicrobial domain. This peptide was designated as $hCAP18_{109\text{-}135}$. Two analog peptides LL/hCAP 18 and FF/hCAP18 (substituted to phenylalanine or leucin, respectively) were also used.

2. ANTIMICROBIAL EFFECT OF CAP18

2.1 Bactericidal effect

Our studies aimed at evaluating their in-vitro activity against *S. sanguis* isolated from patients with BD. We used a synthetic linear peptide, the anti-microbial domain of $hCAP18_{109\text{-}135}$. The factors that may influence activity are to form an amphipathic α-helical structure, local or overall charge distribution and density, and some minimal peptide length. Two analog

The high level of polymorphism and linkage disequilibrium between MHC genes has long hampered the identification of disease causing loci in this region of the genome[5]. In order to overcome this problem, the MHC Haplotype Consortium has initiated a project to re-sequence several complete haplotypes to establish the precise identical-by-descent or ancestral relationships between the most common MHC haplotypes[6]. These data provide a framework and resource for association studies of all MHC-linked diseases, including Behcet's disease and can be accessed via the web site shown in Fig. 3.

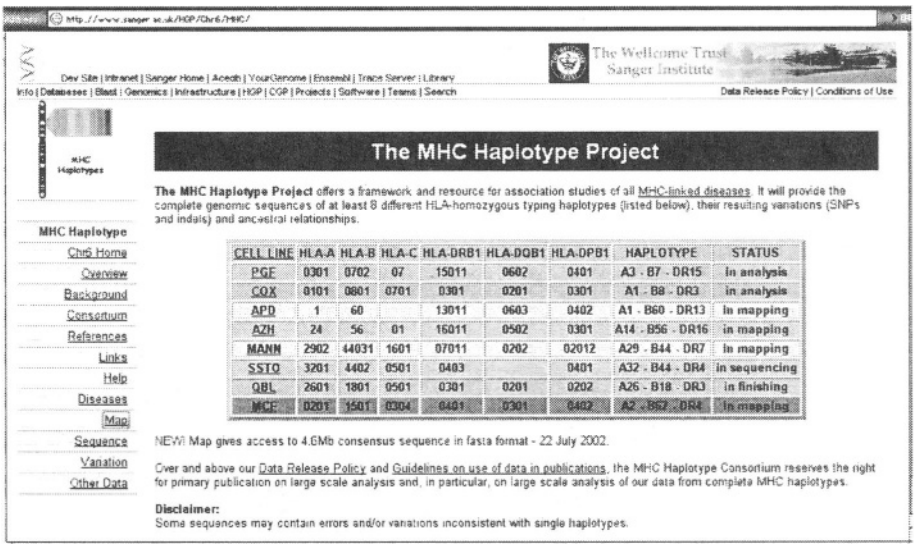

Figure 3. Homepage of the MHC Haplotype Project

4. THE HUMAN EPIGENOME PROJECT

Disease cannot only be caused by genetic change (e.g. DNA mutation), but also by epigenetic change (e.g DNA modification). The most common epigenetic DNA modification is the methylation of cytosines at CpG dinucleotides. Differential CpG methylation has been shown to play a crucial role in many biological processes such as imprinting, gene regulation, chromatin remodeling, genome stability and, of course, disease (particularly cancer). The Human Epigenome Project aims to catalogue genome-wide methylation patterns by identification of methylation variable positions (MVPs)[7,8]. These data will provide the missing link between genetics, disease, and the environment. As pilot study, the Human

Epigenome Consortium is in the process of elucidating the methylation pattern of all expressed MHC genes. These data will be useful to determine whether or not Behcet's disease has also an epigenetic component.

ACKNOWLEDGEMENTS

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Comparison of Proteome Map Between Sera of Patients with Behçet's Disease and Controls

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1. INTRODUCTION

Behçet's disease (BD) is a chronic, multisystemic disorder characterized by recurrent inflammatory reaction. Its etiology or pathogenesis has not been clarified yet[1-3].

In recent years, the field of proteomics has opened new possibilities for the finding of methods of diagnosis and treatment in this disease group. A combination of high-resolution two-dimensional (2-D) polyacrylamide gel electrophoresis and highly sensitive biological mass spectometry has paved the way for high-throughput proteomics[4-7].

In this study, we compared the sera of BD patients with controls by high-resolution two-dimensional electrophoresis with isoelectric focusing in 3-10 immobilized pH gradients. Most spots were identified by reference to the serum map in the SWISS-2D PAGE database.

2. MATERIALS AND METHODS

This study included 20 BD patients visited the BD Speciality Clinic of Severance Hospital, Yonsei University College of Medicine, who fulfilled the criteria of International Study Group for BD and the revised criteria of the BD Research Committee of Japan. At the same time, serum samples were obtained from healthy normal volunteers (n=20) without any skin

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diseases and autoimmune diseases as normal controls. Protein from sera was diluted with sample, and rehydration was performed using 18 cm, pH 3-10 linear immobilized pH gradient(IPG) strip (Amersham Pharmacia Biotech, Piscataway, NJ, USA) for 24 hours. Isoelectric focusing (IEF) was performed with rehydrated IPG strips using PROTEAN IEF cell (Bio-Rad, Hercules, CA, USA) for appropriate time. After IEF, IPG strip were equilibrated in buffer consisting of 6M urea, 2% sodium dodecyl sulphate (SDS), 5M Tris 2 ml, 20% glycerol 4ml, 25% acrylamide 1 ml, and 200 mM MTBP 250 μl for 25 minutes.

After making polyacrylamide gels and adding agarose buffer that includes 0.5% agarose and 0.001% bromophenol blue dye, an IPG strip was embedded. Cathode running buffer of 24.8 mM Tris, 192 mM glycine and 0.1% SDS was added. Gels were run initially at 3 mA/gel for 2 hours and then at 18 mA/gel for approximately 16 hours. After electrophoresis in the second dimension the protein spots were routinely visualized by silver staining. We compared changes of protein spots using MELANIE III (Bio-Rad, Munich, Germany) after scanning with Calibrated Imaging Densitometer (Bio-Rad).

3. RESULTS

We found that the 9 groups of protein spots were changed in comparing between sera of patients with BD and controls by high-resolution two-dimensional electrophoresis (Table 1). The density of protein spots such as haptoglobin, immunoglobulin heavy/light chain, apolipoprotein, fibrinogen gamma A chain, and transthyretin was decreased in sera of patients with BD compared with sera of controls.

Table 1. Changed protein spots in comparison with sera of patients with Behçet's disease and controls by two-dimensional electrophoresis

Protein	Isoelectric point/kDa	Number (%)	Comment
Haptoglobin	6.08/17	3 (15)	Decreased
Immunoglobulin heavy chain	6.5-7.0/38	1 (5)	Decreased
Apolipoprotein	5.78/38	2 (10)	Decreased
Haptoglobin-1	5.76/38	2 (10)	Decreased
Immunoglobulin light chain	5.63/26	1 (5)	Decreased
Fibrinogen gamma A chain	5.56/50	1 (5)	Decreased
Clusterin	4.84/38	3 (15)	Increased
Unidentified	4.8/8	2 (10)	Increased
Transthyretin	5.52/35	20 (100)	Decreased

4. DISCUSSION

As the complete human genome sequence was decoded recently, a lot of studies on analysing the function of human genes are actively in progress. Proteomics are the most suitable method for such projects.

Two-dimensional electrophoresis, described by O'Farrell[8], was the technique that could analyse the protein, separated by the isoelectric point of protein mixture and molecular weight, in terms of quality and quantity. High resolution 2D polyacrylamide gel electrophoresis has been lately used as a standard method of analysing the protein.

In our study, protein spot with 5.52 isoelectric point and 35 kD molecular weight was decreased in all sera of BD patients. It was identified as transthyretin by reference to the SWISS-2D PAGE database. Transthyretin is pre-albumin and decreases during severe liver disease, malnutrition or acute inflammation[9]. This result is consistent with the clinical manifestations of BD and shows that transthyretin may play an important role in the pathogenesis of multisystemic inflammation in BD.

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HLA Typing for Class I and Class II Antigens in Iraqi Patients with Behçet's Disease (Sporadic and Familial Cases)

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1. INTRODUCTION

The aetiology of Behçet's disease (BD) is unknown, but like in other autoimmune diseases, both environmental and genetic factors contribute to its aetiopathagenesis. The familial form of BD is in favour of a genetic predisposition[1-3]. This study was designed to assess the frequency of HLA antigens (class I and II) in association with disease susceptibility to develop such disease in both sporadic and familial cases, and to elucidate the role of genetic factors in the pathogenesis of this disease in Iraqi patients.

2. SUBJECTS AND METHODS

A total of 77 Iraqi Arab patients and 14 families with positive history of BD (92 members) were included in this study, while attending the multi-discipline BD Clinic at Baghdad Teaching Hospital from September 1999-June 2001. They all fulfilled the ISG criteria for diagnosis of BD.

Data allow detailed comparisons regarding sex, age and ethnic origin with the two matched control groups, 55 patients with recurrent oral ulcers (ROU), and 127 healthy volunteers. Typing for HLA class I (A,B,C), and

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class II (DR and DQ) antigens was carried out by using the microlymphocytotoxicity test established by Terasaki and McClelland[4] and modified by Dick and Bender with wide range of antisera.

3. RESULTS

The frequency of HLA-B51(5) in the studied groups is clearly shown in Fig. 1, while comparison of phenotype of HLA antigens, which demonstrates significant differences between the study groups, is illustrated in Table 1. Moreover, distribution of HLA haplotype in siblings of 14 families was not random, but associated with a chi square value of 7.782 which is significant.

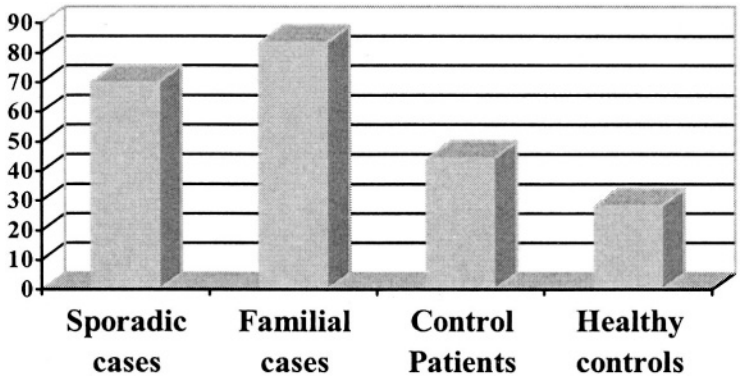

Figure 1. Frequency of HLA-B51(5) in the study groups (%HLA-B51+).

4. DISCUSSION

The view that genetic factors play a major role in the pathogenesis of BD is widely accepted[1,2], hence, searching in familial cases will contribute to understanding more of the causative factors of this disease[2,3]. If environmental factors, such as infection, environmental pollution, lifestyle or diet, influence the development of this disease[5,6]; the incidence of affected married couples should be higher .In spite of the different lifestyles, BD has been observed in many family members since their birth. Therefore, genetic factors seem to be more significant than environmental and other factors in developing familial cases[7], nevertheless B51(5) antigen showed close

association with the disease susceptibility in Iraqi patients (sporadic and familial study; Fig. 1). This result is similar to what has been reported in different countries[2,8,9]. However , higher frequency of DR2 and DQ3 was observed in sporadic cases as well as in familial cases, though these alleles could play a crucial role in the susceptibility of the disease in one way or another[9].

Table 1. Comparison of phenotype of HLA antigens which shows significant differences between the study groups

HLA Antigen	BD patients vs. healthy controls					Patients control vs. healthy control					Patient vs. P.C.	
	RR	EF	PF	X^2_Y	P<	RR	EF	PF	X^2_Y	P<	X^2_Y	P<
HLA-A1	-	-	-	-	-	2.60	0.22	-	5.52	0.05	-	-
2	0.34	-	0.24	7.23	0.01	-	-	-	-	-	-	-
9	4.06	0.23	-	11.72	0.01	4.83	0.27	-	13.44	0.01	-	-
28	0.33	-	0.20	4.11	0.05	0.14	-	0.06	5.804	0.05	-	-
HLA-B												
51(5)	5.89	0.58	-	27.39	0.001	2.02	0.22	-	3.22	-	5.93	0.05
13	-	-	-	-	-	-	-	-	-	-	6.19	0.05
35	0.19	-	0.05	4.068	0.05	-	-	-	5.33	0.05	-	-
55	-	-	-	-	-	13.60	0.15	-	13.10	0.01	-	-
HLA-C												
1	3.23	0.14	-	5.96	0.05	6.45	0.29	-	17.74	0.01	-	-
5	4.3	0.11	-	5.69	0.05	-	-	-	-	-	-	-
HLA-DR												
1	2.17	0.19	-	5.02	0.05	6.3	0.52	-	26.79	0.001	13.51	0.01
2	2.31	0.25	-	6.83	0.01	-	-	-	-	-	4.49	0.05
12(5)	0.04	-	0.8	13.57	0.01	-	-	-	-	-	-	-
10	7.47	0.10	-	6.71	0.01	8.23	0.10	-	6.90	0.01	-	-
HLA-DQ												
1	-	-	-	-	-	3.19	0.50	-	9.74	0.01	-	-
3	2.82	0.39	-	15.40	0.01	-	-	-	-	-	-	-
4	0.19	-	0.17	13.22	0.01	0.28	-	0.34	7.65	-	-	-

RR=Relative Risk, EF=Etiological Fraction, PF=Prevention Fraction, X^2_Y= chi square with Yate's Correction, P= Probability

5. CONCLUSION

For the first time, the genetic background, which plays a crucial role in the susceptibility of this disease, has been studied in details in Iraq. The

major role of the genetic background is supported by the increased occurrence of BD among relatives. There was markedly increased frequency of B51(5), DR2, DQ3 in both sporadic and familial cases of BD. It is of interest in this population study, that the haplotype, which may play crucial role in the susceptibility of BD is A9-B51(5)-CW1-DR2-DQ3, whereas A2, A28, B35, DR12, DQ4 phenotypes decrease the risk of developing BD. HLA DR1 and DQ1 could be considered genetic markers for aetiopathogenesis of the patient control group (ROU).

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Strong Association of *MIC-A*009* of Extracellular Domains and *MIC-A*A6* of Transmembrane Domain in Korean Patients with Behçet's Disease

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1. INTRODUCTION

Behcet's disease (BD; MIM 109650) is a chronic systemic inflammatory disease with polymorphic manifestation. The cause of BD is not known, however, genetic factors, several immunological abnormalities and bacterial/virus infection are believed to play a role. It is known that various symptoms of BD are associated with HLA genes; HLA B51 has been specifically related to eye lesions, HLA B27 to arthritis, and HLA B12 to mucocutaneous lesions. Moreover, the existence of susceptibility genes within a segment of MHC close to the HLA-B genes has been predicted.

MHC class I chain related A (MICA, MIM600169) is a cell surface anchored glycoprotein and may function as stress induced self antigens. MICA may be recognized by a subset of gut mucosal γδ T cells and seems to be confined to gastrointestinal and thymic epithelial cells. The *MICA* gene, which is located near the HLA-B, shows structural similarity to MHC class I molecules and has been shown to be highly polymorphic. A feature of the polymorphism of the *MICA* gene is disproportion in the distribution of synonymous and non-synonymous codons. However, the MICA gene encodes a cell surface glycoprotein that is not associated with β2m, and is conformationally stable independent of conventional class I peptide ligands. With the linkage disequilibrium between MICA and HLAB, the location,

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and the structure and expression of MICA all taken into consideration, it has been postulated that MICA may be a susceptibility factor in BD. Therefore, in this study, to determine whether the genetic variations of *MICA* are a candidate gene for the development of BD and thus being associated with BD risk susceptibility, we analyzed single nucleotide polymorphisms (SNPs) in exons 2, 3, and 4 encoding the extracellular domains, and (GCT)n repetitive polymorphisms in exon 5 encoding the transmembrane domain of MICA of Korean patients with BD.

2. MATERIALS AND METHODS

Genomic DNA was extracted from the peripheral blood samples of 191 patients with BD and 325 unrelated healthy control subjects using the QIAamp Blood kit (Qiagen, USA). The disease was diagnosed according to the criteria proposed by International Study Group. Table 1 shows the primers for screening genetic variations for exons 2, 3 and 4 encoding extracellular domains and exon 5 encoding transmembrane region. PCR amplification of exon 2, 3, 4 and 5 were performed using 200 ng DNA, 10 pmol of each primers, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 50 mM Tris-HCl and 1.0 U of Taq DNA polymerase (Bioneer, Korea). Genotypes for exon 2, 3 and 4, were determined using single strand conformational polymorphism (SSCP) with some modification as reported previously, and single stranded DNA fragments separated in the gel were visualized by silver staining. For microsatellite polymorphisms in exon 5, the amplified product was analyzed using 6% polyacrylamide gel with 8M urea and then stained with silver. The representative samples were confirmed using ABI310 DNA sequencer (PE Applied Biosystem, USA). Statistical analysis was done by the SAS (ver. 8.0, USA).

Table 1. Primers for SNPs and microsatellite polymorphisms in MICA

Domains	Exons	Primers
Extracellular	2	2S3 5'-GAGCCCCACAGTCTTCGT-3'
		2R3 5'-CTGCCCCTAACTTTTCTG-3'
	3	3S 5'-AAGGTGATGGGTTCGGGAAT-3'
		3R 5'-TCTAGCAGAATTGGAGGGAG-3'
	4	4S 5'-GCCAGAGTGAGAACAGTGAAGAGAAA-3'
		4R 5'-GTCACCCTAGGCTCACCAGA-3'
Transmembrane	5	5F 5'-CCTTTTTTTCAGGGAAAGTGC-3'
		5R 5'-CCTTACCATCTCCAGAAACTGC-3'

3. RESULTS AND DISCUSSION

In the combined type of SSCP patterns of 2, 3 and 4 exons in the extracellular domains, the frequency of *MIC-A*009* was significantly higher in the patients group compared with healthy controls [33.8% vs. 11.9%, p=0.001, odd ratio (OR) = 4.0, 95% confidence interval (95% CI) 2.55-6.33; Table 2]. Also, genotype frequencies containing the *MIC-A*009* were higher in patients than in healthy controls. Conversely, the frequency of *MIC-A*008* was significantly lower in the patients than in healthy controls (23.8% vs. 33.7%, p=0.017). The frequency of *MIC-A*A6,* which contains $(GCT)_6$ repeats in the transmembrane domain was significantly higher in the Korean patients group than in healthy controls (44.5% vs. 22.7%, p=0.001, OR = 2.7, 95% CI 1.84 -4.00; Table 3).

Table 2. Frequencies of combination types in extracellular domains of MICA in Behcet's disease patients

	BD	Controls	P values[a]	OR[b]	95% CI[c]
**002*	0.170	0.235			
**004*	0.081	0.089			
**006*	0.000	0.003			
**007*	0.031	0.051			
**008*	0.238	0.337	0.017		
**009*	0.338	0.119	0.001	4.0	2.55-6.33
**010*	0.073	0.117			
**011*	0.024	0.003			
**x*	0.045	0.040			
**del*	0.000	0.006			

[a]P values BD vs. Controls, [b]OR = Odd Ratio (BD vs. Controls), [c]95% CI = 95% confidence interval

Table 3. Frequencies of the (GCT)n microsatellite in the transmembrane domain of MICA in Behcet's disease patients

	BD	Controls	P values[a]	OR[b]	95% CI[c]
**A4*	0.115	0.140			
**A5*	0.259	0.325			
**A51*	0.089	0.150			
**A6*	0.445	0.227	0.001	2.7	1.84-3.99
**A9*	0.092	0.152			
**del*	0.000	0.006			

[a]P values BD vs. Controls, [b]OR = Odd Ratio (BD vs. Controls), [c] 95% CI = 95% confidence interval

These results revealed a strong association of BD in Koreans with *MIC-A*009* and *MIC-A*A6* (p=0.001). However, no association was shown between genetic variations of *MICA* and clinical manifestations of BD. Conclusively, the *MICA* gene is a good functional and positional candidate for a susceptible gene in BD, because the genetic variation of MICA may be attributed to the abnormality or hyperactivity of a functional role of the NKG2D receptor for MICA recognition on NK and γδ T cells in BD patients.

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MICA Transmembrane Region Polymorphism and HLA B51 in Tunisian Behçet's Disease Patients

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1. INTRODUCTION

It has been well established that HLA-B51 antigen is a genetic marker associated with Behçet's disease (BD) in several ethnic groups[1]. However, an increased prevalence of microsatellite polymorphisms located within a 46-kb-segment centromeric of HLA-B gene suggested that other loci situated in this region may be BD susceptibility genes[2,3]. MICA (MHC class I chain-related gene A) is a polymorphic gene located within this critical region. Analysis of the triplet repeat polymorphism in its transmembrane region (MICA-TM) in Japanese and Greek patients revealed that A6 allele may be more closely associated with BD than HLA-B51[4,5]. However, analysis of other ethnic groups has not always confirmed this association[6-9]. In this study, we proposed to analyse the MICA-TM and HLA-B51 allele distribution in Tunisian BD patients.

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2. MATERIAL AND METHODS

2.1 Study population

41 Tunisian patients (28 males and 13 females) satisfying the international diagnostic criteria for the diagnosis of BD and 43 sex- and age-matched healthy Tunisians controls were included in this study.

2.2 HLA class I typing

As only genomic DNA samples from healthy controls were available, HLA-B51 typing of both groups was performed using allele-specific PCR[10]. Serological HLA class I typing performed on BD patients confirmed total concordance between serological and molecular techniques.

2.3 Analysis of the triplet repeat polymorphism in the TM region of MICA gene

Polymerase chain reaction (PCR) was performed using primers flanking the transmembrane region as previously described[4]. PCR products were subjected to electrophoresis on a denaturating polyacrylamide gel, transferred on a Hybon-N+ membrane, incubated with one of the primers previously radio-labelled and visualised by autoradiography. Direct sequencing was performed on DNA samples for homozygous subjects in order to confirm genotyping.

2.4 Statistical analysis

Gene and phenotype frequencies were estimated by direct counting. Statistical analysis was performed using chi square method and Fisher's exact probability test. Statistical analysis was assigned to p value <0.05.

3. RESULTS

3.1 HLA-B51 frequency

As expected, HLA-B51 allele was significantly increased in BD patients as compared to healthy controls (48.7 % vs 25.5%, $p<0.05$).

3.2 MICA-TM allele distribution

All five distinct alleles (A4, A5, A5.1, A6 and A9), previously described, were found in patients and controls. The phenotype frequency of A6 allele was slightly increased in BD patients compared to healthy subjects (85.3% vs 74.4%, p>0.05). No significant association between BD and A4, A5, A5.1 and A9 alleles was found. 95% and 100% of HLA-B51 patients and controls carried A6 allele, respectively (p>0.05), suggesting a strong linkage disequilibrium between these two alleles. In HLA-B51 negative subjects, A6 allele was present in 76.1% of patients and 65.5% of controls (p>0.05). No difference was observed in A4, A5, A5.1 and A9 allele frequencies in BD patients stratified for the effect of HLA-B51.

4. CONCLUSION

None of MICA microsatellite alleles was significantly increased in Tunisian BD patients. Only HLA-B51 was primarily associated to BD in this population. These data, similar to those found in other ethnics[6,9], further support the hypothesis that the amino-acids, common to all HLA-B51 encoding alleles and absent in other HLA-B antigens, probably confer high affinity binding for peptides that may contribute to BD development. However, the possibility that HLA-B contributes to the pathogenesis as an additional or complementary risk factor cannot be excluded.

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HLA-B51 Frequency in Iranian Patients with Behçet's Disease

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1. INTRODUCTION

The etiology of Behçet's disease (BD) is still unknown but it follows the general pattern of autoimmune disorders. Both environmental and genetic factors contribute to its pathogenesis[1]. Although the genetic susceptibility seems to be multifactorial, the most important association is with the class I histocompatibility gene B5[2]. HLA-B51, a split antigen of HLA-B5, has generally been associated with BD in different countries notably those along the silk route[3].

The aim of this study was to determine the frequency of HLA-B51 in Iranian patients with BD, and to compare it with control patients.

2. MATERIALS AND METHODS

In a prospective study, 599 consecutive new patients referred to the BD clinic, were typed for the presence of HLA-B51 and HLA-B5 serologically in a 9-month period from April 2001 to January 2002. Their frequencies were compared in patients diagnosed of BD with those in whom BD was ruled out (control patients). A confidence interval at 95% (CI) was calculated. The comparisons were made by chi square test. The strength of association was estimated by calculating the odds ratio (OR).

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3. RESULTS

Among 599 patients, 201 were BD patients and 398 were controls. The frequency of B51 was 39% (CI: 6.7) in BD and 18% (CI: 3.8) in controls. The difference was statistically significant ($p<0.000001$). The OR was 2.87 (CI: 1.96-4.21). The frequency of B5 was 48% (CI: 6.9) in BD and 25% (CI: 4.3) in controls, with a statistically significant difference ($p<0.000001$). The OR was 2.72 (CI: 1.91-3.9). The B51 subtype was present in 78% (CI: 8.3) of B5-positive BD patients, and in 69% (CI: 9.1) of B5-positive controls. The difference was not statistically significant ($p=0.16$).

4. DISCUSSION

This study revealed that Iranian patients with BD have a higher frequency of both HLA-B51 and HLA-B5 compared with control patients. Although the frequency of B51 subtype was slightly higher in B5-positive BD patients compared with controls, it was not statistically significant. This confirmed the previous data on 48 Iranian patients with BD, in whom the higher HLA-B5 positivity rate was found to be caused by an increase in both the HLA-B*5101 and HLA-B*5108 alleles[4].

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Frequency of HLA in Patients with Behçet's Disease and Association with Occlusive Retinal Vasculitis

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1. INTRODUCTION

The etiology of Behçet's disease (BD) is still uncertain, but it is well known that both genetic and environmental factors contribute to its pathogenesis. HLA B5 is the most important genetic factor associated with BD in 50-70% of cases in various ethnic groups, wherever this association was examined. The aim of this study was to evaluate the frequency of HLA in BD patients with and without retinal vasculitis and investigate its association with occlusive retinal vasculitis.

2. MATERIAL AND METHODS

Thirty Caucasian BD patients (23 men, 7 women; mean age 31.2±6.2 years with a range of 19-48 years) were typed for HLA by standard lymphocyte microcytotoxicity technique. All patients fulfilled the International Study Group criteria for BD. Patients were divided into two groups with and without retinal vasculitis. Control group included 1000 healthy donors of Caucasian origin. Fluorescent angiography was used to confirm occlusive form of retinal vasculitis. Statistical analysis was performed using the chi square method.

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3. RESULTS

Table 1 shows the frequency of HLA in BD patients with and without retinal vasculitis.

Table 1. HLA in BD patients with and without retinal vasculitis

Antigen	Control n=1000	Patients with retinal vasculitis, n=14			Patients without retinal vasculitis, n=16		
	Abs. (%)	Abs. (%)	p	RR	Abs. (%)	p	RR
A1	270 (27.0)	3 (21.4)	0.7699	0.74	3(18.8)	0.5791	0.62
A2	457 (45.7)	9 (64.3)	0.2645	2.14	8(50)	0.9285	1.19
A3	230 (23.0)	2 (14.3)	0.7481	0.56	2(12.5)	0.5470	0.48
A9	213 (21.3)	3 (21.4)	1.0000	1.01	4(25)	0.7582	1.23
A10	110 (11.0)	2 (14.3)	0.6612	1.35	2(12.5)	0.6935	1.16
A11	118 (11.8)	1 (7.1)	1.0000	0.57	1(6.3)	0.7098	0.50
Aw19	296 (29.6)	2 (14.3)	0.3740	0.40	2(12.5)	0.1721	0.34
A28	83 (8.3)	3 (21.4)	0.1083	3.01	-	0.6339	0.00
B5	155 (15.5)	12 (85.7)	<0.0001	5.53	11(68.8)	<0.0001	4.44
B7	173 (17.3)	1 (7.1)	0.4850	0.37	1(6.3)	0.3332	0.32
B8	161 (16.1)	-	0.1438	0.00	1(6.3)	0.4911	0.35
B12	242 (24.2)	3 (21.4)	1.0000	0.85	3(18.8)	0.7737	1.38
B13	55 (5.5)	1 (7.1)	0.5509	1.32	1(6.3)	0.5991	1.15
B14	68 (6.8)	1 (7.1)	1.0000	1.05	1(6.3)	1.0000	0.91
B15	113 (11.3)		0.6669	1.31	2(12.5)	0.7007	1.12
B17	87 (8.7)	1 (7.1)	1.0000	0.81	1(6.3)	1.0000	0.70
B18	108 (10.8)	1 (7.1)	1.0000	0.64	1(6.3)	1.0000	0.55
Bw22	55 (5.5)	-	1.0000	0.00	2(12.5)	0.2249	2.45
B27	76 (7.6)	-	0.6173	0.00	4(25)	0.0312	4.08
B35	175 (17.5)	4 (28.6)	0.2876	1.89	4(25)	0.5034	1.57
B40	79(7.9)	-	0.6175	0.00	1(6.3)	1.0000	0.78

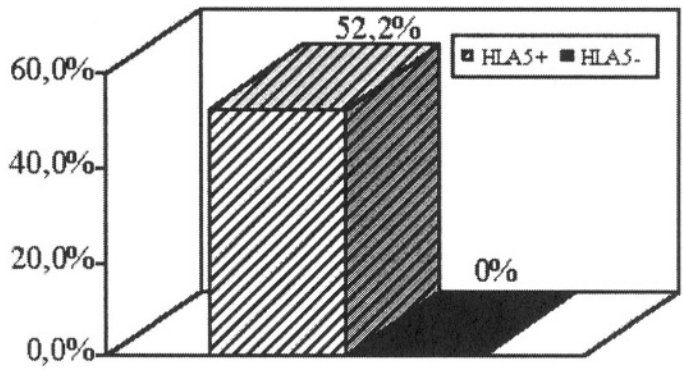

Figure 1. Frequency of occlusive retinal vasculitis in BD patients with or without HLA-B5

The frequency of HLA B5 was remarkably high in the patient group compared to controls (respectively 76.7% and 15.5%, p<0.000l, OR=4.95). Occlusive form of retinal vasculitis was found only in BD patients with positive HLA-B5, p=0.0242 (Fig. 1).

4. CONCLUSION

This study suggests that the presence of HLA-B5 antigen is predisposed to BD and may be a genetic marker of severe forms of retinal vasculitis.

The *ICAM1469*E* Is Associated with Susceptibility to Ocular Lesions and Vasculitis in Korean Patients with Behçet's Disease

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1. INTRODUCTION

Behcet's disease (BD) is a chronic systemic inflammatory condition of unknown cause. One of the manifestations of the disease is vasculitis, an inflammation of large and small arteries and veins characterized by perivascular infiltration by lymphocytes and mononuclear cells.

ICAM-1, which serves as a receptor for Mac-1 and LFA-1, is a member of the immunoglobulin superfamily and plays a key role in the transendothelial migration of neutrophils and T-cell activation. The increased expression of both soluble and tissue *ICAM-1* in BD suggests an important role in the inflammatory events resulting in vessel damage. In order to determine whether *ICAM1* gene polymorphisms convey susceptibility for ocular lesions and vasculitis in BD, we analyzed the prevalence of the polymorphisms *ICAM1K469E* and *ICAM1R241G.*

2. MATERIALS AND METHODS

Genomic DNA was extracted from peripheral blood leukocytes from 197 patients with BD including 151 with ocular lesions and 44 with vasculitis.

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Control DNA was obtained from 248 individuals without BD. *ICAM1K469E* and *ICAM1R241G* polymorphisms were detected with *Bst*U1- and *Bsr*G1-PCR-RFLP, respectively.

3. RESULTS

The frequency of the *ICAM1469*E* was significantly higher in BD patients compared with controls (0.41 vs 0.31, *P=0.003*, OR=1.25, 95% CI 1.09-1.51), and was higher in patients with ocular lesions (0.41) and with vasculitis (0.44). The *ICAM1241R* was not found in any of the 248 controls, but was present in one patient with BD who was heterozygous for *ICAM1241*R*.. This patient was a 56-year-old man with a 12-year history of the disease who presented with ocular lesions and who had a genotype of *ICAM1469*E/*E.*

4. DISCUSSION

Certain polymorphisms of the *ICAM1* gene are associated with BD, and *ICAM1469*E* is associated with an increased risk of ocular lesions and vasculitis in Koreans. The degree of association between *ICAM1* gene polymorphisms and BD is dependent on ethnic origins. The *ICAM1469*E* confers a susceptibility in Korean and Jordanian patients[1] while *ICAM1241*R* is representing a risk factor in Italian patients[2]. The frequency of *ICAM1 241*R* is higher in European healthy controls (3.1-18.0%) compared to those observed in Far East Asia, including Korea (0.0%), and Japan (0.0%), Palestine and Jordan (1.5%).

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IMMUNOLOGY

Chemokines in Behçet's Disease, a Field to Be Explored as a Potential Basis for Therapy

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1. INTRODUCTION

I read specifically about Behçet's disease more than ten years ago when I started studying the implications of chemokines in the pathology of chronic inflammatory conditions like rheumatoid arthritis and related diseases. The features of the disease are impressive, but even more impressive are the clinical observations that enabled Adamantiades and then Behçet to recognize several complex pathophysiological signs as the expression of a unique syndrome[1]. Among chemokines, IL-8 (CXCL8) is described most prominently in the rich literature addressing the immunological features of the disease[2-5], and elevated levels of IL-8 (CXCL8) in serum are reported to be a better marker of Behçet's disease activity than the levels of C-reactive protein and the erythrocyte sedimentation rate[5], which are often monitored to score chronic inflammatory conditions. Although neutrophils are often prominent in the inflammatory infiltrates of Behçet's disease, the lesions also contain monocytes, which are regarded as sources of IL-8 (CXCL8) and several inflammatory cytokines[2,6], and lymphocytes. The presence of mononuclear cells, which do not respond to IL-8 (CXCL8), clearly suggests that other chemokines are pathophysiologically relevant.

IL-8 (CXCL8) and several other chemokines being discovered initially, were associated with inflammation. They were shown to be induced by bacterial toxins like LPS and inflammatory cytokines, in particular IL-1 and TNF[7,8] in many different tissue cells, and in the leukocytes infiltrating inflammatory tissue sites[8]. They are called *inflammatory chemokines* and their main function is to recruit leukocytes for host defence in infection and

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inflammation. A functionally different set of chemokines was discovered several years later. They are expressed constitutively in discrete areas of the lymphoid tissues and direct the traffic and homing of lymphocytes and dendritic cells within the immune system, and are called *homing chemokines.* Inflammatory chemokines act on a defined set of receptors, such as CCR1, CCR2, CCR3, and CCR5, which are broadly expressed in phagocytes, T lymphocytes, NK cells and immature dendritic cells, CXCR1 and CXCR2 which are restricted to neutrophils, and CXCR3 which is found in T lymphocytes and NK cells[9,10]. Together these receptors account for the recruitment of all types of leukocytes that can accumulate in inflamed tissues. The system of leukocyte recruitment for host defence and inflammation is redundant, as indicated by the fact that most receptors recognize more than one chemokine and that several chemokines act on more than one receptor (see Table I).

Table 1. Human CXC and CC chemokine receptors and their corresponding chemokine ligands (CXCL and CCL). The chemokines are indicated by the number of their gene according to the systematic nomenclature[11,12]

CXCR	CXCL
1	6 8
2	1 2 3 5 6 7 8
3	9 10 11
4	12
5	13
6	16

CCR	CCL
1	3 5 7 8 13 14 15 16 23
2	2 7 8 13
3	5 7 8 11 13 15 24 26 28
4	17 22
5	3 4 5 8
6	20
7	19 21
8	1
9	25
10	27 28

2. CHEMOKINES IN INFLAMMATORY DISEASES

Several chronic inflammatory diseases like rheumatoid arthritis, lupus erythematosus, chronic bronchitis, sarcoidosis, and inflammatory bowel disease have been studied more extensively than Behçet's disease. Immunochemical and cytofluorimetric analysis of tissues, exudates, and

body fluids highlights the role of chemokines in the pathological process, and the patterns of expression of chemokines and receptors reflect the peculiarities of the underlying disease. Disease models in wild-type and gene-deleted mice have been used extensively to study the relative role of different chemokines in terms of potency and efficacy, and the potential synergy of different chemokines acting on single or multiple receptors[13].

The possibility of inhibiting inflammation by preventing chemokine activity was suggested soon after the discovery of IL-8 (CXCL8)[7]. The studies of structure-activity relations that were undertaken with IL-8 (CXCL8) and related chemokines to identify sequence domains involved in receptor recognition and activation[14-16] led to the identification of a sequence of three residues, Glu-Leu-Arg (ELR motif), immediately preceding the first cysteine, that are conserved in all chemokines acting via CXCR1 and CXCR2, and are essential for receptor activation[8,17]. It was then shown that CXCR1 and CXCR2 could be blocked with analogues of IL-8 (CXCL8) obtained by truncation or minimal modification of the amino-terminal triggering sequence, and that the same principle could be applied to other CC and CXC chemokines[8,17]. The appeal of the antagonists grew considerably with the recognition that all chemokines act via heptahelical, G_i-protein coupled receptors[11], and with the discovery that human immunodeficiency viruses (HIV) bind to chemokine receptors, and that viral entry is prevented by chemokines and chemokine antagonists[18,19]. All structure-activity relation studies underscore the importance of the amino-terminal domain of CXC and CC chemokines for receptor recognition and activation. Two separate or partly overlapping sites of interaction with the receptors can be distinguished, one in the amino-terminal sequence preceding the first cysteine and the other within the exposed, conformationally rigid loop after the second cysteine. It has been proposed that chemokines interact with their receptor in two steps: The receptor binds first what we call the *docking domain* restricting the mobility of the chemokine which becomes properly orientated for receptor activation via the amino-terminal *triggering domain*[20].

Treatment of rodents with chronic joint inflammation (collagen-induced arthritis in mice, the chronic polyarthritis of the MRL-*lpr* mouse, and adjuvant-induced arthritis in rats) with antagonists obtained by amino-terminal modification of MCP-1 (CCL2)[21] or RANTES (CCL5)[22] subsequently showed that inflammatory diseases can be prevented or cured by blocking the activity of certain chemokines.

These observations were extremely important, and the development of chemokine antagonists became a major goal in the pharmaceutical industry. The main focus of the research effort, as reflected by numerous patent applications, is on antagonists for CXCR1, CXCR2, CXCR3, CCR1, CCR2,

CCR3, and CCR5, the receptors for inflammatory chemokines. Several mostly polycyclic compounds have been identified in broad screening programs as blockers of single or multiple receptors[23]. Clinical efficacy data will soon be available for several compounds that have already passed pre-clinical development. The principle of preventing leukocyte recruitment is generally applicable to different types of chronic inflammatory diseases and may be extended to therapeutic use in transplant rejection, arteriosclerosis, and autoimmune conditions. Once clinical efficacy has been established in common inflammatory conditions, open studies will be possible in more rare indications, and Behçet's disease will be one of them.

So far drug discovery has concentrated on the search of mono-selective compounds. However, the concerted expression of several chemokines in inflammatory conditions and transplant rejection suggests that inhibitors of multiple receptors may be interesting drugs. For instance, autoimmune inflammation may be treated efficiently with drugs simultaneously blocking three receptors CCR1, CCR2, and CXCR3, and the combined blockade of CCR1, CCR2, and CCR3 may be a good therapeutic strategy for allergic inflammation. Such compounds, however, will be more difficult to develop clinically, and we will have to wait for the clinical results with mono-selective drugs before trying combinations or substances blocking more than one receptor.

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Expression of CCR5 in Behçet's Disease

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1. INTRODUCTION

Behçet's disease (BD) is a chronic inflammatory, multisystemic condition. Although its etiopathogenesis has not yet been clarified, the immune system is involved and activated in the course of the disease. Activation of circulating T and B lymphocytes also occurs and these immunoactive cells infiltrate into the affected regions followed by a second phase of neutrophil chemotaxis[1].

Th1 type immune reaction includes several chemokines and their receptors, whereas chemokine receptor 5 (CCR5) is mainly expressed. Pharmacological analysis of chemokine receptors is at an early stage of development. Disease indications have been established in HIV acquired immune deficiency syndrome. Additional indications are emerging among inflammatory and immunologically mediated diseases. An imbalance of Th1/Th2 association may play a critical role in active inflammation. CCR5 is a good marker of these inflammatory reactions[2].

The purpose of our study is to show CCR5 expression in oral and genital ulcers, and to explain pathogenesis of BD with a Th1 immune response.

2. MATERIALS AND METHODS

Fifteen patients with BD [9 male, 6 female (mean age 34.2 years)] were investigated. A detailed history of drug use, such as glucocorticosteroids or

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other immunosuppressive treatments was obtained, such patients were not included in this study. Biopsies were taken from active lesions (seven oral ulcers and eight genital ulcers) after patient's consent. Two independent and blinded observers evaluated immunohistochemically stained serial sections. For semiquantitative analysis the stained cells were examined and graded. Their number was expressed as percent of total cell population.

3. RESULTS

All tissue samples were found to be at least grade 1 positive for CCR5. Five out of 15 samples were grade 3 positive whereas 6 out of 15 were grade 2 positive. No significant difference in expression of CCR5 between oral ulcer and genital ulcer tissue samples was detected.

4. DISCUSSION

We examined the expression of CCR5 in BD. We found CCR5 expression in BD tissue samples of both oral and genital ulcers. The elevated expression of CCR5 in the tissues of BD suggests that this receptor and its ligands play a role in the pathogenesis.

CCR5 has been found to play a key role in early infection with HIV-1. CCR5 deficiency is associated with relative resistance to HIV-1 infection and there is major thrust to develop anti-CCR5 based therapies for HIV-1. However, it is not known whether CCR5 is critical for a normal antiviral T cell response[3,4].

In this study we correlated the expression of CCR5 in active period of BD. Infection with an unknown virus may result in rapid activation and overlapping expression of a number of chemokine genes. Nansen et al.[5] showed that infection with vesicular stomatitis virus of mice caused rapidly lethal encephalitis and resulted in chemokine gene expression. Virus activated CD8+ T-cells which were found to express CCR5. Further investigations on the role of viruses in BD and relation between CCR5 expression and the disease onset are required.

Herfarth et al.[4] studied polymorphisms of chemokine receptors in Crohn's disease which is a chronic inflammatory disease of the intestine that is characterized by mononuclear cell infiltration. CCR5 polymorphism was found to be an important determinant of overall disease susceptibility. This study also supports our results since there are several similarities between Crohn's and Behçet's disease.

Mack et al.[6] studied rheumatic diseases and found CCR5 positive cells in sinovial effusion with arthritis. It suggests an important role of CCR5 in the process of joint inflammation .The interaction between chemokines and their receptors is an important step in the control of leukocyte migration.

We investigated the expression in both genital mucosa and oral mucosa but could not find any significant difference between them. A possible polarization of T lymphocytes toward the Th1 immune reaction type has been suggested. IFN-α is a key cytokine produced by Th1 cells. Recent attention has focused on IL-12 plasma levels. The disease activity is correlated with IL-12 levels. Expression of CCR5 indicates the activation of immune system in BD and this may cause cytokine release. CCR5 may play a pathophysiological role in the course of the disease. Although we studied only a limited number of patients with BD, the expression of CCR5 in patients with active BD suggests that BD is more likely to be mediated by Th1 response. On the other hand CCR5 may be a new target for therapy. This study supports that anti CCR5 treatment modalities or the use of selective CCR5 antagonists should be used for down-regulating pro-inflammatory cytokine response in BD.

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Immunophenotype and Th1/Th2 Cytokines in Patients with Adamantiades-Behçet's Disease

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1. INTRODUCTION

Adamantiades-Behçet's disease (ABD) is a chronic systemic inflammatory vasculitis, characterized by oral and genital ulcers and by cutaneous, ocular, arthritic and neurologic involvement[1]. Although the aetiology of ABD remains unknown, genetic predisposition, immune dysfunction, infectious agents and environmental factors seem to contribute to the pathogenesis of this disease. On the other hand TCRγδ+ T-cells play a role in the innate immunity and are involved in inflammatory responses as well as in the pathogenesis of infectious diseases[2]. Additionally, the immune response driven by the induction of specific type 1 or 2 cells is of critical importance in the pathogenesis of infectious, autoimmune and rheumatic diseases[3]. In the present study, the immunophenotype and Th1/Th2 cytokine profile in the peripheral blood lymphocytes were evaluated in patients with ABD.

2. PATIENTS AND METHODS

We studied 52 ABD patients, 34 males and 18 females, of age ranging from 17 to 60 years. All patients fulfilled the International Study Group

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criteria. The patients were classified according to the disease activity in two groups: Twenty-eight patients with active disease (group A) and 24 patients in remission (group B). 45 healthy individuals, matched for age and sex served as normal controls (NC). Direct immunofluorescence in whole blood and analysis in an EPICS-XL (Beckman-Coulter) flow cytometer was used for determination of CD2+, CD3+, CD3+CD4+, CD3+CD8+, CD3-CD16/56+, CD19+, CD3+TCRγδ+, CD5+CD19+ lymphocyte subpopulations. The percentage of T-cells producing either IFN-γ and IL-2 (type 1 immune response Th1) or IL-4 and IL-10 (type 2 immune response Th2) was measured by flow cytometry after T-lymphocyte stimulation with PMA and ionomycin in short term cultures. Statistical analysis was performed with the non-parametric Wilcoxon signed-ranks test (SPSS).

3. RESULTS

The study showed a statistically significant increase of the percentage of T-lymphocytes expressing TCRγδ (5.7±5.0 vs 2.2±0.8, p=0,010) irrespective of disease activity (Fig. 1). It should be noted that a statistically significant high number of ABD patients (24/52, 46.3%) showed an increased percentage of TCRγδ T-cells (≥5%, where 5 equals the mean value + 2 SD of the mean value of the controls), whereas such a high percentage was not found in any control subject. No difference between ABD patients and NC was found in any other lymphocyte subpopulations (Fig. 2).

For ABD patients and in comparison to the controls a statistically significant increase of the percentage of T lymphocytes positive for IL-2 (20.5±10.3 vs 3.3±2.2, p<0.0001) for the patients with active disease and 11.0±1.8 vs 3.3±2.2 (p<0.0001) for the patients in remission was found, in comparison to normal controls. It should be noted that the percentage of IL2+CD3+ T-cells was significantly lower in the patients on remission in comparison to the patients with active disease (20.5±10.3 vs 11.0±1.8, p<0.0001). No significant differences were found between T-cells positive for IFN-γ, IL-4, IL-10 in ABD patients compared to NC (Fig. 3).

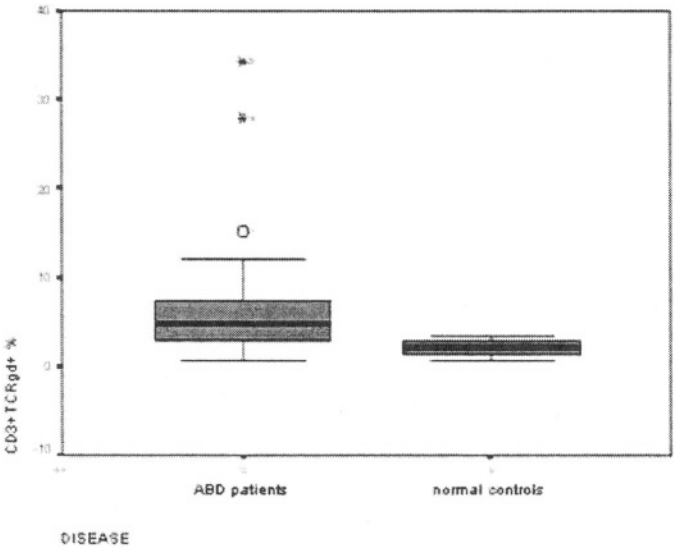

Figure 1. Boxplot presentation of TCRγδ+ T lymphocytes in ABD patients and NC

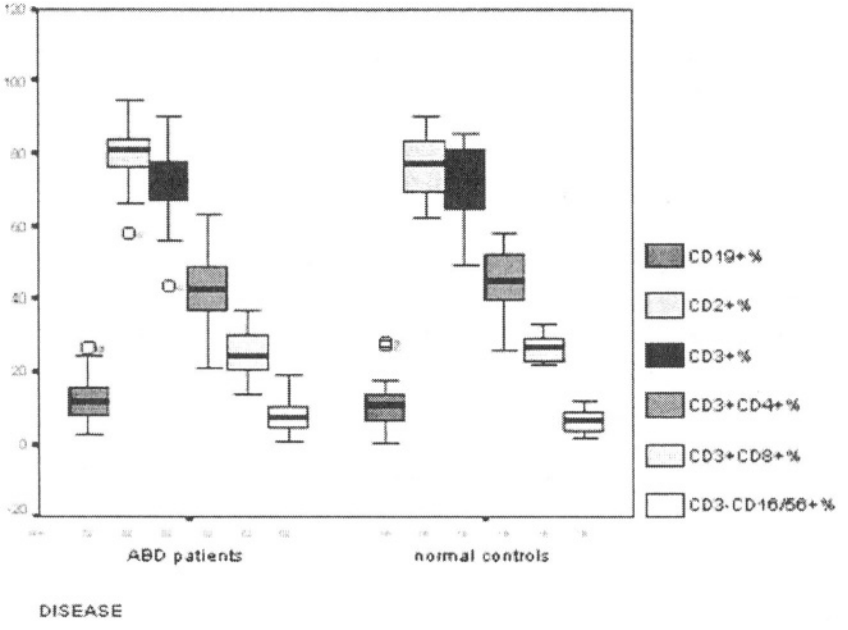

Figure 2. Boxplot presentation of lymphocyte subsets in ABD patients and NC

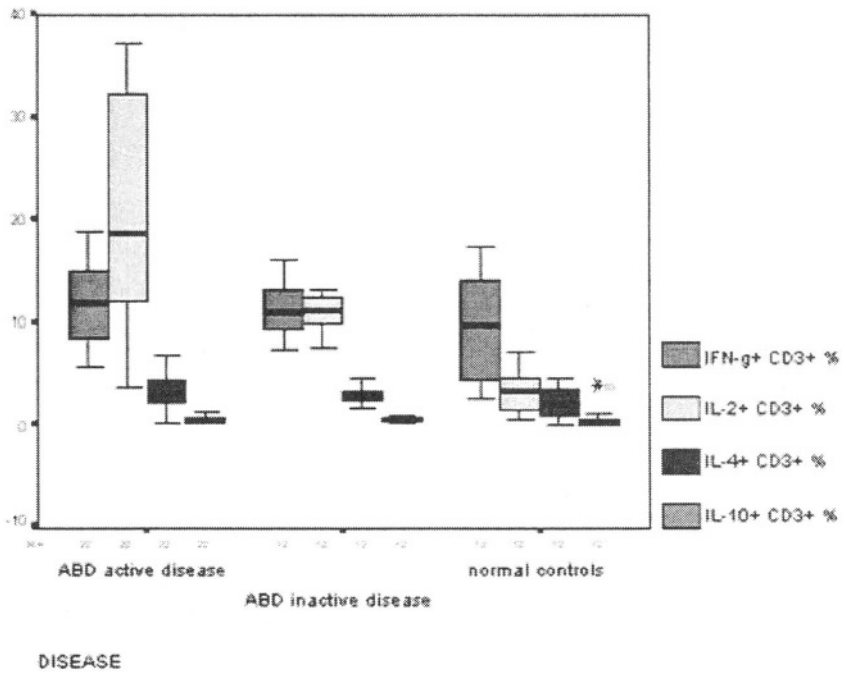

Figure 3. Boxplot presentation of cytokine producing T cells in ABD patients with active and inactive disease and NC

4. CONCLUSION

In a high percentage of ABD patients a significant increase of TCR-γδ T lymphocytes was found irrespective of disease activity. A previous study by Suzuki et al.[4] reported similar findings. Type 1 immune response (indicated by the increased expression of IL-2 in T CD3+ lymphocytes) was also observed. The work by Frassanito et al.[5], support the Th1 polarization of the immune response as well. These observations may help in understanding the immunopathophysiology of the disease. They may also be of use in designing therapeutic interventions for ABD patients.

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Cytokine Production of Peripheral Blood Mononuclear Cells Stimulated with *Streptococcus Sanguis* Antigen in Patients with Behçet's Disease

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1. INTRODUCTION

Behçet's disease (BD) is a syndrome of unknown aetiology consisting of recurrent oral and genital aphthous ulcerations, ocular manifestations such as uveitis, cutaneous involvements and other inflammatory responses encompassing all systems of the body including cardiovascular, respiratory, gastrointestinal and central nervous system. The aetiology and pathogenesis of BD have remained unclear, but it has been assumed that an infectious agent, immune mechanism and genetic factor are involved in the onset of the disease.

The streptococcal influence on BD might well be emphasized, especially the *Streptococcus sanguis* antigen. *S. sanguis* was found at the site of the lesion[1]. Increased IgG and IgA antibodies to *S. sanguis* were found in patients with BD[2]. *S. sanguis* stimulated T-cell proliferation, up-regulation of the TH1 cytokines IL-2 and IFN-γ mRNA, and TH2 cytokine IL-6[3].

Recent attention has focused on TH1 and TH2 cytokines. Persons with active BD have significantly more IL-2-producing CD4 T-cells than inactive case subjects and control subjects[4], and IL-12 plasma level and disease activity are correlated[5]. These results suggest that Th1 cells may play an important role in the immunopathogenesis of BD. But others have reported that the cytokine production profile has a mixed Th1/Th2 in BD[6].

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In this study, we have investigated the production of cytokines IL-4, IFN-γ in cultured supernatant after incubating inactivated *S. sanguis* whole cell antigen with peripheral blood mononuclear cells (PBMCs) isolated from healthy controls, and patients in both active and inactive stage of the disease.

2. MATERIALS AND METHODS

2.1 Subjects

Twelve patients (age range 26-44 years, 3 males and 9 females) were diagnosed as having BD on the basis of the diagnostic criteria of the International Study Group for BD. The activity of BD was assessed by the 1994 criteria of disease activity of BD, which has been proposed by the BD Research Committee of Japan (Table 1).

2.2 Preparation of PBMCs

PBMCs from healthy normal controls and BD patients in both active and inactive stages of disease were separated using Ficoll-hypaque density gradient centrifugation.

2.3 Preparation of *S. sanguis* whole cell antigen

S. sanguis strain 113-20, isolated from oral cavity of a patient with BD, was obtained from Dr. F. Kaneko (Fukushima Medical College, Japan). The bacteria were grown in brain-heart infusion broth at 37 °C for 24 hours in an anaerobic condition. The cells were washed in PBS and suspended in 0.5% formalin for 3 days at 4°C. After washing, they were stored in -20°C.

2.4 Cell culture and cytokine measurement

PBMCs (1×10^6/well) were cultured in 96-well microtiter plates containing RPMI 1640 medium (GIBCO, Grand Island, NY, USA) supplemented with penicillin G (100 U/ml), streptomycin (10 μg/ml), L-glutamine (0.3 mg/ml), and 10% fetal bovine serum. *S. sanguis* whole cell antigen was added to the cultures at the concentration of 0.1, 1, 10 μg/ml. The cells were incubated for 0, 6, and 72 hours at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Then, the supernatants were harvested and kept frozen at –20°C. The concentration of cytokines was measured by ELISA (IL-4, IFN-γ; Pierce Endogen, Rockford, IL, USA).

3. RESULTS

3.1 Cytokine production capacity by PBMCs in response to *S. sanguis* antigen

Stimulation of PBMCs with *S. sanguis* whole cell antigen and subsequent measurement of IL-4, IFN-γ in supernatants 72 hours later revealed increasement in the production of IFN-γ but not in that of IL-4.

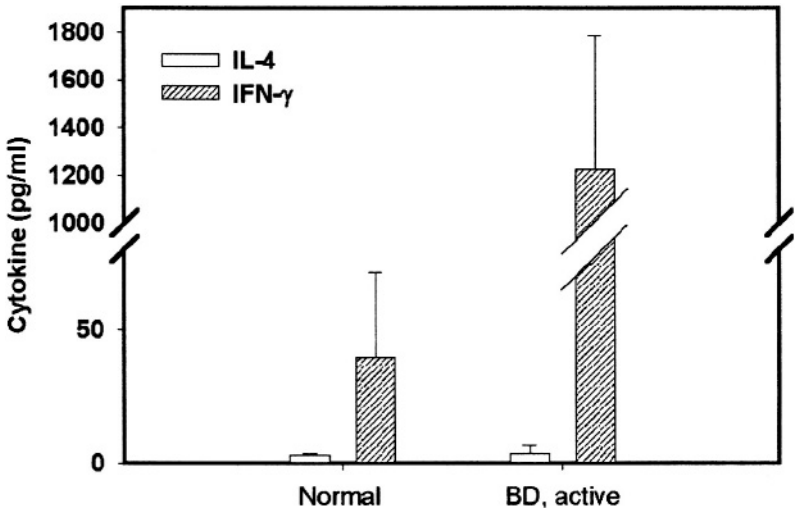

Figure 1. The increase in the cytokine production capacity by PBMCs in response *to S. sanguis* whole cell antigen in BD as compared with normal controls

3.2 IFN-γ production capacity by PBMCs with regard to the stimulating concentration of antigen

Incubating PBMCs obtained from normal controls and patients with BD in the milieu containing *S. sanguis* whole cell antigen at the concentration of 0.1 μg/ml, 1 μg/ml, and 10 μg/ml, and subsequent measurement of IFN-γ in the supernatants after 72 hours showed statistically significant difference between the two groups, and same happened even at the concentration of 0.1 μg/ml.

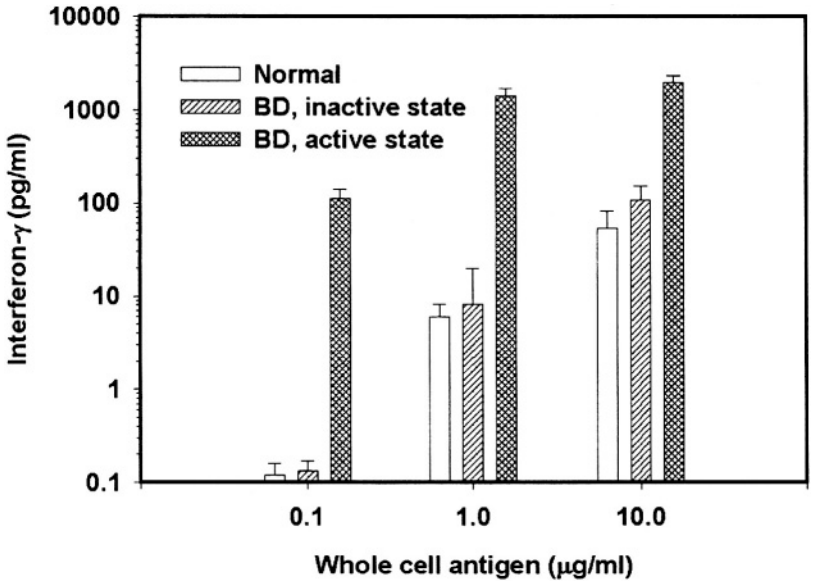

Figure 2. IFN-γ production capacity by PBMCs with regard to the stimulating concentration of *S. sanguis* whole cell antigen in BD

3.3 IFN-γ production capacity by PBMCs with regard to time lapse after stimulation

Stimulation with the antigen at the concentration of 10 μg/ml was followed by measurement of IFN-γ after 0, 6, and 72 hours following the start of incubation. PBMCs from patients with active disease had significant up-regulation of IFN-γ production as compared with normal controls and patients in inactive stage of disease. Taking time course into consideration, IFN-γ production increased with time starting 6 hrs after the incubation with the antigen.

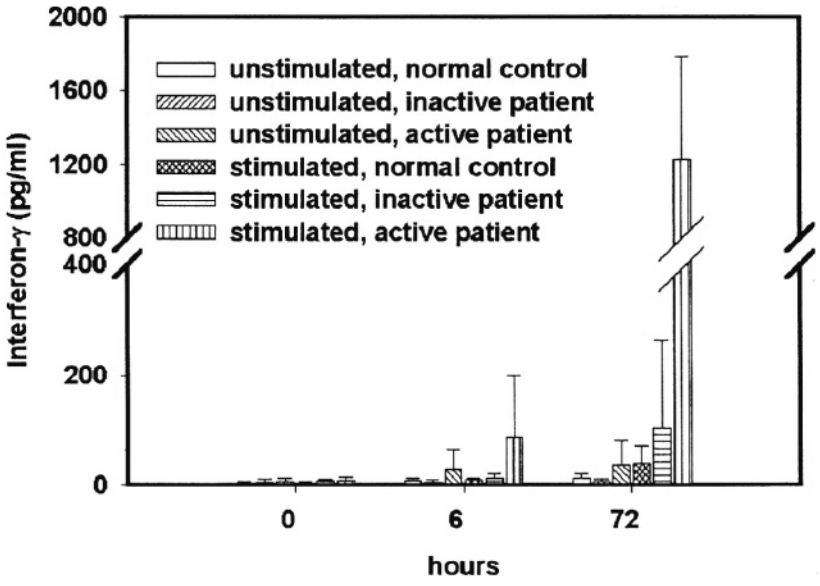

Figure 3. IFN-γ production capacity by PBMCs with regard to time lapse after the stimulation with *S. sanguis* whole cell antigen in BD.

4. SUMMARY

After 72 hrs, the production of IFN-γ was significantly increased in patients with active BD as compared with controls or patients with inactive disease. However, there was no significant increase of IL-4. Taking time course into consideration, IFN-γ production increased with time starting 6 hrs after the incubation with the antigen and the reaction was also dose-dependent with respect to the amount of antigen.

5. CONCLUSION

IFN-γ production in patients with active BD differs in response to stimulation with *S. sanguis* antigen from that of healthy controls and patients with inactive disease.

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Serum Levels of Soluble TNF-α Receptor-II (P75), Circulating γδ T-Cells and Adamantiades-Behçet's Disease Activity

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1. INTRODUCTION

Adamantiades-Behçet's disease (ABD) is a chronic relapsing systemic inflammatory disorder, characterised by recurrent oral and genital ulcers, ocular lesions, skin manifestations, arthritis and vascular, intestinal and neurological involvement[1]. The etiopathogenesis has yet to be fully elucidated, however, genetic predisposition, immune abnormalities, infectious agents, and environmental factors have been implicated[2]. Recent studies suggest that functional B-cell and T-cell aberrations, as well as various cytokines including INF-γ, IL-1, IL-2, and TNF-α, are involved with the inflammatory process in this condition[2-4].

TNF-α is a potent pro-inflammatory cytokine produced primarily by activated monocytes/macrophages, as well as by activated T-cells, including a subpopulation expressing the γδ chain of the T cell receptor (TCRγδ). γδ T-cells have not only been found in an increased number in patients with ABD, but do also produce extreme amounts of TNF-α[3,4]. On the other hand, human TNF-α actions are mediated by two high affinity membrane receptors, namely the TNF-α receptor-I (or p55, MW 55 kD), and the TNF-α receptor-II (or p75, MW 75 kD). Both types are shed from the cell surface by proteolytic cleavage or being produced in the endosomal compartment as

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soluble forms (sTNFR-I and sTNFR-II, respectively). sTNFR-I and sTNFR-II inhibit TNF-α action *in vitro* and *in vivo,* thus playing an important regulatory role in the cytokine interplay[5-7]. To gain further insight into TNF-related pathogenetic mechanisms in ABD[8,9], we measured serum levels of sTNFR-II, as well as the circulating numbers of peripheral γδ T-cells, in a large number of patients with different levels of disease activity.

2. PATIENTS AND METHODS

Peripheral blood was obtained after informed consent from 73 consecutive outpatients with an established diagnosis of ABD (International Study Group criteria)[10]. There were 48 males and 25 females (mean age of 36.6 ± 12.6 years) with a mean disease duration of 15.37 ± 9 years. Patients with evidence of concomitant infection at the time of sampling, as well as patients receiving anti-TNF-α treatment for ocular lesions were excluded[11]. Disease activity was determined as previously described[9]; namely all patients were classified in three groups: a) active disease (n=23), defined as the presence of major oral aphthae and genital ulceration, arthritis in at least two joints, ocular manifestations, neurological manifestations and/or large vessel involvement, b) mildly active disease (n=26), defined as the presence of minor aphthae, genital ulceration, skin lesion or monoarthritis, and c) inactive disease (n=24), defined as the absence of any clinical manifestations at the time of sampling.

Serum levels of sTNFR-II were measured by a quantitative ELISA (HyCult Biotechnology, The Netherlands) in all ABD patients, as well as in 13 healthy age- and sex-matched controls. Numbers of peripheral blood γδ T-cells were determined in 34 representative ABD patients and 20 healthy controls by two-colour flow cytometry, using appropriate anti-CD3 and anti-TCRγδ monoclonal antibodies as described in detail elsewhere[12]. C-reactive protein (CRP) serum levels, serving as an additional marker of ABD disease activity, were also measured by nephelometry in a representative subgroup of 30 patients with ABD. The unpaired or paired t-test was used for comparisons between mean values when appropriate. To establish correlations between individual values of serum sTNFR-II, numbers of γδ T-cells, and/or CRP serum levels linear models were employed.

3. RESULTS

As shown in Fig. 1, serum levels of sTNFR-II were significantly higher in patients with active ($p=0.02$) or mildly active ($p=0.007$) disease,

comparing to control individuals. In contrast, no significant difference was observed between patients with inactive disease and controls. Moreover, a significant difference between patient with active and inactive disease was present ($p<0.02$), suggesting that elevated levels of this molecule could predict active disease.

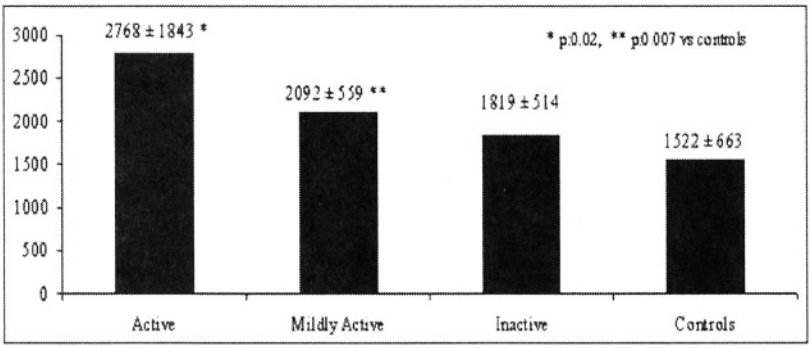

Figure 1. Serum sTNFR-II levels (mean ± SD pg/ml) in ABD patients and healthy controls

As shown in Fig. 2, numbers of peripheral blood γδ T-cells were significantly increased in ABD patients, irrespectively of disease activity, comparing to controls. Although numbers of peripheral blood γδ T-cells were higher in patients with active disease than in those with mildly active or inactive disease, differences did not reach significance.

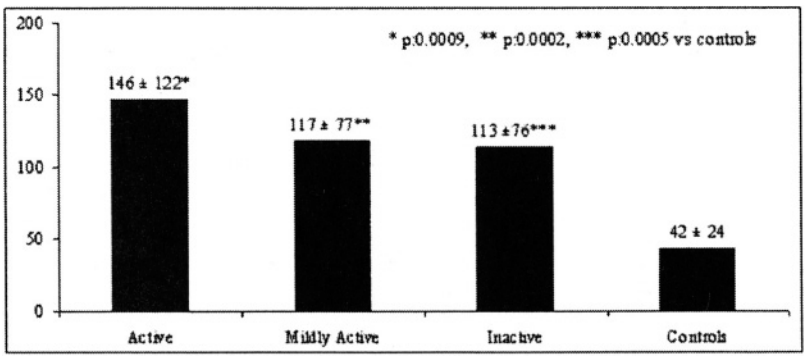

Figure 2. Peripheral blood γδ T cells numbers (mean ± SD cells/μl) in ABD patients and healthy controls

Finally, we found that CRP serum levels were higher in patients with active disease (n=10, mean ± SD 14.4 ± 11.3 mg/l), comparing either to

patients with mildly active (n=10, 7 ± 9.5, NS), or inactive disease (n=10, 3 ± 2, p=0.006) As shown in Table 1, a strong correlation of sTNFR-II with CRP levels within individual patients was observed. In contrast, γδ T-cells numbers did not correlate at any level either with individual CRP or sTNFR-II serum levels.

Table 1. Correlations between sTNFR-II serum levels, CRP serum levels and γδ T-cells numbers within patients with ABD

sTNFR-II versus CRP	(n=30)	$R^2 = 0.49$	$p < 0.00001$
Γδ Tcells versus CRP	(n=13)	$R^2 = 0.09$	NS
sTNFR-II versus γδ Tcells	(n=34)	$R^2 = 0.02$	NS

4. DISCUSSION AND CONCLUSION

Given that TNF-α itself is a potent inducer of sTNFR production through its binding to the surface TNFR[6], it is likely that increased sTNFR-II in ABD reflects TNF-α overexpression. Several studies have reported that increased sTNFR-II serum levels correlate with disease activity in various inflammatory disorders in which a central pathogenetic role of TNF-α has been proven[7-9]. In patients with systemic lupus erythematosus, in whom CRP is unable to differentiate disease activity, sTNFR-II correlates with clinical disease activity to a remarkable extent, even better than anti-DNA antibodies[13,14]. Along these lines, our results suggest that elevated levels of this molecule, predict indeed the presence of active disease probably reflecting the neutralization of increased TNF-α bioactivity.

Our findings on peripheral γδ T-cells are in accordance with previous reports, in which these T-cells have been also found in an increased number, not depending on the activity of ABD[3,4]. Since these T-cells overproduce TNF-α, one may expect that a positive correlation between γδ T-cells and sTNFR-II serum levels would exist in ABD. However, such correlation was not found, probably because we measured the total numbers of γδ T-cells rather than their activated subset. In any case, whether the expansion of T-cells expressing TCRγδ in ABD is a primary pathogenetic element or an epiphenomenon remains to be elucidated in further studies.

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T-γδ Receptor Restriction in Peripheral Lymphocytes of Patients with Behçet's Disease

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1. INTRODUCTION

Behçet's disease is an inflammatory disease of unknown origin that is characterized by oro-mucocutaneous and ocular inflammation. Previously, increased peripheral blood levels of T-γδ lymphocytes were described in patients with Behçet's disease. The role of these T-γδ lymphocytes is unknown as well as the restriction pattern of the γδ receptor.

2. PATIENTS AND METHORS

In this study we investigated T cell subsets and T cell receptor Vδ families in peripheral blood of 17 patients with Behçet's disease (and 8 controls) using FACS analysis with Vγ and Vδ specific antibodies.

3. RESULTS

No differences were found between T lymphocyte subsets and T-αβ and T-γδ receptor expression in patients with Behçet's disease and controls. In 8 patients a high restriction of Vδ3 usage was found (> 5%). The results from Vδ analysis (Vδ subsets of $\gamma\delta^{+}$T cells in %) is summarized in Table 1.

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Table 1. Vδ analysis (Vδ subsets of $\gamma\delta^+$T cells in %) in patients with Behçet's disease

Patient	Vδ1	Vδ2	Vδ3
1	50.1	32.3	17.1
2	23.9	40.8	26
3	63.7	12.1	18.5
4	72.9	21.2	6.0
5	35.5	10.6	50.5
6	1.6	86.7	6.0
7	28.2	23.0	6.8
8	11.6	76.9	8.5
Range	1.5-21.6	69.0-93.3	<1-2.6

4. CONCLUSION

We found high numbers of $V\delta3^+$ T cells in 8 patients with Behçet's disease. The role of this T cell receptor repertoire in Behçet's disease is unknown and is subject of further investigation.

Lymphocyte Subsets and Activated Neutrophils in Iraqi Patients with Behçet's Disease

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1. INTRODUCTION

Despite extensive research, the aetiology of Behçet's disease (BD) remains mysterious, though evidence has accumulated that genetic, environmental and hormonal factors may play crucial roles in the development of this disease. Nevertheless immunological mechanisms are deeply involved in its pathogenesis[1-3]. Neutrophils reside on the top of the list and show a hyperactivity in this disease[4] since they are capable of promoting tumour necrosis factor (TNF-α). The latter perms neutrophils and prolong their own life span, resulting in abnormal accumulation of activated neutrophils in the site of inflammation[4].

Polyclonal B-cell activation with various autoantibodies have been detected by many groups, though total resting B-cells are normal in this disease[5,6]. Changes concerning the CD4+/CD8+ ratio may be further involved. On the other hand, HLA-B51(5) molecules may participate at these events[1,3].

In this study, activity of neutrophils and cell markers [CD3, CD4, CD8, DR-antigen T-cells, CD 19 (B-cell), and CD66 (activated neutrophils)] were investigated in patients with BD.

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2. SUBJECTS AND METHODS

The study comprises three groups: (1) Patient study group, which included a total of 35 Arab Iraqi patients who fulfilled the ISG criteria for diagnosis of BD while attending the multiscipline BD Clinic at Baghdad Teaching Hospital. (2) Control groups, which included 20 control patients with only recurrent oral ulcerations (ROU) and 15 healthy volunteers.

Typing for HLA class I was carried out by using microlympho-cytotoxicity test established by Terasaki and modified by Dick and Bender.

Direct immunoflourescence assay for identification of leukocyte surface antigens (CD-Ag) using specific fluorescein-labeled monoclonal antibodies including mouse antihuman CD3, CD4, CD8 (T-cells), CD 19 (B-cells), DR-Ag, and CD66 (neutrophils)[7].

3. RESULTS

Periodical changes in lymphocyte subsets and activated neutrophils of BD patients were observed compared to the other study groups, which is reflected by increase in percentage of DR-Ag positive T-cells (activated cells), elevated CD 19+ B-cells, and CD66+ activated neutrophils as clearly shown in Tables 1 and 2.

Table 1. CD3, CD4, CD8, CD4/CD8, DR-antigen, CD19 and CD66 expression in patients with Behçet's disease and other study groups (mean CD%±SD)

Study groups	CD3+ T-cells	CD4+ T-cells	CD8+ T-cells	CD4/CD8 ratio	HLA-DR+ activated T-cells	CD19+ B-cell	CD66+ Neutrophils
Total patients	A	*b	a	***b	**a	**a	**a
N=35	70.8±16.6	33.8±14.2	44.2±20.7	0.82 ± 0.43	37.8 ± 23.9	25.8±11.7	31.8±14.8
HLA-B51(5)+	B	**b	a	***b	***a	**a	**a
N=19	68.8±15.3	36.2±15.5	47.1±22.2	0.76 ± 0.46	46.1 ± 22.6	25.6±11.9	32.9±14.1
HLA-B51(5)-	A	***b	a	***b	a	***a	**a
n=16	73.2±19.1	28.2±10.0	36.6±13.4	0.86 ± 0.29	22.7 ± 7.6	26.6±10.9	30.9±14.5
Relative	B	b	a	***b	**a	a	**a
n=20	64.4±13.2	46.4±10.5	41.4±12.4	0.89±0.31	33.1±19.7	19.3±8.6	35.9±18.8
Patients control	B	*b	a	**b	**a	***a	*a
N=20	64.9±11.4	38.8±18.8	38.8±13.8	0.97±0.49	35.6±18.3	28.3±13.2	28.3±11.6
Healthy control N=15	69.4±12.5	52.1±10.0	35.7±9.4	1.4±0.21	18.5±5.6	15.6±5.8	19.2±9.1
Normal values	73±6.5	44±7.9	33±7.4	1.4±0.6	-	14±4.2	-

a= increased , b= decreased , * P<0.05, **P<0.001, ***P<0.001

Table 2. Comparison with healthy groups

Study groups	CD3	CD4	CD8	CD4/CD8	HLA-DR	CD19	CD66
HLA-B51(5)+ vs. HLA-B51(5)-	N.S b	N.S a	N.S.a	N.S.b	**a	N.S.b	N.S.a
HLA-B51(5)+ vs. R.	N.S a	*b	N.S.a	N.S.b	N.S.a	*a	N.S.b
HLA-B51(5)+ vs P.C.	N.S a	N.S b	N.S.a	N.S.b	N.S.a	N.S.b	N.S a
HLA-B51(5)- vs R.	N.S a	***b	N.S.b	N.S.b	N.S.b	**a	N.S.b
HLA-B51(5)- vs P.C.	N.S a	*b	N.S.b	N.S.b	*b	N.S.b	N.S.a
R. vs. P.C.	N.S b	N.S.a	N.S.a	N.S.b	N.S.b	**b	N.S.a

4. DISCUSSION

The results of this study are comparable to those of other studies[3,5,8,9]. These results support the hypothesis that the immuno-pathogenesis of BD is possibly T-cell dependent, however, the initial reaction is a cell-mediated delayed hypersensitivity with mononuclear cell infiltration and decrease in CD4+/CD8+ ratio. Neutrophil hyperfunction and significant increase in CD 19+ B-cells led us to the assumption that these move hand in hand with T-cell activation, hence polyclonal B-cell activation with various auto-antibodies has clearly been observed[10].

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L-Selectin Expression on Leukocytes of Patients with Behçet's Disease

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1. INTRODUCTION

Behçet's disease (BD) is a multisystemic immune-mediated inflammatory disorder targeting primarily blood vessels. Inflammation requires transmigration of leukocytes from the circulation to the tissues which involves expression of several adhesion molecules on endothelium, platelets and leukocytes[1].

L-selectin is a cell membrane surface receptor on neutrophils, monocytes, and lymphocytes, and is responsible for the attachment of leukocytes to inflamed endothelium[2]. After activation of leukocytes, L-selectin is rapidly shed from the cell surface, hence the term sL-selectin[3]. The sL-selectin retains functional activity and inhibits attachment of leukocytes to cytokine-activated endothelium at high concentrations, thus regulating leukocyte attachment to inflamed endothelium[3]. The initial attachment of the blood leukocytes is mediated in part by L-selectin while subsequent firm adhesion is being mediated by β2-integrins[4]. L-selectin is therefore believed to modulate the first step of neutrophil migration out of the vasculature (i.e. neutrophil rolling in the microcirculation)[5]. Cytokines such as IL1 and TNF induce E-selectin, P-selectin, ICAM-1, VCAM-1 and ligand for L-selectin[4]. This phenomenon is important since BD is known to be associated with significantly increased production of TNFα, IL6, IL-1, and IL-8 by peripheral blood monocytes on exposure to lipopolysaccharides[6].

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The aim of the present work is to study L-selectin expression on leukocytes of patients with BD and to investigate a possible relation to the state of activity and/or the clinical variants of the disease.

2. PATIENTS AND METHODS

The study included fifty patients fulfilling the Inclusion Criteria of the International Study Group of BD[7], enrolled from the Alexandria BD Registry which specifies 850 patients by 2002. Besides, 50 healthy control subjects, matched for age and sex, were included. Only patients who were recently diagnosed and were not on immuno-modulatory therapy for at least one month prior to blood sampling were included. Every subject was subjected to:

1. Thorough history taking and full clinical examination for diagnosis of the disease, exclusion of concomitant diseases and assessment of the state of activity.
2. Ophthalmological and neurological assessment using all needed imaging techniques.
3. L-selectin expression on leukocytes by direct immunofluorescence technique using monoclonal antibody CD62L (DakoPats, Denmark) and flow cytometry (FACS Calibur, Becton & Dickinson)[8].

3. RESULTS

3.1 Demographic and clinical profiles

Among 50 patients, 41 were males and 9 females. The mean age was 32.32 ± 7.4 years. Thirty nine patients had ocular BD (20 enrolled during active state of the disease and 19 during non-active), fifteen had neuro-Behçet's (NB) (7 during activity and 8 non-active), and 4 patients had both ocular and NB.

3.2 L-selectin expression

The mean L-selectin expression on leukocytes of patients with BD (n = 50) was 13.60 ± 11.30% and in control subjects 29.42 ± 9.92% ($t = 7.586$; $p < 0.001$). The mean L-selectin expression on leukocytes in patients with ocular BD was 11.02 ± 7.54% and in those with NB 18.17 ± 16.58%. In active OB the mean expression was 5.21 ± 4.3% and in non-active OB 17.13

± 4.8% (t = 8.152; P = 0.0001) while in active NB 4.78 ± 2.7% and in non-active NB 29.87 ± 14.41% (t = 4.5; p = 0.001). There was a statistically significant difference in L-selectin expression on leukocytes in patients with non- active OB vs normal controls (t = 3.21; p = 0.024) while in NB, there was no significant difference between inactive NB and control subjects (t = 0.29; p = 0.821) (Table 1).

Correlation studies revealed no effect of age, nor duration of disease on L-selectin expression (r = -0.180; p = 0.210 and r = -0.120; p = 0.935 respectively). Also, the gender did not have any impact on L-selectin expression either among patients or in control group (Table 1).

Table 1. L-selectin expression on leukocytes of different groups studied

Groups	N	L-Selectin expression (%) Mean ± SD	P	Groups compared
Total BD	50	13.26 ± 11.30	0.000	Total BD vs controls
Male BD	41	12.57 ± 11.05	0.703	Male BD vs females
Female BD	9	16.38 ± 12.55		
Controls	50	29.42 ± 9.92		
Male controls	41	29.49 ± 10.52	0.918	Male controls vs female controls
Female controls	9	29.11 ± 7.46		
Ocular BD	39	11.02 ± 7.54		
Neuro BD	15	18.17 ± 16.58		
Active OB	20	5.21 ± 4.30	0.024	Active OB vs controls
Non active OB	19	17.13 ± 4.80	0.0001	Non active OB vs. active OB
Active NB	7	4.78 ± 2.70	0.821	Active NB vs controls
Non active NB	8	29.87 ± 14.41	0.001	Non active NB vs. active NB

BD=Behçet's disease, Contr.=Controls, NB = Neuro Behçet's disease, OB = Ocular Behçet's disease

4. DISCUSSION

This study shows that L-selectin expression on leukocytes was significantly decreased in our patients with BD compared with the control group. Our findings are in accordance with the results of Kaku et al.[9], who reported a dramatic decrease in expression of LECAM-1 and CD44 expression on peripheral leukocytes in 24 patients with ocular BD. These findings are similar to what has been found in patients with atherosclerosis[10]. Also, a similar phenomenon has been described in patients subjected to trauma namely elective limb surgery with a mean operation time of 122 minutes in whom neutrophils L-selectin expression revealed a significant drop 24 hours after trauma which was even more pronounced with increasing severity of the post traumatic course[11].

Our results may reflect the nature of the disease which is a chronic inflammation. This lower expression of L-selectin may be explained by its rapid shed from neutrophils after chemotactic stimulation[1]. This view is supported by the well known neutrophil hyperfunction which is one of the characteristic features of BD[12] with excessive effector functions such as superoxide production[13] and chemotaxis in vitro[14]. Also, increased circulating proinflammatory cytokines and chemokines stimulate neutrophils in BD[15]. Furthermore, Takeno et al.[16] have demonstrated spontaneous production of cytokines by neutrophils from patients with BD.

Similar to scarcity of reports dealing with L-selectin expression on leukocytes of BD patients[9], there is few information on serum sL-selectin which has been found to be increased in patients with widespread (systemic) BD and not in those with merely mucocutaneous involvement in the work of Haznedaroglu et al.[17]. This latter finding may reflect shedding of L-selectin from the leukocyte surface and thus explain our finding of decreased L-selectin expression in our patients with BD.

There was no significant difference in L-selectin expression in male vs female patients with BD in our study. The work of Miyamoto[18] who demonstrated that estrogens protect against cellular infiltration by reducing the expression of E-selectin and IL-6 in endotoxin induced uveitis does not contradict our findings since his work was an experimental one. In fact, it may explain the lower prevalence and milder form of uveitis encountered in females in most BD series[19-21] which may be due to a down regulation of these inflammatory genes by estrogens.

The results of the present study in relation to decreased L-selectin expression on leukocytes have been consistent in the two clinical subvarieties of BD that is ocular BD and NB. Still, a major difference between ocular BD and NB has been observed in the present study in relation to L-selectin expression during the inactive state: In ocular BD, the percentage of L-selectin was higher in non-active state than in active state but still statistically significantly lower than normal control values; in NB, on the other hand, the low L-selectin expression in active state was reverting during non-active state to values nearly equal to those of normal control subjects.

These findings may be explained by a continuous antigenic stimulation perpetuating an over-reaction to microbial stress proteins[22]. This over-reaction is yet facilitated by genetic predisposition such as the MICA gene which is in linkage disequilibrium with HLA-B51 and also is a cell stress response gene sharing nucleotide sequences with the human HSP70 promoter[22]. This interaction between the genetic and the environmental elements may not be the same for the various clinical forms of the disease. Many factors have been proposed to explain the different immunological

profiles associating variable organ affection, such as diversities related to variations in the properties of vascular bed in different tissues, to cytokines inducing translocation of proteinase 3, thus influencing the type and site of inflammation, or variations of the endothelial cells in different organs in their ability to respond to inflammatory cytokines and the variability in their tropism to various insults. In fact, this immunological heterogeneity with the different clinical aspects and/or organ lesions in BD has been previously described by the same group in relation to TGF-β1 as well as ANCA and total antioxidant activity[25,26]. This immunological heterogeneity may explain the different behaviour of the disease with more frequent attacks in ocular BD. Even more, it may explain the different prognostic outcomes and therapeutic response[27].

The continuously decreased L-selectin expression in ocular BD may also explain our findings in the present study concerning the lack of correlation between L-selectin expression with age of the patients and the duration of the disease.

These results apart from confirming the role of adhesion molecules in the pathology of BD during activity, point to a heterogenous immunological behaviour in relation to inflammation in the disease which would be responsible for its different clinical and prognostic outcomes. Our findings which describe a rather newly divulged area in the immunological profile of BD deserve more elucidation for a better understanding of the immunopathology of the disease. In fact, as has been proposed by Thomas Lehner[22], continuous efforts should be undertaken to identify the hierarchy in the cytokine-chemokine network, and its master switch in the different clinical situations of BD for a more effective immuno-therapeutic application.

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Autoimmunity to S-Antigen and Retinal Vasculitis in Patients with Behçet's Disease

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1. INTRODUCTION

Retinal vascular involvement is a very common finding in ocular form of Behçet's disease (BD). Although its pathogenesis is not fully understood, it seems that retinal antigens may play a role in ocular inflammation, contributing to tissue damage and self-maintenance of inflammation[1].

The aim of this study was to investigate immune response to S-antigen in BD patients with retinal vasculitis.

2. MATERIAL AND METHODS

S-antibodies and activated lymphocytes to S-antigen were investigated in serum of 12 BD patients (9 men, 3 woman, mean age 29.7±6.1 years with a range of 23-42 years) at active period of retinal vasculitis by passive hemagglutination reaction and lymphocyte blast transformation test. All patients fulfilled the International Study Group criteria for BD. Control group included 21 healthy donors. Chi-square tests and Student's t-test were used for statistical analysis.

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3. RESULTS

S-antibodies were found in four BD patients (33.3% vs 9.5% control group, ns, Fig. 1) and their level was not too high (1:16-1:64, mean level log2–4.8±0.96). Activated lymphocytes to S-antigen were determined in six BD patients (50% vs 14.2% in control group, p=0.0440) (Fig. 2).

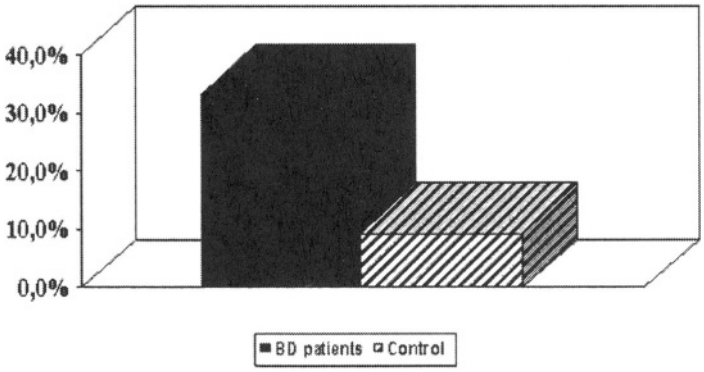

Figure 1. Frequency of S-antibodies detection in patients with BD and with retinal vasculitis

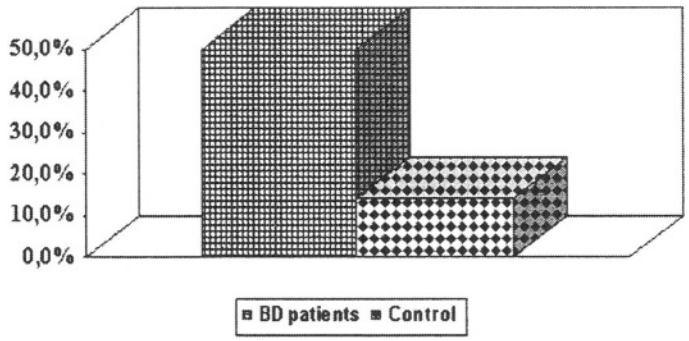

Figure 2. Frequency of S-antigen activated lymphocytes detection in BD patients with retinal vasculitis

4. CONCLUSION

These results suggest that activity of retinal vasculitis in BD patients is associated with lymphocytes activation to S-antigen. We have not found significant differences of S-antibodies in studied and control group.

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Nerve Growth Factor in Behçet's Disease

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1. INTRODUCTION

The role of cytokines in the pathogenesis of uveitis and, especially, of the ocular involvement in Behçet's disease (BD) has not been elucidated yet. IFN-γ, IL-2 and IL-6 have been demonstrated in ocular tissue and in aqueous samples of patients with uveitis, while IFN-γ IL-1, IL-2, IL-6 and TNF-α have been shown to induce inflammation in experimental animals after intraocular injection[1,2]. Nerve growth factor (NGF) is a neurotrophin exerting biological activity on immune system[3-5]. It is also one of the cytokines involved in autoimmune diseases and its increase has been noted in experimental uveitis[6]. The aim of this preliminary study was to evaluate the circulating levels of NGF, IL-2, IL-10, IFN-γ, and TGF-β in BD-affected patients with ocular involvement and to compare the values with normal subjects.

2. PATIENTS AND METHODS

Ten BD-affected patients, 8 males and 2 females, average age 37.9 ± 9.56 years (range: 25-50 years), with a mean disease duration of 12 ± 4.83 years (range: 3-19 years), were included in the study. All patients were affected by diffuse uveitis with retinal vasculitis, eight tested positive for HLA B51 antigen, and four had also displayed neurologic involvement.

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Blood samples were collected from all patients and NGF, IL-2, IL-10, IFN-γ, and TGF-β were evaluated by specific ELISA. At the time of blood collection no ocular or extraocular symptoms were present. Three patients were taking cyclosporine A (mean dose: 3 mg/kg/day) and steroids (mean dose: prednisone 14 mg/day), two cyclosporine A (2,5 mg/kg/day) in combination with azathioprine (75 mg/day) and steroids (prednisone 9.25 mg/day), one cyclosporine A (2.5 mg/kg/day) alone, and one steroids (prednisone 5 mg/day) alone. Three patients were out of therapy. Ten healthy controls, sex and age matched, were also investigated for the presence of blood cytokines.

Statistical analysis was performed by Mann-Whitney U test to compare the cytokines concentrations in BD patients with controls and by Spearman rho test to correlate the NGF levels with each cytokine value.

3. RESULTS

NGF and all the other cytokines were dosable in the serum of BD-affected patients. The mean concentrations in BD patients resulted as follows: IL-2 357.97 ± 194.46 pg/ml, IL-10 410 ± 161,2 pg/ml, IFN-γ 3968.25 ± 2785.13 pg/ml, TGF-β 4511.9 ± 1428.4 pg/ml, NGF 37 ± 34.75 pg/ml. Statistical evaluation showed a significant decrease of NGF levels in patients with BD (37 ± 34.75 pg/ml) when compared to healthy controls (98.40 ± 83.40 pg/ml) (p=0.0426). A significant increase of IL-2 (357.97 ± 194.46 versus 136.50 ± 117.08 pg/ml) (p=0.05) and IFN-γ, circulating levels (3968.25 ± 2785.13 pg/ml versus 1382.52 ± 272.38 pg/ml) (p=0.05) was also observed in patients with BD. No significant changes were present in IL-10 and TGF-β circulating levels between patients and controls, and no significant correlation was observed between NGF levels and each of the evaluated cytokines. No correlation was also observed concerning the previous neurologic involvement and NGF levels.

4. DISCUSSION

Our study has demonstrated that even in inactive phase of BD, i.e. in patients taking therapy and / or in those out of therapy, it is possible to find an increase in circulating levels of pro-inflammatory cytokines, such as IL-2 and IFN-γ. Conversely we have found a decrease of NGF serum levels in inactive BD, while levels higher than controls have been associated with different autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis) in the past[5-7]. Furthermore, considering that high

NGF serum levels have been associated with the severity of systemic illness in BD-affected patients[8], a possible role for this cytokine in the postulated autoimmune process in BD may be suggested. Further studies are needed to confirm the increase of NGF in the active phase of the disease and to evaluate the possible role of this cytokine as a marker for disease activity and severity.

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Low Serum Mannose-Binding Lectin Levels in Behçet's Disease

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1. INTRODUCTION

Microorganisms like HSV and some *Streptococcus* serotypes have been implicated in the pathogenesis of BD[1]. The *"innate"* immune responses are the first defence against external *"dangers"* such as microorganisms and are activated in BD[2].

The C-lectin pathway which is one of the three complement pathways is known to be activated by bacterial carbonhydrates[3]. The serum level of the mannan-binding lectin (MBL) which initiates the pathway is profoundly reduced by certain structural gene and promoter region mutations[4]. Low serum MBL levels and MBL gene mutations are implicated in the pathogenesis of recurrent infections and some autoimmune disorders such as rheumatoid arthritis (RA) and systemic lupus erythematosus[4,5].

In this study, we evaluated the associations between serum MBL levels and the presence and clinical manifestations of BD.

2. MATERIALS AND METHODS

MBL levels are measured in the sera of 45 patients with BD, 28 patients with recurrent oral ulcerations (ROD) (F/M: 17/11, mean age:35.8 years), 35 patients with RA (F/M: 30/5, mean age: 51.4 years) and 44 healthy controls

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(HC) (F/M: 26/18, mean age: 31.3 years). All patients with BD (F/M:29/16, mean age: 33.4 years) fulfilled the International Study Group's criteria.

MBL levels were measured by ELISA (Antibody Shop, Copenhagen). Briefly, diluted serum samples were incubated in microwells precoated with a specific antibody against MBL. Unbound components were removed by washing and wells were incubated with biotinylated antibody. The reaction was stopped with sulphuric acid and the optical density was read at 450 nm.

Data are presented as median, range and p values. Mann-Whitney U test was used for comparisons. BD and control groups were divided into 2 groups according to low (<500 ng/ml) MBL levels and non-parametric comparisons were performed by chi-square test.

3. RESULTS

A wide range of serum MBL levels was observed in all groups (range BD: 48-5390 ng/ml, ROU:50-5070 ng/ml, RA: 48-5220 ng/ml, HC: 56-5110 ng/ml). No significant differences were observed in the median serum MBL levels between the four groups (BD: 2500 ng/ml, ROU: 3065 ng/ml, RA: 2540 ng/ml and HC: 2431 ng/ml). Very low levels of MBL (<500 ng/ml) were detected in a significantly higher subset of patients with BD (31%, 14/45) compared to HC (11%, 5/44), ROU (11%, 3/28) and RA (11%, 4/35) (BD vs HC, p=0.04) (Fig. 1). No correlations were observed between the low MBL levels and clinical manifestations and disease activity in patients with BD (Table 1). Different treatment regimens had also no effect on MBL serum levels.

Table 1. MBL serum levels of BD patients with different clinical manifestations and treatment regimens

	MBL<500 (n=14) (%)	MBL≥500 (n=31) (%)
Uveitis	3/14 (21)	7/31 (22)
Erythema Nodosum	11/14 (78)	15/31 (48)
Vascular involvement	2214 (14)	6/31 (19)
Arthritis	5/14 (35)	11/31 (35)
CNS involvement	2/14 (14)	1/31 (3)
Genital ulcers	10/14 (71)	25/31 (80)
Pathergy positivity	8/14 (61)	21/29 (72)
Family history (ROU+BD)	8/14 (57)	13/31 (41)
Active disease	11/14 (78)	30/31 (96)
Colchicine treatment	13/14 (92)	25/31 (80)
Immunosuppressive treatment	7/14 (50)	14/31 (45)

* The clinical parameters did not differ statistically in the two groups

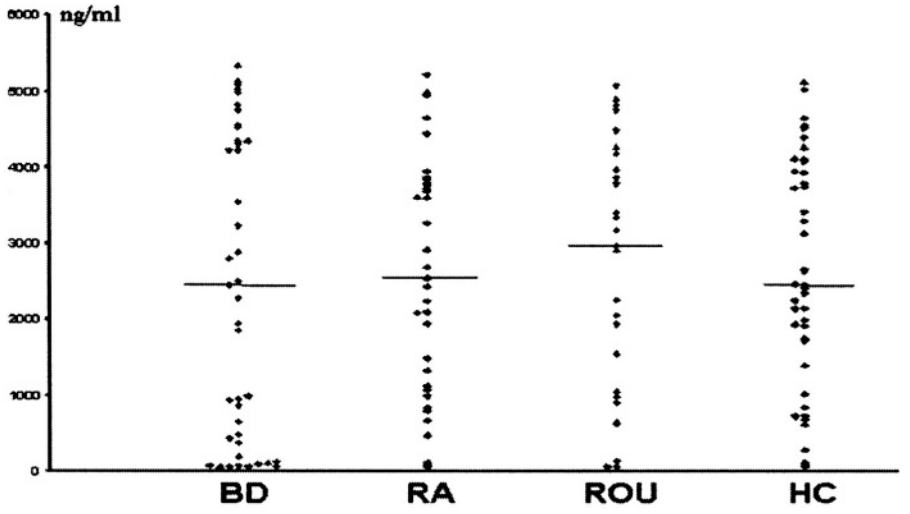

Figure 1. MBL serum levels in patients with BD, RA, ROU and HC

4. CONCLUSION

Very low levels of MBL were detected in a significantly higher number of BD patients compared to HC, ROU and RA. This observation needs to be confirmed with genetic analysis as low MBL levels are shown to correlate with MBL gene mutations[4].

These results suggest that MBL deficiency might contribute to the pathogenesis of BD by impairing *"innate"* immune responses against microorganisms.

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CONCEPTS FOR RESEARCH

ISBD Basic Research Perspectives

A preliminary "outline" for basic research workshop studies

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This report aims to outline main basic research areas in Behçet's disease (BD) pathogenesis. For each area a current summary is followed by *"possible"* new research perspectives, suggested to be explored.

1. INFECTIOUS ETIOLOGY

Various clinical and basic data strongly point out an infectious triggering in BD pathogenesis. However, a BD-related specific response to any particular microorganism *(e.g. Herpes simplex virus-HSV, S. sanguis)* or a species (*Streptococci* in general) is still not proven. HSV studies have mainly been done in Korea and streptococcal work in Japan, with no confirmation from other populations. The question whether antibacterial or antiviral treatments will be effective in BD is also still controversial.since only a few studies exist on this topic. A part of the infectious theory is involvement of heat shock proteins (HSP) in the pathogenesis with "molecular mimicry" mechanisms. However, there are differences in the HSP responses in the UK, Japan, and Turkey regarding the peptide specificity, activated cell types (CD4, γδ+T cells) and disease specificity. This model also has problems especially in explaining tissue predilection of BD. One of the few animal models of BD, although uveitis as the sole manifestation, is developed with human HSP60 peptides, and both oral triggering of uveitis and tolerance induction are observed in this model. Thus, HSP60 is a strong candidate for immune modulation therapies in BD patients.

Alternatively, microbial agents could be non-specific immune triggers in BD. Immunologic abnormalities might effect the mucosal surfaces, which

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would be immunologically "silent" in germ-free conditions, and it might require local microorganisms of any type at a necessary local concentration and/or virulence for ulcer development.

1.1 Perspectives

Studies of a reproducible immune activation model (such as flow cytometric investigation of cell surface molecules of CD25, CD69, or intracellular Th1 cytokine staining) with different antigenic stimuli (preferentially prepared by one center) should be done in different populations. The investigations should include both active and inactive cases and also different clinical subsets of BD, since "in-vivo pre-activation" during clinical relapses might influence the results. These studies should also include microorganisms thought to be unrelated to BD (e.g. *E. Coli,* fungi etc.). For an alternative approach, local environment of oral or genital mucosa could be crucial in ulcer development and might be related to hygienic factors. Periodontal health of BD patients compared to ethnic controls is insufficiently investigated. Similarly, determination of whether BD incidence and severity has been decreasing throughout the last decades, and, if this happens to be so, whether this is due to earlier diagnosis, better treatment, or better oral hygiene conditions requires proper epidemiological studies.

2. INFECTIOUS AETIOLOGY

Innate immune responses are mainly studied in infectious conditions by now. No data related to complement abnormalities or increased susceptibility to infection in BD is reported, However, as BD might have an early aberrant immune response, this process might be linked to an abnormality in innate immunity, such as defects in phagocytosis, release of certain enzymes, or receptors such as toll-like receptors (TLR), mannose-binding lectins and their signalling pathways.

2.1 Perspectives

Role of innate immune responses against microorganisms or non-specific pro-inflammatory agents such as "urate" crystals could be explored to see whether a BD-specific, early and/or aberrant response is present in neutrophils, NK or NKT cells. Activation of innate receptors both on the cell surface and/or intracellular signalling mechanisms could also give clues

about BD-specific immune defects, which might also be related to less-efficient bacterial or viral clearance. Recently described HSP60/70 binding to TLRs form a new link between autoantigens and innate responses, which should be explored in BD.

3. IMMUNE HYPER-REACTIVITY

A strong argument favors early immune activation in BD with pathergy and urate reactions. Neutrophil responses in HLA-B51 transgenic mouse model and recent observations of increased interferon (IFN)γ responses to low dose superantigens support this model.

3.1 Perspectives

Peripheral blood mononuclear cell mRNA expressions of possible early activation-related (intracellular signalling, cell surface adhesion molecules, etc.) proteins should be investigated. A candidate gene approach aiming at single molecules, or a general approach using new technologies such as microarrays could be used. With similar techniques, sequential analysis (24-48 h) of BD-related pathological tissue specimens such as pathergy or urate test samples could also give insights to molecular mechanisms.

4. IMMUNE MEDIATORS

BD is suggested to be a Th1 type disorder with elevated levels of various pro-inflammatory cytokines and chemokines. However, the place of BD among various vasculitides and autoimmune disorders might be due to a unique mediator profile with upregulation of selected cytokines and chemokines. Among these only IL-8 is studied extensively for the moment.

4.1 Perspectives

Single analysis of various pro-inflammatory mediators is both very expensive and only giving information to a limited extent. New techniques such as microarray systems should be explored.

5. ROLE OF HLA-B5

HLA-B51, although not completely responsible for the genetic susceptibility to BD, is still the major genetic factor in BD. The classical role of HLA class I antigens such as B51 is the presentation of endogenous antigens synthesized within the cell to CD8+ cytotoxic-suppressor T cells. However, B51 restricted T cell responses are still unknown in BD.

5.1 Perspectives

As HLA-B51 binding peptides are known, B51 restricted T cell clones with their cytokine patterns shall be studied in BD. These possible 9 aminoacid peptides should also be analysed for their presence in the aminoacid sequences of any exogenous or endogenous proteins as possible *"Behçetogenic"* antigens. Also, further studies on HLA-B51-molecular mimicry (retinal-S antigen) hypothesis in different populations would be important. An aberrant generation of HLA-B51 heavy-chain dimers, similar to HLA-B27, could be an alternative model of pathogenic mechanism, too. This model also explains significant CD4+ T cell expansions in a disease, which is associated with a class I HLA antigen. Interaction of HLA-B51 with the relevant killer-immunoglobulin-like receptors (KIR) on the surface of NK, CD8+ and γδ T-cells may be the link between innate immunity and BD, and the role of B51/KIR binding in the pathogenesis is another issue waiting to be studied.

6. AUTO-IMMUNE RESPONSES

Human HSP60, retinal-S antigen, alpha-tropomyosin and endothelial lymphocyte cell surface antigens are among the candidates for an auto-immune response in BD. Self antigens cross reacting with streptococcal Bes-1 protein may also be important in the pathogenesis.

6.1 Perspectives

The role of above mentioned antigens in perpetuating or suppressing inflammatory responses should be further explored. Studies in different populations are also lacking to confirm their universal role in BD. New methods such as the SEREX may also help to identify novel antigens causing immune-reactivity during the inflammatory responses of BD.

7. VASCULAR PATHOLOGY

BD might be a true vasculitis with immune damage to endothelium, resulting from immune-complexes, or specific antibodies such as anti-endothelial cell antibodies. Alternatively, a neutrophil-related damage of vasa vasorum might be implicated in vascular pathology.

7.1 Perspectives

Further characterization of endothelial cell surface antigens and their role in intracellular pro-inflammatory signaling cascades should be explored. The unique nature of BD among vasculitides for venous pre-dominance is also unexplained and can be linked to the differences of arterial and venous endothelium, their separate responses to damaging agents or differences in their own vascular supply. Defects in haemostatic mechanisms (Factor V Leiden, protrombin: 20210 mutations) are possibly also linked to the venous pathology, but not studied sufficiently in every population.

8. GENDER DIFFERENCES

Although gender differences in BD are not clear in every population, a male-related susceptibility or female-related protective factor seems to be present in BD, especially regarding to the disease severity. This can be a general severity factor linked to increased functional activity of neutrophils or other immune cells, or specifically linked to vascular involvement by affecting endothelial cells. The protective role of estrogens on LPS-induced experimental uveitis model and neutrophil superoxide production is previously demonstrated.

8.1 Perspectives

In vitro immune responses of neutrophils, T cells and endothelial cells in the presence of different hormons should be explored. Animal models induced by HSP60 or retinal-S antigen should also be manipulated hormonally.

9. OTHER GENETIC AND ENVIRONMENTAL FACTORS

A considerably high sibling recurrence rate ratio (λs) in BD supports a strong genetic impact on the pathogenesis. However, the contribution of HLA-B51, the strongest genetic susceptibility factor defined so far (see also item 5), is estimated to be less than 20%. Currently unknown pathogenic mechanisms can be found by the identification of other BD-susceptibility genes using candidate gene approach or whole-genome screens. Recently described linkage to the telomere of chromosome 6 in familial BD patients is an exciting new area with an expectation of a novel pathogenic gene.

9.1 Perspectives

Association of HLA-B51 with BD in many different ethnic groups can be exploited for testing the hypothesis of spreading BD-related susceptibility genes through the Silk Road, via methods used for the analysis of ancient populations.

Familial aggregation of BD, with documentation of λs, needs to be replicated in other populations or subgroups of patients. Twin studies are also lacking in BD. An overall concordance rate of 25% can be estimated with the available 8 monozygotic twin pairs, and a rate of 50% in HLA-B51 positive pairs. A twin-registry would be very helpful to collect a larger series of both monozygotic and dizygotic twins for the heritability analysis.

Subgroups of patients with an increased genetic risk or Mendelian inheritance patterns, such as juvenile cases or patients with a very extended multicase family, should be defined in BD. Gender differences (see also item 8), can also be explained by epigenetic effects, and male or female-only patients could constitute another subgroup for epigenetic analysis.

Candidate gene approach is still the preferred way for the search of other susceptibility genes because of the easy access of cases and controls. However, association studies in cases and controls are prone to various drawbacks, including admixture, population stratification, genetic drift, etc. Thus, positive association results are rarely replicated in other populations. Family-based association studies, such as transmission disequilibrium test using trios of cases and parents, can be an appropriate solution. Since BD usually starts in the 3rd or 4th decade, it would be possible to reach both parents in most cases. It would also give us an opportunity to test the genetic linkage in the presence of an association. These trios would also be useful for fine-mapping strategies with linkage diseqilibrium analysis.

With the completion of the first whole genome screening, we have now more than 5 new loci, which need to be fine-mapped for the identification of novel BD-susceptibility genes, and also to be replicated and confirmed in another sets of multicase families. Increased awareness of the familial aggregation would be very helpful in finding new extended multicase families for future studies.

A web page could be established in the ISBD web site dedicated to genetic studies, especially for an easy access of the association study results with details of methods. It would make it easier to replicate positive findings in other populations.

MUCOCUTANEOUS MANIFESTATIONS

Complex Aphthosis: Evaluation for Behçet's Disease?

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1. INTRODUCTION

Complex aphthosis is a term that may be unfamiliar, either from a dermatologic, stomatologic, or a periodontal disease perspective. It is very common for patients to be referred for consideration of Behçet's disease as a diagnosis when patients have almost constantly three or more oral aphae or oral and genital aphthae. The purpose of this paper is to test our hypothesis that complex aphthosis is a diagnosis of exclusion.

We evaluated patients referred to us at the Wake Forest University School of Medicine Department of Dermatology over a period of six years for the purpose of excluding Behçet's disease.

1.1 Terminology

Recurrent aphthous stomatitis is the most common cause of oral ulcerations, affecting approximately 25% of the general population. Although common in childhood and young adults, the incidence of severity decreases with age. Three types have been identified: minor aphthae (80% of cases); major aphthae; and herpetiform aphthae.

Complex aphthosis is defined separately. In 1985 Jorizzo and associates[1] defined complex aphthosis as three or more almost constantly present oral aphthae alone, or oral and genital aphthae, in the absence of Behçet's disease. (Fig. 1).

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- ≥3 almost constant oral aphthae, alone, or
- oral and genital aphthae, AND
- absence of Behçet's disease

Figure 1. Complex Aphthosis – Definition

Types of complex aphthosis include: **Primary Idiopathic**, which is a diagnosis of exclusion; and **Secondary** (Fig. 2). Underlying diseases that are well known to be associated with almost constant three or more oral aphthae or recurrent oral and genital aphthae include inflammatory bowel disease; cyclic neutropenia; human immunodeficiency virus infection; vitaminic deficiencies including iron, folate and B_{12}. Technically Behçet's disease could be considered a Secondary Type of complex aphthosis, however, by definition, Behçet's disease is an exclusion for the diagnosis of complex aphthosis.

- Primary Idiopathic Type
- Secondary Type
  - Inflammatory Bowel Disease
  - Cyclic Neutropenia
  - Human Immunodeficiency Virus Infection (HIV)
  - Vitamin Deficiencies
    i. Iron/Total Iron Binding capacity
    ii. Folate
    iii. Vitamin B_{12}

Figure 2. Types of Complex Aphthosis

2. METHODS

A chart review was performed on 81 patients who were referred for evaluation of Behçet's disease to the Department of Dermatology at Wake Forest University School of Medicine over a 6 year period from 1995-2001.

Our first obligation to these 81 patients was to conduct a complete dermatologic history and physical examination, a biopsy of an aphthous lesion, and to perform polymerase chain reaction (PCR) and other appropriate studies to exclude herpes simplex virus when indicated (for all

genital lesions and for some lesions in other locations). If the lesions were typical oral aphthae, PCR might not have been performed.

3. RESULTS

During the course of our initial evaluation, we found eleven patients who had simple recurrent aphthous stomatitis, with no further disease detected. This included eight patients with aphthae minor (simple aphthae minor), two patients with aphthae major, and one patient with herpetiform aphthae.

Six patients were excluded who did not have aphthae. These patients included one with Sjogrens Syndrome, two with pemphigus vulgaris, one patient with lichen planus, one patient with factitial disease, and one patient who had simple aphthosis with the primary systemic disease dermatomyositis.

An additional sixty-four patients were then defined as having aphthae that qualified as complex aphthosis or Behçet's disease.

Ten of these patients met full criteria for Behçet's disease based on the international criteria[2]. We also exclude inflammatory bowel disease (as for the O'Duffy criteria).

Fifty-four patients remained who had complex aphthosis (from the original eighty-one patients). Those patients were subjected to our evaluation for excluding secondary causes of complex aphthosis.

3.1 Evaluation

Evaluation for excluding secondary causes of complex aphthosis included a hemogram (complete blood count with differential platelet determination) to exclude patients with cyclic neutropenia; human immunodeficiency virus testing if indicated and only after consent was obtained from the patient; a search for vitaminic deficiencies including determinations of folate, vitamin B_{12} levels and iron and iron-binding capacity determinations.

When history and physical examination and preliminary screening laboratory tests suggested inflammatory bowel disease, the patients were referred to the appropriate gastroenterology specialists for gastrointestinal evaluation for diagnosis of inflammatory bowel disease.

As a result of this evaluation, we were able to identify twelve patients with secondary complex aphthosis as follows: one patient with cyclic neutropenia; one patient with HIV infection that was not suspected previously, and ten patients with inflammatory bowel disease.

We are not including six patients who had identified vitaminic deficiencies because correction of the vitaminic deficiency in each case had no impact on the aphthosis. Therefore these six patients with vitaminic deficiencies were included in the primary idiopathic complex aphthosis group.

3.2 Primary idiopathic complex aphthosis

Primary idiopathic complex aphthosis is the diagnosis of exclusion given to the remaining forty-two patients. Clinical characteristics of these patients are presented (Fig. 3).

- 42 Patients
- 12 Males/30 Females (1:2.5 Ratio)
- Mean Age at Onset - 31.3 years
- ≥3 almost constant oral aphthae only - 29 patients
- Oral and genital aphthae - 13 patients
- Mean duration of follow-up in our clinic to exclude Behcet's disease - 4 years

Figure 3. Primary Idiopathic Compex Apththosis

During this average four year follow-up period, none of the forty-two patients progressed to meet criteria for Behçet's disease.

3.3 Management

Complex aphthosis is a plaguing problem that often interferes with the patient's ability to eat. It could be viewed as being comparable to the kinds of symptoms that patients with Behçet's disease experience from a mucosal standpoint.

All 42 patients received some adjunctive topical therapy (Figs. 4, 5). The most common therapy used was topical application of superpotent topical corticosteroids. Occasional patients with oral pharyngeal aphthae were given inhalant corticosteroids but were told not to inhale. With this method the corticosteroid was efficiently distributed to an area that was not accessible to topical application with a fingertip.

Patients over the last several years received topical tacrolimus as an adjunctive therapy, and some patients received viscous lidocaine for

symptomatic relief, although we were quite careful not to anesthetize the posterior pharynx during mealtime due to concern of aspiration.

We employed a therapeutic ladder for systemic therapies (Figs. 4, 5). Our view is that patients with primary idiopathic complex aphthosis should not receive aggressive therapies normally reserved for patients with Behçet's disease who have ocular disease or other significant systemic manifestations. Among our referral group, patients had been treated prior to referral with high doses of prednisone, cyclosporine, methotrexate, chlorambucil, cyclophosphamide, which, in our opinion, are too aggressive for these patients with only mucosal disease.

- Topical
  Various Combinations
  - Superpotent topical corticosteroids
  - Spray inhalant corticosteroids for oropharyngeal lesions
  - Topical tacrolimus
  - Viscous lidocaine
- Systemic
  Therapeutic ladder
  - Colchicine
  - Dapsone/sulfapyradine
  - Colchicine & dapsone
  - Thalidomide

Figure 4. Primary Idiopathic Complex Aphthosis: Management

We begin with colchicine 0.6 mg twice daily increased to three times daily as tolerated from a gastrointestinal standpoint[3]. Initially before the six year study, our approach was to then use dapsone, 100 mg per day, as monotherapy for those who could tolerate or were unresponsive to colchicine. All patients received a glucose-6-phosphate-dehydrogenizing screen at baseline because of the potential to receive this treatment option. Patients who were deficient in this enzyme did not receive sulfone or sulfa drug therapy.

We had several patients who, when proceeding on the therapeutic ladder from colchicine to dapsone, did not discontinue their colchicine. Their response to the combination of these two medications was better than either one alone. Although not subjected to double-blind study scrutiny, we have a subjective impression that colchicine and dapsone have a synergistic effect. Therefore many patients in this case series received colchicine/dapsone combination therapy.

Patients who failed colchicine/dapsone therapy and still had major symptomatic disease, were offered thalidomide therapy[4]. Recently thalidomide has been available as an FDA approved prescription drug in the USA, with very strict monitoring termed the STEPS program. This requires monthly visits and careful monitoring. Occasional patients needed an even more aggressive therapy such as with low dose weekly methotrexate or prednisone.

Percent of patients who achieved therapeutic endpoint with each therapy:	
Topical alone	4/42 (9.5%)
Colchicine	14/42 (33%)
Dapsone	2/42 (5%)
Colchicine & Dapsone	12/42 (28%)
Thalidomide	10/42 (24%)
Total	42 Patients

Figure 5. Therapeutic Ladder

The main toxicity of colchicine was gastrointestinal toxicity. 40 patients tried colchicine, 21 had gastrointestinal side effects. The dose was lowered for tolerance or the drug was discontinued.

With dapsone, the number one complication was haemotologic toxicity-specifically a drop in hemoglobin. 27 patients were treated with dapsone, starting at 100 mg per day. 6 of the 27 had a drop in hemoglobin that was significant enough to require a dose adjustment. One patient reported headache, and 2 patients reported miscellaneous toxicities.

Of the 17 patients who started thalidomide, 2 patients had neuropathies on nerve conduction tests 6 months to a year after initiating therapy. One patient reduced the dose, one discontinued the treatment. Additional complaints in thalidomide treated patients were one patient with constipation, one with headache, and one with a dermatologic eruption.

4. CONCLUSION

We outline an approach to the common problem of patient evaluation and treatment of patients referred for Behçet's disease who have severe aphthosis but not Behçet's disease.

To summarize, of 81 patients referred to our department over a 6 year period for evaluation of Behçet's disease:

- 6 patients had diseases that were not in the aphthosis category;
- 10 patients proved to have Behçet's disease using International criteria (and O'Duffy re: exclusion of inflammatory bowel disease);
- 11 patients had recurrent aphthous stomatitis;
- 54 patients met the criteria for complex aphthosis. Of these:
  - 12 had secondary type aphthosis;
  - 42 idiopathic primary complex aphthosis.

Algorithim for evaluation of patient referred for Behçet's disease:

1. Are the lesions aphthae?
2. Does the patient have simple aphthosis or complex aphthosis?
3. Using International Criteria, does the patient have Behçet's disease?
4. Is there underlying relevant disease?

From an evaluation standpoint the first question when a patient is referred for exclusion of Behçet's disease is: are the lesions aphthae? In 6 of our patients they were not. The next question would be: is this simple aphthosis or complex aphthosis? This question is answered by the frequency and the intensity of the lesions. If the patient has almost constantly more than 3 oral aphthae or recurrent oral and genital aphthae, clinical criteria to define complex apthosis are met. The next question is answered by excluding Behçet's disease using standard criteria, and evaluation must both be at baseline as well as ongoing. The remaining patients who do have complex aphthosis must be evaluated to exclude relevant underlying disease.

By using an appropriate therapeutic ladder, beginning with topical therapy and progressing to colchicine plus dapsone to thalidomide as an endpoint, most patients are able to achieve control that is acceptable to them. In our patient group this meant that about 25% of the patients ended up on thalidomide as their definitive long term therapy and over 50% of the patients were treated with colchicine or colchicine plus dapsone as their definitive endpoint of therapy.

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Complex Aphthosis

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1. INTRODUCTION

Virtually all patients with Behçet's disease (BD) suffer from recurrent aphthous stomatitis (RAS)[1-3]. RAS can be classified as simple versus complex aphthosis[1,4-7]. RAS has many synonyms; canker sores, aphthosis, aphthous ulcers, or recurrent oral ulcers. The word "aphthae" means ulcers. The author prefers the term recurrent aphthous stomatitis[1,6,7]. The lesions of RAS are discrete, round to oval erosions or shallow ulcers of the non-masticatory oral mucosa. They typically have a perilesional erythematous halo and are covered by a grayish fibromembranous slough. Sites of predilection include the buccal and labial mucosae, the lateral and ventral tongue, the floor of the mouth, and the soft palate and fauces. Lesions of the masticatory mucosa of the hard palate and maxillary and mandibular attached gingivae and dorsal tongue are unusual.

Patients usually develop RAS during childhood or adolescence. Recurrences are less frequent and milder with increasing age. The prevalence of RAS varies with the population studied. Some populations such as medical and dental students have a prevalence rate of 50%. It is estimated that 20% of the general population will have RAS during their childhood or early adult life.

2. SIMPLE AND COMPLEX APHTHOSIS

The presence of the lesions of RAS is critical to the diagnosis of BD[1,2]. The diagnosis is rarely made in the absence of oral aphthosis. Aphthosis can

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be classified as simple aphthosis or complex aphthosis (Table 1). Patients with BD typically have complex aphthosis. Simple aphthosis is a common, episodic, short-lived type of RAS affecting 20-50% of the population in their youth[1,6,7]. Complex aphthosis is an uncommon, persistent, chronic type of RAS which may be associated with systemic diseases[1,4,5,8]. Complex aphthosis patients may have anogenital aphthae. The presence of oral plus anogenital aphthae does not constitute a diagnosis of BD. The condition might be considered a forme fruste of BD[5], but the diagnosis of complex aphthosis is preferable to making an inaccurate diagnosis of BD.

Table 1. Classification of Recurrent Aphthous Stomatitis

Simple Aphthosis	**Complex Aphthosis**
Common	Uncommon
Episodic	Episodic or continuous
Short-lived lesions	Persistent
Few lesions	Few to many lesions
3-6 episodes/year	Frequent or continuous ulcerations
Prompt healing	Slow healing
Minimal pain	Marked pain
Little disability	Disabling
Limited to oral cavity	May have genital lesions

The recurrent aphthae of both simple and complex aphthosis are classified morphologically as minor aphthous ulcers (MiAU), major aphthous ulcers (MjAU), and herpetiform ulcers (HU)[6,9]. Some authors note an increased prevalence of MjAU in patients with BD when compared to all patients with RAS[10].

Successful management of patients with complex aphthosis requires an accurate diagnosis, classification of the disease, and recognition of causal or associated conditions. It is incumbent on the clinician to evaluate the patient with complex aphthosis for these conditions. Correction of the underlying condition such as gluten-sensitive enteropathy (GSE) by a gluten-free diet can result in a substantial diminution of disease activity or a remission[11].

Ulcus vulvae acutum represents an acute severe episode of oral and vulvar aphthae often associated with an infectious gastroenteritis such as tuberculous enterocolitis, typhoid fever, or *Yersinia* enterocolitis. Upon recovery, simple aphthosis may remain as the only remnant of the disease. Patients with rare combinations of signs and symptoms in the context of complex aphthosis have been reported as the MAGIC syndrome (mouth and genital ulcers with inflamed cartilage), the FAPA syndrome (fever, aphthosis, pharyngitis, and adenitis), and cyclic neutropenia. Aphthous-like oral ulcerations have been reported in HIV-positive patients. Lesions tend to be large and disabling. These lesions of complex aphthosis tend to occur in

individuals with CD4+ counts<100 cells/mL. The differential diagnosis of this profound immunosuppressive state includes infectious or drug-induced oral ulcers. The diagnosis of HIV-associated aphthous-like oral ulcers is one of exclusion.

Anemia and hematinic deficiencies have been associated with lesions of RAS for many years. Several studies have confirmed the presence of a subset of patients who may be deficient in iron, folic acid, zinc, vitamins B1, B2, B6, and B12 and whose disease remits or improves dramatically with replacement of their deficiencies[12-14]. Hematological screening should be considered for all patients with complex aphthosis, those patients with persistently troublesome signs and symptoms, and any patients with signs or symptoms of malabsorption or nutritional deficiency. Screening includes a complete blood count with red blood cell indices; serums levels of iron, zinc, and vitamin B12; red blood cell or serum folate; and anti-endomysial, anti-gliadin antibody, or tissue transglutaminase studies.

Gastrointestinal diseases have been associated with lesions of RAS for many years. Indeed, according to DuBois and van den Berghe[15], the word "sprue," signifying the gastrointestinal disease, is derived from the Dutch word "spruw" which means aphthosis. The association of lesions of RAS with GSE/sprue has been recognized previously[11]. The malabsorption associated with GSE can lead to deficiencies of B vitamins and folate. Some authors report that both oral and gut lesions resolve with a gluten-free diet. Furthermore, some patients with lesions of RAS may not have symptoms of GSE, but yet the oral lesions will improve with a gluten-free diet[16]. Thus, patients with RAS may have symptomatic or asymptomatic GSE with gluten hypersensitivity and/or nutritional deficiencies, either or both of which may be related to the development of the lesions of RAS. However, Hunter and co-authors[17] report that, in the absence of documented GSE, a double-blind controlled study of patients with RAS did not confirm that a gluten-free diet or a gluten-supplemented diet consistently yielded benefit or worsening for patients, but did show a large placebo effect.

The lesions of RAS may be associated with inflammatory bowel disease (IBD), such as ulcerative colitis and Crohn's disease. Simple or complex aphthosis may antedate, coexist, or serve as a marker for increasing intestinal disease activity. Patients with IBD not only have lesions of RAS but may also have erythema nodosum, papulopustular lesions or lesions of pustular vasculitis, and inflammatory ocular disease such as iritis and uveitis. Thus the distinction between multisystem IBD and BD may be difficult[18-21].

3. MAYO PATIENTS WITH COMPLEX APHTHOSIS

The records of 244 patients with complex aphthosis who had been seen personally by the author were evaluated. There were 152 females (62.3%) and 92 males (37.7%). The ages ranged from 15 months to 81 years of age. The peak decade was 20-29 years of age. Patients who suffered oral aphthous ulcerations approximately 50% of the time or had continuous oral ulcerations or had oral and genital ulcerations or suffered major disability from aphthosis were included. Causes for aphthosis were sought for each patient. The response to therapy was assessed. Twenty-five patients with complex aphthosis and BD were excluded. More than 60% of patients were 10-39 years of age.

Presentation as simple aphthosis converting to complex aphthosis occurred in 125 (51.3%) while the disease of 119 (48.7%) presented as complex aphthosis. Genital lesions were present in 34 (13.9%) of patients. Females suffered from genital lesions more often than males (16.4% versus 9.8%). Almost ¾ of patients had MiAU (73.4%) while 22.5% had MjAU and 4.1% had HU type of RAS lesions.

A substantial number (almost 60%) of patients had associated conditions relevant to their complex aphthosis problem (Table 2). Anemia and/or hematinic deficiencies were present in 61 (25.0%), gastrointestinal disease in 41 (16.8%), and hematopoietic and immunodeficiency conditions in 12 (4.9%) of patients. The onset was associated with smoking cessation in 10 (4.1%), drug reactions in 8 (3.3%), and chronic trauma in 6 (2.5%) of patients. Twelve patients (4.9%) had pseudo-Behçet's disease, 8 patients (3.3%) had cicatrizing oropharyngeal disease, while 4 (1.6%) had erythema multiforme associated with complex aphthosis. It is notable that 25 patients with complex aphthosis and BD were seen during this same period.

Table 2. Conditions Associated with Complex Aphthosis

Condition	Number	%
Anemia and/or hematinic deficiencies	61	25.0
GI diseases	41	16.8
Hematopoietic and /or immunodeficiency	12	4.9
Smoking cessation	10	4.1
Drug reactions (NSAIDs, Captopril)	8	3.3
Chronic trauma	6	2.5

Treatment of patients with complex aphthosis was successful in many patients with replacement of hematinic deficiencies, treating primary diseases such as Crohn's disease and GSE, modifying provocative factors such as drug reactions and trauma, and utilizing drugs such as systemic

corticosteroids and nonsteroidal anti-inflammatory drugs such as colchicine, dapsone, pentoxifylline, and antibiotics.

4. SUMMARY

Clearly, complex aphthosis alone does not constitute BD. Furthermore, the patient with complex aphthosis should be evaluated for associated conditions or diseases, some of which are "correctable causes" of RAS. The oral lesions of BD are aphthous in nature and are best classified as complex aphthosis. While some patients with complex aphthosis will develop BD, some will remain as sufferers of complex aphthosis for years until a cause is identified or the disease enters a spontaneous or therapeutically induced remission. Complex aphthosis is the major pseudo-Behçet's disease encountered in a referral practice[22].

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Comparison of Oral Aphthae in Behçet's Disease and Idiopathic Recurrent Aphthous Stomatitis

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1. BACKGROUND

The purpose of this study was to evaluate the difference between oral aphthae in Behçet's Disease (BD) and recurrent idiopathic aphthous stomatitis (RAS).

The resemblance between the two entities has intrigued dermatologists for a long time, especially A. Touraine who suggested that they might be two extremes of the same entity: on one end RAS, being a localized benign form, and on the other the general form named "La grand aphtose de Touraine", including BD.

2. INTRODUCTION

Typical oral aphthae is a round or ovoid yellowish or gray necrosis surrounded by red margin. It is painful and will heal spontaneously after 1 to 4 weeks without treatment. The size is normally from few millimeters to 1 or 2cm. Only few cases leave scars after healing. The giant aphthosis is rare but the healing usually leaves scars. In every attack the number varies from one to several. It may be located in every part of the oral mucosa.

Recurrent aphthous stomatitis (RAS) is characterized by recurring oral aphthae. The etiology of RAS is not clear. Considerable research attention has been devoted to elucidate the causes of RAS: local and systemic

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conditions, genetic, immunologic, and infectious microbial factors have been identified as potential etiologic factors. However, to the present date no principal etiology has been discovered[1]. There are identifiable predisposing factors in some cases: hematinic deficiency, low serum iron or ferritin, and folate deficiency, while some authors defined only vitamin B12 deficiency[2], cyclic neutropenia, gastrointestinal diseases, endocrine factors, immunodeficiency, and systemic lupus erythematosus. Other factors like trauma, certain food hypersensitivity, infectious agents, stress, drugs, and BD are all different causes[3]. In some cases of recurrent aphthae no predisposing factor can be found and thus can be nominated as idiopathic RAS. A positive family history is found in about one-third of patients. Those are familial idiopathic RAS.

3. METHODS

In a prospective study 56 new cases of BD and 133 new cases of RAS were evaluated consecutively. Sex, age of onset, frequency of aphthous lesions, their duration, their mean number at each attack, the pathergy test, HLA-B5, HLA-B51, and HLA-B27 were evaluated in both groups. Comparison was made by Student t test and chi square test.

4. RESULTS

The mean age in the BD group was 33.9 years. The standard deviation (SD) was 9 and the confidence interval (CI) was 2.4. In RAS group the mean age was 31.4 years (SD=9.2, CI=1.6). The comparison between the two groups was done by the Student t test. The t was 0.994 with a p value (p) of 0.32, which was not significant.

The mean time from onset in the BD group was 5.9 years (SD=6.3 and CI=1.6). In RAS patients the mean time from onset was 6.4 years (SD=6.7, CI=1). Comparison by Student t test showed t=0.450, and the p value p=0.65. The difference was not statistically significant.

The mean frequency of attacks in the BD group was every 28 days, SD was 29.7, and CI was 8. In RAS group, the mean frequency of attacks was every 36 days, SD=35, and CI=6.1. The difference was not statistically significant (t by Student t test was 1.315 and p was 0.19).

The mean number of ulcers in each attack in the BD group was 3.6 (SD=2.3 and CI=0.6). In RAS, the mean number of ulcers was 3.1 (SD=2.1, CI=0.4, t=1.514, p=0.13). The difference was not significant.

The mean duration of attacks in BD was 9.5 days, SD was 5.3, and CI was 1.4. In RAS the mean duration of attacks was 10 days, SD was 4.93, and CI was 0.7. The t value was 0.669, and the p value was 0.50. The difference was not significant.

In BD group, male patients were 63% (CI=12.6). In RAS group, male patients were 49%, CI=8.5, chi square=2.937, and p=0.09. The difference was not significant.

HLA-B5 in BD group was present in 64% of patients, CI was12.6. In RAS group it was present only in 22.6% of patients, CI was 7.2, chi square was 30.195, $p<0.0001$. The difference was highly significant.

HLA-B51 was present in 37.5% of patients in the BD group (CI=12.6). In RAS it was 18%, CI was 6.5, chi square=8.222, and p=0.004. The difference was again highly significant.

HLA-B27 was present in 9% of the BD group (CI=7.5). In RAS it was 2.3%, CI=2.4, chi square=2.839, and p=0.09. The difference was not significant.

Pathergy test in BD was positive in 57% (CI=13). In RAS it was 5% (CI=3.7, chi square=63.640, and the p value was $p<0.0001$). Here again the difference was highly significant.

5. DISCUSSION

There was no significant difference between BD and RAS regarding sex, age of onset, number of attacks, number of lesions in each attack, and the duration of the ulcers. There was a significant difference in positive pathergy test, HLA-B5, and HLA-B51.

In a previous study we showed that the genetic factor in familial RAS in general population was not the same as in familial BD patients[4]. In another study from Korea a much higher prevalence of HLA-B51 was found in Korean BD patients compared to patients with RAS or healthy controls[5].

6. CONCLUSION

Clinically it is impossible to differentiate oral aphthosis of BD from that of RAS. There is a high resemblance between the two entities. Positive pathergy test and HLA-B51 are the main differentiating factors.

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Bipolar Aphthosis. A Forme Fruste of Behçet's Disease

Long term follow-up of 26 cases

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1. INTRODUCTION

Much confusion exists regarding the classification of bipolar aphthosis. While criteria for Behçet's disease are lacking, bipolar aphthosis is considered a forme fruste of Behçet's disease by some authors, and a different disease entity[1-4] by others.

In our study, we aimed to evaluate the course of bipolar aphthosis, and to examine the relationship between bipolar aphthosis and Behçet's disease.

2. PATIENTS AND METHODS

Clinical data of 26 patients with bipolar aphthosis followed-up at the multidisciplinary Behçet's disease Unit at Ankara University Faculty of Medicine between the years 1986 to 2001 were retrospectively evaluated. All of the patients had oral and genital ulceration but no other signs or symptoms of Behçet's disease. During the follow-up period the patients were evaluated every 3 months and whenever indicated, and any sign or symptom that occurred between two visits was recorded. International study group criteria were used for the diagnosis of Behçet's disease.

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3. RESULTS

Out of the 26 patients with bipolar aphthosis, 18 patients were female and 8 patients were male. The patients were 10 to 55 years of age (mean age 32.69 years). The duration of follow-up ranged from 2 to 16 years, and the mean duration of follow-up per person was 5.50 years (±5.55 SD). Age at the onset of oral ulcerations ranged from 6 to 52 years (mean: 25.42) and age at the onset of genital ulcerations ranged from 10 to 45 years (mean: 30).

During the follow-up period, 4 patients (15.3%) had additional manifestations of Behçet's disease and fulfilled the diagnostic criteria for Behçet's disease. One patient had uveitis 6 years after the initial evaluation. Another patient had arthritis and papulopustular lesions after 3 years, and one patient experienced recurrent attacks of uveitis and arthritis after 5 years. One patient had erythema nodosum after one year during pregnancy. These patients had experienced bipolar aphthosis for 8 years as a mean before developing the complete signs of Behçet's disease. On the other hand in the majority of cases, no other manifestation of Behçet's disease was noted after a long follow-up period ranging from 2 to 15 years.

4. DISCUSSION

In our study out of the 26 patients with bipolar aphthosis, 4 patients had additional manifestations of Behçet's disease and fulfilled the diagnostic criteria of the International study Group for Behçet's disease indicating that patients with bipolar aphthosis should be closely monitored for the development of Behçet's disease. Further studies are needed for better understanding of the pathogenesis of these diseases and to clarify the relationship between bipolar aphthosis and Behçet's disease.

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The Effect of Smoking on the Clinical Features of Adamantiades-Behçet's Disease

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1. INTRODUCTION

Adamantiades-Behçet's disease (A-BD) is a chronic multisystemic inflammatory disorder affecting orogenital areas, skin, eyes, joints, and vascular and central nervous system[1,2]. This vascular disease is characterised by remissions and exacerbations. The aetiology of the disease remains to be elucidated although genetic, environmental, and immunological factors have been implicated[1,2]. However, it is indisputable that vasculitis is a hallmark of the disease[3]. The epidemiology of this disorder has been recently extensively investigated[4].

Smoking has been associated with many disease states. It reduces the size of oral aphthous ulcers, improves the symptomatology of ulcerative colitis[5] and symptoms of A-BD in case reports[6,7] and in one formal study[8].

2. MATERIALS AND METHODS

One hundred and eighteen patients (74 male and 44 female) fulfilling the International Study Group Criteria for Behçet's Disease were studied. In a standard questionnaire smoking habits and alcohol consumption were investigated in retrospective study. Also, demographic data, clinical features, familiar incidence, and treatment were recorded.

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Subjects were classified by smoking status into two categories: never, current smokers (the ex- smokers were included in the non-smoking category), and by alcohol consumption into two categories: no, yes.

We defined oral aphthous ulcers as frequently when the patient had any more than once per month otherwise we defined it as rarely.

Statistical analysis: conditional logistic regression[9] was used, the odds ration (OR) was adjusted by gender, age and alcohol consumption and the 95% confidence internal (CI) was calculated using SAS statistical package[10].

3. RESULTS

Table 1 shows the mean value and the corresponding standard error of demographic characteristics (age, age at disease onset and disease duration) of our patients by gender and smoking status.

Table 1. Demographic characteristics of 118 patients with A-BD by gender and smoking habits

	Male		Female	
	Smokers	Non-smokers	Smokers	Non-smokers
	n= 39	n= 35	n= 19	n= 25
	Range(x ± SE)	Range(x ± SE)	Range(x ± SE)	Range(x ± SE)
Age (yrs)	20 – 68	13 - 74	24 - 53	11 – 60
	(36.28 ± 1.69)	(35.40 ± 2.54)	(40.37 ± 1.92)	(36.56 ± 2.56)
Age at disease onset (yrs)	5 – 67	4 – 43	6 – 46	6 – 47
	(24.28 ± 1.94)	(21.34 ± 1.83)	(28.63 ± 2.41)	(24.68 ± 2.40)
Disease's duration (yrs)	1 – 33	2 – 45	2 – 36	2 – 45
	(11.10 ± 1.28)	(14.51 ± 1.85)	(10.89 ± 2.25)	(12.20 ± 2.33)

Table 2 presents the distribution of patients relating to the presence of oral aphthous ulcers by age, gender, alcohol consumption and smoking status. An univariate analysis was applied to patients having frequent (56%), and rare presence of oral aphthous ulcers. A statistically significant association was found between rare presence of oral aphthous ulcers and smoking (64%) (P=0.01).

Table 3 demonstrates mutually adjusted data concerning demographic and life–style variables of our 118 patients with A-BD with respect to the frequency of oral aphthous ulcers. Only smoking is a significant predictor of decreased risk (OR=0.31; P=0.006), whereas the potentially positive association with alcoholic beverages is non–significant (P=0.45). The frequent presence of oral aphthous ulcers in female patients remains suggestive (OR=2.22, P=0.06).

Table 2. Distribution of the 118 patients with A-BD concerning the frequency of oral aphthous ulcers by age, gender, alcoholic beverages and smoking habits

	Oral aphthae		
	Frequently n =66	Rarely n=52	P
Age(mean ± SE)	37.8 ± 1.57	35.3 ± 1.58	> 0.15 *
Gender Female	30	14	0.06 **
Male	36	38	
Alcohol consumption			
Yes	17	17	0.53 **
No	49	35	
Smoking habits			
Yes	25	33	0.01 **
No	41	19	

* P–value for t-test, ** P–value for chi-square test

Table 3. Multiple Logistic Regression-derived Odds Ratios (OR) and 95% Confidence Interval (CI) for 118 patients with A-BD in relation to the frequency of oral aphthous ulcers (rarely or frequently)

Variable	Categories or unit	Odds Ratio (OR)	95% Confidence Interval (CI)	P – value (two tailed)
Gender	Male	Baseline		
	Female	2.22	0.96 – 5.14	0.06
Age (yrs)	Continuous	1.02	0.99 – 1.06	0.21
Alcoholic	No	--		
beverages	Yes	1.43	0.56 – 3.65	0.45
Smoking habits	No	--		
	Yes	0.31	0.13 – 0.71	0.006

4. DISCUSSION

Our results show clearly a statistically significant (P=0.01) inverse association between smoking and the presence of oral aphthous ulcers in A-BD (64%). The potentially positive association with alcohol consumption was not significant (P=0.45).

Our study is the first retrospective one in a significant number of patients. Silveira and Mc Grath[6] reported marked improvement in some clinical features of the disease with smoking in two patients with A-BD. Recently, a patient with A-BD experienced improvement of oral aphthous ulcers by use of nicotine patches[7]. However, another study reports that in 3 out of 12 smoking patients the smoking worsened the oral aphthous ulcers[11]. In a prospective study studying a week of nicotine consumption, smoking improved oral aphthous ulcers in A-BD, and in 2 out of 47 patients also

genital ulcers and erythema nodosum[8]. Two other patients showed improvement of oral apthous ulcers with cigarette smoking[12].

Increased migration and overall function of neutrophils has been demonstrated in A-BD[13]. The levels of neutrophil–generated oxygen free radicals and superoxide anion production were increased in A-BD[14]. Interleukins (IL-2, IL-8, IL-10) and TNF-α were also increased in patients with A-BD[15,16]. Several studies have shown that cigarette smoking inhibits several of the reported cellular functions in A-BD. Smoking inhibits the spontaneous migration of neutrophils[17], the oxygen free radicals from neutrophils[18], and the chemotaxis of neutrophils[19]. On the other hand, it has been demonstrated that smoking inhibits the production of IL-2 and TNF-α[20] and also the proinflammatory mediators in patients with ulcerative colitis[21]

The reported favorable results of smoking on the clinical manifestations of A-BD are derived not only from nicotine but also from alpha and beta unsaturated aldehydes[19].

In conclusion, cigarette smoking improves oral aphthous ulcers in A-BD through various mechanisms.

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Muco-Cutaneous Lesions of Behçet's Disease

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1. INTRODUCTION

Muco-cutaneous lesions of Behçet's Disease (BD) are the main clinical dermatologic manifestation of BD, and the most important signs in the diagnostic criteria of BD[1,2].

2. MUCOUS MEMBRANE LESIONS

1) Recurrent oral aphthosis is the most important lesion of BD (96.1±0.6%). It was divided in major and minor, and herpetiform aphthosis. There are also punctiform, miliaria, and giant aphthosis. The shape and the size of aphthae can vary from one to the next attack in the same patient[1].

2) Genital aphthosis was seen in 63.9±1.5% of patients. In females, they are larger[2]. In males, they are on the scrotum, and on the penis. Sometimes aphthous lesions can be localized around the anus.

3) Erythema may also be seen on the mucous membranes[2].

3. SKIN LESIONS

Skin lesions are the second most frequent manifestation of BD. They were seen in 67.4±1.5% of the patients.

The pathergy phenomenon, which is a skin hyperreactivity to trauma, is seen frequently in BD (59.9±1.6%). It is not constant and has a time

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variation in the evolution of the disease. The most important cutaneous lesions of BD are pseudofolliculitis, pustular lesions[1], papulo-pustular lesions or acneiform eruptions. Other lesions are cutaneous aphthosis, small nodules, and Sweet's-like lesions or Behçet's cellulitis[4]' which is neither infectious nor a superficial thrombophlebitis. Pyoderma gangrenosum-like lesions are extremely rare.

4. SUBCUTANEOUS LESIONS

Subcutaneous Lesions: Erythema nodosum is seen in 22.8±1.3% of cases. They present more often erythema and edema around the lesions than the classic erythema nodosum.

Histology of all lesions shows a vasculitis, mainly leukocytoclastic or lymphocytic.

5. CONCLUSION

Muco-cutaneous lesions are not specific, but a sound knowledge of them does contribute to diagnosis.

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Dilsen's Pathergy Test in Behçet's Disease: Positive Correlation with Clinical Manifestations

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1. INTRODUCTION

Pathergy test has been considered as important criteria of international study group criteria for the diagnosis of Behçet's disease. It has been reported that there is lack of correlation between the mucocutaneous manifestations and systemic disease expressions.

2. PATIENTS AND METHODS

Thirty five patients with Behçet's disease, who were either newly diagnosed or free from treatment for at least two months before, were included. Scoring of clinical manifestations at time of pathergy testing using the clinical manifestation index correlated with Dilsen's pathergy test grading.

3. RESULTS

Using the correlation rule, we found that grading of pathergy test were directly proportional to the score of clinical manifestation. Application of t-test showed this correlation to be statistically significant.

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4. CONCLUSION

The reason behind this positive correlation is using grading of pathergy test rather than just positive ordinary pathergy test. So pathergy grading can be used as a guide for the activity of BD and response to therapy.

Is Behçet's Disease a Kobner Positive Disorder?

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1. INTRODUCTION

The Kobner reaction was originally described by Kobner in 1877 and is the development of isomorphic pathological lesions in distant wounds of patients with pre-existing cutaneous diseases. The term Kobner reaction is applied when a dermatosis develops at a site of trauma. Although best known in psoriasis, it has subsequently been observed in various other diseases such as vitiligo, pityriasis rosea, or lichen planus[1-3].

In several cases of Behçet's disease aphthous ulcers could be seen in the apparently normal skin of the patients following several traumas, chewing, pressure etc. The interval between exposure to such provocation factors and appearance of the isomorphic reactions varied from case to case[4].

Kobner reaction may follow simple irritation, physical injury, wounds, sunburn or vaccination. The trauma has to reach or act on the papillary layer of the dermis, but epidermal injury is also neccessary. Simple vasodilatation, vasoconstriction or suction injury that suppress the epidermis may not evoke the reaction. Some mediators during active disease might serve as protective factors. The Kobner reaction can be enhanced or inhibited by certain chemicals and cytotoxic agents. After the injury, an interval of 8 days is usually necessary.

For pathogenetic mechanisms involved in the isomorphic response, several possibilities should be considered: a viral origin, an immunological origin, a vascular origin, and a neural origin. Some mediators released from inflammatory cells during the active and inactive stages may serve as protective factors.

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2. PATIENTS AND METHODS

In a prospective clinical study we aimed to assess the mucocuteneous reaction with trauma, and to show the Kobner reaction. We investigated 20 "volunteer" Behçet's disease patients (ages 18-40, the average age 30 years) with aphthae and genital ulcers who experienced more than 3 episodes a year. The 20 Behçet patients and 10 control subjects were traumatized with different type of materials (microscope slide, small needle, cotton swap) to determine the positive Kobner reaction. Trauma sites were evaluated at 72 h, 7^{th}, 12^{th}, 15^{th} day for development of new ulcers, induration and erythema.

3. RESULTS

There was no significant positive Kobner reaction except for 2 cases. The two patients who were found to be Koebner positive devoloped new ulcers at trauma site. Four patients showed new ulcers in different localisation. The Behçet's disease Kobner reaction was not always in the linear confugiration and was not induced by trauma in every case. There was no significant difference between Behçet disease and control groups.

4. DISCUSSION

Although we could not show Kobner reaction in all cases after trauma, it may precipitate new ulcers in susceptible persons. An accidental bite of the mucosa, dental injection, tootbrush bristle or sharp food should be avoided. Scratching the genital mucosa leads to the development and precipitation of new ulcers. Traumatic piercing of the mucosa is less likely in keratinised epithelium, and ulcer is rare in keratinised mucosa.

The Koebner reaction can be inhibited by cytotoxic agents during treatment. Topical corticosteroids may have a protective effect and may may promote healing and shortening the clinical course of the ulcers. Recently, we recommended corticosteroid sprays which have shown efficacy in controlling the new ulcer.

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Extensive Pyoderma Gangrenosum-Like Lesion in Two Cases of Behçet's Disease, Responding Only to Cyclosporin

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1. INTRODUCTION

Pyoderma gangrenosum (PG) like lesion is a Behçet's disease (BD) skin lesion[1] which is very exceptionally seen. We report here 2 cases with unusual severe and extensive skin lesions, having an intensive hypersensitivity to needle injection by inducing new lesions.

2. CASE REPORT

The first case is a 42-year-old man with a history of 5 years of recurrent bipolar aphthosis, skin pustulosis, ocular lesions, and articular involvement. After 3 years of remission, he developed large and extensive ulcerations on the legs, buttock, and back. Biopsy of the lesion showed vasculitis. Local treatment with injection of triamcinolone acetonide induced new ulcerations after each injection. Cyclosporin was the only successful treatment. He developed a central nervous system involvement secondary to cyclosporin.

The second case is a 20-year-old male with a severe oro-pharynx and genital aphthosis, pustular lesions, and articular involvement. He developed large ulcerations on his leg. The biopsy showed vasculitis. Lesions responded only to cyclosporin.

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3. DISCUSSION

PG is a neutrophilic dermatitis with the same hypersensitivity to trauma as BD. In PG some cases are associated with bowel diseases as in BD. Also, PG can produce in some cases localization of neutrophilic lesions in other organs such as CNS, heart, and lymph nodes which resembles BD to some extent.

4. CONCLUSIONS

The severe involvement, in one of our cases, prompts to be careful in the manipulation of PG like lesions. The only successful treatment in the two cases was cyclosporin.

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A Case of SAPHO Syndrome with Pyoderma Gangrenosum and Inflammatory Bowel Disease Masquerading as Behçet's Disease

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1. CASE REPORT

A 45-year-old man presented with a 2-year history of abdominal pain, diarrhea and pyodermatous lesions in the lower leg in August 1998. His past history included uveitis, palmo-plantar pustulosis, Behçet's disease, pustulotic osteoarthropathy and ankylosing spondylitis. He was diagnosed as having Beçet's disease because of the occurrence of aphthae, acne, erythema nodosum, muscle pain, arthralgia and the positivity for HLA-B51 in 1981. In 1988 he underwent joint replacement for sternocostoclavicular arthropathy.

A dermatological examination revealed pyodermic ulcers and atrophic ciribriforme scars on the legs, and acne scars on the face (Fig. 1). The skin biopsy specimen from the leg lesion was consistent with pyoderma gangrenosum; dense neutrophilic infiltration throughout the dermis without granulomatous changes (Fig. 2). Small bowel examinations demonstrated longitudinal ulcers, cobblestone appearance and fistulisation of the ileum. Barium enema revealed stenosis of the sigmoid and descedens colon. Partial ilectomy and colectomy were performed under a diagnosis of Crohn's disease, although the resected materials showed histopathologic features of nonspecific inflammatory changes without granulomae. He had relief of the abdominal pain and diarrhea.

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In September 1999, redness and swelling suddenly occurred on the left sternocostoclavicular area without any causative pathogens, associated with diarrhea and polyarthralgia. He was successfully treated with predonisolone and methotrexate.

A diagnosis of SAPHO syndrome associated with pyoderma gangrenosum and inflammatory bowel disease (IBD) was made possible by the whole episodes of his illnesses.

2. DISCUSSION

It is intriguing to note that our patient had presented with the variety of clinical symptoms suggestive of Behçet's disease until the final diagnosis was made. Other differential diagnoses included PAPA syndrome (pyogenic sterile arthritis, pyoderma gangrenosum, and acne), acquired hyperostosis syndrome and bowel-associated dermatosis-arthritis syndrome.

SAPHO syndrome is an acronym of synovitis, acne, pustulosis, hyperostosis, and osteitis, which is characterized by rheumatoid factor-negative osteoarthropathy associated with various dermatologic manifestations[1,2]. The association of SAPHO syndrome with IBD or pyoderma gangrenosum has been reported recently[1,3]. We believe in the presence of a close pathogenic link among various symptoms in our patient.

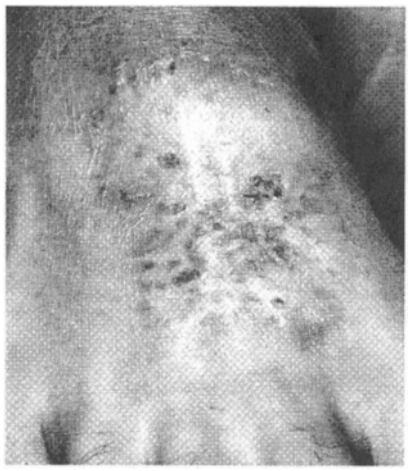

Figure 1. Atrophic ciribriforme scars on the lower leg

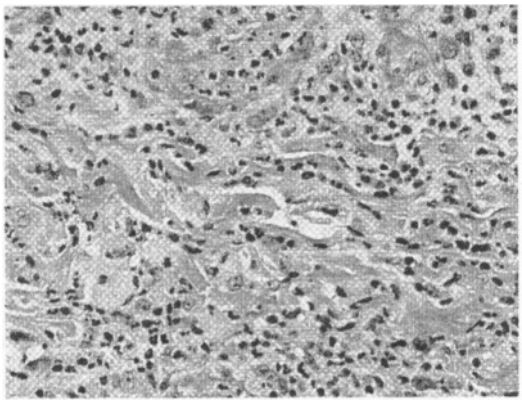

Figure 2. Dense neutrophilic infiltration throughout the dermis without granulomatous change.

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Cytokine Expression Within Mucocutaneous Lesions of Behçet's Disease: Involvement of Proinflammatory and Th1 Cytokines

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1. INTRODUCTION

During the last decade, the renewed interest in the role of cytokines in several dysimmune disorders stressed the possible implication of these molecules in the pathophysiology of Behçet's disease (BD). Cytokine levels were investigated in sera, in biological fluids and in supernatants of cultured peripheral blood mononuclear cells (PBMC) of patients with BD[1-11]. These investigations reported increased levels of pro-inflammatory cytokines as well as T helper 1 (Th1) cytokines. Unfortunately, cytokine patterns in peripheral blood or in culture supernatants of polyclonally stimulated PBMC may not precisely reflect the pathophysiological process occurring within BD lesions. In the present study, a set of Th1, Th2, and pro-inflammatory cytokines has been analyzed within typical mucocutaneous lesions of patients with active BD. The data were compared to those obtained by the analysis of cytokines in the sera taken from the same patient using ELISA tests.

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2. MATERIAL AND METHODS

2.1 Study population

The study population consisted of 20 patients with active BD fulfilling the International Study Group criteria. All patients had at least one mucocutaneous lesion at the time of the study. Patient biopsies were carried out from 13 oral ulcers, 6 genital ulcers, and 3 pseudofolliculitis after 2 or 3 days of evolution. In all patients, a pathergy test was realized and a biopsy performed when the test was positive after 24hours. Fifteen sex- and age-matched healthy volunteers were included as controls for serum cytokine analysis. Biopsies of skin and oral mucosa were obtained from four healthy controls.

2.2 Cytokine quantification

Serum levels of IL-1, IL-4, IL-6, IL-8, IL-10, IL-12, IFN-γ and TNF-α were measured by an immunoenzymatic assay using commercial antibodies (Pharmingen). Quantification of IL-4, IL-8, IL-10, IL-12(p40), IL-13, IFN-γ and MCP-1 mRNAs was performed using quantitative real time PCR according to PE Biosystems procedure. Data were normalized referring to the expression of an endogenous control, the 18S ribosomal RNA. Non-parametric Mann-Whitney test was performed to compare median cytokine concentrations of the study groups with sub-groups. Statistical significance was assigned to p-values lower than 0.05. Correlation between levels of the different cytokines was assessed using Spearman's rank correlation coefficient.

3. RESULTS

3.1 Serum cytokine analysis

Serum cytokine levels were higher in BD patients than in control group. However, only IL-10 level was significantly increased in active BD ($p=0.0002$). Increased levels of IFN-γ and IL-12 were significantly associated with the presence of oral ulcers ($p=0.02$) and vascular lesions ($p<0.05$).

3.2 Intralesional expression of cytokine mRNAs

None of the studied cytokines was detected in normal skin obtained from healthy controls whereas only IL-10, IL-8, and MCP-1 mRNAs were detected at low levels in the normal oral mucosa. In BD biopsies, MCP-1, IL-8, IL-10, IFN-γ, IL-12, IL-4, and IL-13 mRNAs were detected in 100%, 96.7%, 90.3%, 77.4%, 45.1%, 6% and 6% of samples, respectively. For each cytokine, mRNA levels were compared according to the lesion type. IFN-γ and MCP-1 mRNAs were significantly higher in oral ulcers compared to genital ulcers ($p<0.05$). IL-8 mRNA levels were significantly higher in oral and genital lesions than in pathergy test lesions ($p<0.01$). IL-4 and IL-13 expressions were detected only in two out of 11 positive pathergy tests.

3.3 Association between *in situ* cytokine gene expression and some features of BD patients

For each type of lesion, we searched for an association between intralesional and serum cytokine levels. No correlation was observed. Moreover, we analyzed the eventual association of the pattern of intralesional cytokine expression with a particular genetic background (HLA-B51 or microsatellite alleles of MICA). No significant association was found.

4. DISCUSSION

In the present study, we found that several cytokines were over-expressed in the active stage of BD. Interestingly, there was no association between serum and *in situ* levels. In sera, only IL-10 was significantly raised in patients with active BD as compared to healthy controls suggesting that serum IL-10 may constitute a marker of BD activity, although controversial results were previously reported on this matter[9,10]. It is however interesting to note that increased levels of IFN-γ and IL-12 were significantly associated with the presence of oral ulcers and vascular lesions indicating a possible role for these cytokines in the physiopathology of such lesions. BD lesions were characterized by the accumulation of MCP-1, IL-8, IL-12, and IFN-γ mRNAs. With the exception of IL-10, the typical Th2 cytokines (IL-4, IL-13) were absent in BD lesions in spite of the relatively high peripheral levels of IL-4. These data highlight the importance of chemokines as well as Th1 responses in BD lesions. Increased IL-10 level suggests a role of this cytokine in preventing a more severe inflammatory pathology in these

tissues. Furthermore, some differences observed in cytokine expression between different BD lesions suggest that the pathophysiological mechanisms of these lesions vary from one tissue to another. Finally, no association between tissue cytokine expression and a particular genetic profile was found suggesting the absence of involvement of genetic factors in the expression of a particular cytokine pattern.

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OCULAR MANIFESTATIONS

Origin and Outcome of Macular Edema in Behçet's Disease

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1. INTRODUCTION

The chronic macular edema (CME), frequent in ocular Behçet, is a treat to the visual outcome. CME is caused by an accumulation of intramacular fluid, due to abnormality in retinal vascular permeability which is a complication of uveitis[1] and retinal vasculitis[2]. CME can cause significant anatomical and physiological changes in macula and impair the visual acuity[3,4]. We have investigated the origin and outcome of CME in patients with Behcet's disease.

2. MATERIALS AND METHODS

This case series study has been performed in 1999. Seventeen patients, who had at least one and half year of follow up at our clinic and presented macular edema were consecutively selected and included in the study. They were followed up for two more years with ophthalmic examinations and fluorescein angiographies (FA). Patients having initially optic atrophy, retinal vascular necrosis, and macular scars were not included.

Macular edema was diagnosed by 3M of Goldman and Haag-Streit biomicroscopy. Consecutive FA's were performed to confirm the existence, origin, and outcome of macular edema. The term evident retinal vasculitis is applied where the sheathing of the retinal vessels has been

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ophthalmoscopically visible. No evident retinal vasculitis has been stated when vascular leakage had been observed on FA.

Six patients had complete Behçet's disease and eleven patients presented incomplete Behçet's disease[5,6].

All patients were under immunosuppressors and corticosteroid therapy.

The immunosuppressors which were used were as follows:

- Cyclosporin A,
- Cyclosporin A + Methotrexate,
- Cyclophosphamide,
- Cyclophosphamide + Methotrexate,
- Methotrexate,
- Immuran.

3. RESULTS

Seventeen patients, thirty-four eyes were included in the study. Ten were males and seven females. The mean-age of the patients (at the first consultation in our clinic) was 26.3 years (ranging from 13 to 41 years). The evolution of non-ocular symptoms before consulting us was 4.7 years (ranging between 0.5 and 25 years). The duration of ocular symptoms before consultation was 14.7 months (ranging between 1 week and 3 years). At the last visit, the follow up period of the patients was 6.05 years (ranging between 1,5 and 17 years).

29 eyes presented macular edema; 19 eyes at first consultation at our clinic, and 10 had developed macular edema during the follow up period. In 12 patients macular edema was bilateral and in 5 cases it was unilateral. In five eyes of five patients macular edema was never observed.

In the 29 eyes with macular edema, 18 eyes presented hyalitis and evident vasculitis, 10 eyes presented hyalitis and no evident vasculitis (leakage on FA), and one eye presented retinal vasculitis but no hyalitis.

In the five eyes without macular edema none presented retinal vasculitis, and only one eye had hyalitis.

Only in four eyes of the 29 eyes with macular edema, the edema had disappeared by the last visit. All four eyes initially presented retinal vasculitis and hyalitis. At the last visit none of these four eyes presented vasculitis, and only one eye presented hyalitis. The initial and final vision, and symptoms of these four eyes are indicated in table 1.

Six eyes of four patients presented macular scar at the last visit. All six eyes presented retinal vascular necrosis, retinal atrophy, and optic atrophy. The mean duration of the ocular disease in these four patients was 10.5 years compared to 6.9 years in the other patients.

Table 1. The initial and final vision and symptoms of the four eyes out of 29 eyes with macular edema in which macular edema disappeared, Shariati Hospital, 1999-2001

Case	Initial VA	Hyalitis	Vasculitis	Final VA	Hyalitis	Vasculitis
3	0.4	+	+	1.0	-	-
5	0.1	+	+	1.0	+	-
13	0.3	+	+	0.7	-	-
16	0.9	-	-	1.0	-	-

Seven eyes in five patients presented an important macular edema, extensive fluorescein leakage on FA, and severe reduction of vision at the last visit. The mean duration of the ocular disease in these patients was 7.8 years. The initial and final visions of these eyes with severe reduction of vision are indicated in table 2.

Table 2. The initial and final vision of seven eyes with severe reduction of vision and macular edema.

Case	Laterality	Initial VA	Final VA
8	R	6 m	2 m
	L	0.4	4 m
9	R	0.1	1 m
	L	0.5	0.3
10	L	1.0	0.2
11	R	1.0	0.1
12	L	0.5	1 m

R=right, L=1eft, m=rneter

4. DISCUSSION

In our investigation, in only four eyes out of the 29 eyes which CME and ocular Behçet, the macular edema disappeared with the systemic treatment of corticosteroids and immunosuppressors. Many therapies have been proposed to treat CME in uveitis and vasculitis patients, such as local injections of corticosteroids, oral doses of acetazolamide or vitrectomy.

In the investigation of Jennings et al.[7], although the vision improved in 6 of the 12 patients, the posterior subtenon injection of corticosteroids in uveitis patients with CME led to a concomitant improvement in a blood barrier permeability only in two eyes. Acetazolamide has been tried by several investigators to reduce the CME in uveitis patients. Cox et al.[8] reported a prospective cross-over study of acetazolamide on CME in 1988. They showed improvement in both visual acuity and CME in 3 out of 6 patients. Farber et al.[9] who compared the visual acuity of the patients with acetazolamide and placebo patients observed no significant difference between the two groups. Whitcup et al.[10] showed that acetazolamide therapy

results in a small decrease in CME in patients wich chronic uveitis but does not improve the visual acuity. Lashei et al.[11] showed that acetazolamide was not effective in CME of ocular Behçet's patients.

Pars plana vitrectomy appeared to decrease CME in patients with uveitis [12-15]. Kiryu et al.[16] showed that pars plana vitrectomy of CME secondary to sarcoid uveitis plays a role in the treatment of macular edema. Slit lamp biomicroscopy showed that CME had resolved in 14 of 18 eyes with macular edema. However, in an active retinal vasculitis such as ocular Behçet's disease with constant, and permanent leakage of serousity from the retinal vessels the efficacy of such treatments should be proven.

5. CONCLUSION

In our patients with ocular Behçet's disease, retinal vasculitis was the cause of macular edema. Long standing macular edema can provoke considerable reduction of vision and non-reversible lesions such as macular degeneration.

In most cases macular edema persisted despite our severe medical treatments. Other interventions should be undertaken to prevent chronic macular edema and its consequences.

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Fluorescein Angiography and Optical Coherence Tomography in Ocular Behçet's Disease

A preliminary study

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1. INTRODUCTION

Cystoid macular edema (CME) is a major cause of visual loss in uveitis, and its diagnosis is very important[1,2]. At present, one of the most widely used investigations for confirming the presence of CME is fluorescein angiography (FA). This is an invasive test, with side effects ranging from nausea in up to 20% of cases to its rare complications of anaphylaxis and death[3-5].

Optical coherence tomography (OCT) is a new method for high-resolution cross-sectional imaging of the retina[6]. It has been used to study a number of macular conditions including central serous chorioretinopathy, age-related macular degeneration, macular holes, macular edema, epiretinal membranes, and optic disc pit-associated maculopathy[7-12]. This technique provides non-invasive, non-contact, in vivo, cross-sectional images of the retina with a maximal longitudinal resolution of 10 microns. It is useful in the objective evaluation of macular thickness and quantitative assessment of CME.

To evaluate the potential of OCT in determining macular involvement in ocular Behçet's disease, we carried out a cross-sectional study comparing the relative efficiency of OCT compared with the current standard of FA.

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2. MATERIALS AND METHODS

Thirty-three eyes of 18 Behçet patients, who were examined at the Ankara University Department of Ophthalmology between October 2000 and February 2002, were included in this study. Of the 18 patients, 11 were male and 7 were female. The patients were aged between 18-60 years (mean age 32.4). All of the patients were diagnosed by the Behçet Center at Ankara University based on the diagnostic criteria for Behçet's disease. All patients underwent a complete ocular examination, indirect ophthalmoscopy, colour fundus photography, digital FA and OCT scanning.

The images obtained from the OCT scanning (OCT 1 scanner, Humphrey Instruments, CA, USA) had a laminar substructure with two bands of high-intensity signal. In previous publications, the distance between the inner aspects of these bands has been assumed to give a measure of retinal thickness.

The digital fluorescein angiograms (Zeiss Optical AG, Germany) were taken subsequently after a 5 ml dose of 20% sodium fluorescein had been injected intravenously.

The patients were categorized into two groups based on the positive or negative findings of ocular Behçet's disease on FA.

3. RESULTS

On FA, 12 of the 33 eyes had leakage in the macula which increased in the late stage (Table 1). In addition, one had macular hemorrhage with surrounding leakage; one showed hemorrhage due to branch retinal vein occlusion, branch retinal vein occlusion (BRVO), and leakage; and one had macular hole and leakage at the macular capillaries. In 2 eyes, there was an increase in the tortuosity of the macular capillaries, and in one eye there was an increase in the FAZ together with macular ischemia. Two eyes had pigment epithelial atrophy. The remaining 13 eyes had normal FA.

On OCT, there was macular edema and thickening in 10 eyes and macular hole in one eye. The remaining 22 eyes had normal OCT scans.

When we compare FA findings with OCT; 8 eyes with fluorescein leakage (Fig 1A,B), one eye with capillary tortuosity, and one eye with hemorrhage due to BRVO (Fig 2) showed macular edema and thickening on OCT. One eye with macular hole was clearly seen on OCT (Fig 3). Four eyes with leakage, 2 with pigment epithelial atrophy, one with capillary tortuosity, one with macular ischemia, one with macular hemorrhage with surrounding leakage were normal on OCT. The remaining 13 eyes were normal both on FA and OCT.

Table 1. Macular findings on FA and OCT in 18 Behçet patients (33 eyes)

		SEX	FA-OD	FA-OS	OCT-OD	T-OD	OCT-OS	T-OS
1	HK	M	*	macular edema, hypofluorescence due to hemorrhage, surrounding hyper fluorescence	*	*	normal	135
2	HS	M	normal	*	normal	183	*	*
3	AA	M	leakage from the capillary, increase in capillary tortuosity increase in FAZ	leakage from the capillary, increase in capillar tortuosity, increase in FAZ	edema	300	normal	157
4	HB	M	normal	normal	normal	*	normal	122
5	SE	M	increase in capillary tortuosity	leakage at the late stage	normal	165	edema	256
6	MD	M	edema	edema	normal	169	normal	169
7	AÇ	F	normal	normal	normal	177	normal	169
8	FS	F	hyper-hypofluorescent mottling due to PE atrophy	*	normal	130	*	*
9	CD	M	normal	Normal	normal	168	normal	182
10	DB	F	leakage from the capillary, vasculitis	macular hole, surrounding leakage, vasculitis	edema	155	hole	*
11	DS	F	late leakage from the capillary, vasculitis	hyper-hypofluorescent mottling due to PE atrophy	edema	750	normal	95
12	Eİ	M	leakage from the capillary	normal	edema	630	normal	150
13	UG	M	macular ischemia. increase in capillary tortuosity vasculitis	leakage from the capillary	normal	150	edema	900
14	NÖ	F	normal	increase in capillary tortuosity	normal	180	edema	635
15	BE	M	normal	normal	normal	140	normal	180
16	DK	M	hypofluorescence due to BRVO, leakage from the capillary	normal	edema	180	normal	160
17	GÇ	F	normal	CME- leakage	normal	160	edema	375
18	HE	F	mild leakage from the capillary, increase in FAZ	leakage from the capillary at the late stage, İncrease in FAZ	edema	390	normal	170

FA: Fluorescein Angiography, OCT: Optical Coherence Tomography, T: Thickness (μm), *:Not evaluated

BRVO: branch retinal vein occlusion, PE: pigment epithelial, CME: cystoid macular edema

In 15 eyes, FA was positive for macular edema and in 18 eyes the FA was negative. Of the 15 eyes which were FA positive, 11 had positive findings on OCT. All 18 FA negative eyes, were also negative on OCT.

Figure 1. A) FA shows late leakage in the right eye. Macular edema and thickening is evident on OCT. B) FA shows late leakage in the left eye of the same patient. However, OCT is normal for this eye.

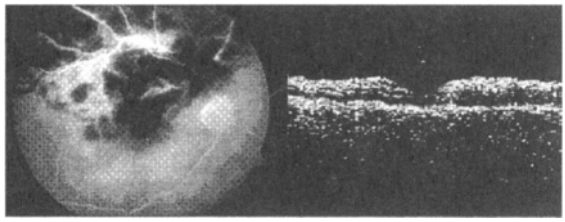

Figure 2. FA shows macular edema and hemorrhage due to BRVO and macular edema on OCT.

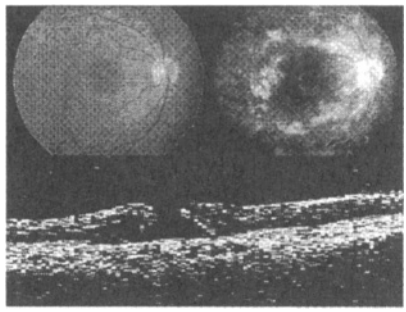

Figure 3. Full-thickness macular hole is clearly seen on OCT.

When FA was taken as the reference standard, the sensitivity of OCT was 68 %, and specificity 100 % in detecting the macular edema. The positive predictive value was 100% and the negative predictive value was 77%.

The mean macular thickness measured in 32 eyes was 254±197μm (range: 122-900 μm). One eye had macular hole. The mean macular thickness of 10 eyes which had significant macular edema on OCT was 457±242 μm (range: 155-900 μm). The mean macular thickness of 22 eyes

which did not have significant macular edema on OCT was 157±22 μm (range: 122-182 μm).FA and OCT results can be seen in Table 1.

4. DISCUSSION

Fluorescein angiography identifies breakdown of the blood-retinal barrier. The fluorescein leakage indicates where thickening is likely to occur in the future. It does not give a measure of thickening itself. OCT has the potential to measure changes in retinal thickness. As the uveitic CME occurs at the fovea, a scanning strategy centered on this area minimizes the possibility of significant thickening remaining undetected[13].

Antcliff et al.[13] found the specificity as 100% and sensitivity as 96% on OCT when FA was taken as the reference standard on 121 eyes of 58 uveitis patients. Although our series composed of only Ocular Behçet's disease patients as opposed to heterogenous types of uveitis forming Antcliff et al.'s series[13], we had far lower sensitivity but the same specificity results.

In previous studies of macular thickness in normal volunteers, OCT had an average SD of 11 μm for repeated scans of the same eyes (coefficient of variation, 7.5%).[7] Our patients who did not have significant macular disease had a SD of 22 μm, which is probably due to the limited but nevertheless acceptable number of patients in our study. Also, as Antcliff et al.[13] have stated, measurement of intraretinal cystoid space size depends on patient fixation and therefore greater variation in cystoid space size can be expected. In our study, the SD of patients who had significant macular edema on OCT was 242 μm.

Macular edema can be detected by FA. However, for a quantitative evaluation OCT is necessary. In the evaluation of macula when vitreous condensation is present, OCT might be superior to FA. It is showed that OCT is useful in not only identifying epiretinal membranes, persistent CME and juxtafoveolar membranes but also detecting the response to the treatment in uveitis patients. Thus OCT is essential for assessing the quantitative response to treatment in particular[14-15].

Despite the importance of OCT in detecting macular edema, it seems to have some drawbacks. One is the rather small imaging area of OCT. As vasculitis is one of the major features of Behçet's disease uveitis, OCT is inadequate for its assessment. FA is explicitly superior to OCT in detecting leakage and dynamic changes at the vascular system. Moreover, it gives information about the periphery such as the ischemia, leakage, vasculitic appearances and relevant diseases, which are very important in determining the treatment.

In conclusion, we underscore the essence of FA as the primary investigating tool. Moreover, we accentuate the importance of OCT, which is much easier to perform relatively to FA, as an associate device for the assessment of the macula quantitatively. OCT is useful in the evaluation of the macula when vitreous condensation is present. This was a preliminary study to determine the role of OCT in the evaluation of ocular Behçet's disease.

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Cells of the Vitreous Body in Behçet's Disease

A case report

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1. INTRODUCTION

Little is known about the identity of cells accumulating in the anterior chamber and in the vitreous body during uveitis in Behçet's disease. In other entities of idiopathic uveitis T-cells are the prominent cells infiltrating the vitreous body[1,2].

2. PATIENT AND METHODS

2.1 Patient

Vitreous cells were obtained from a 28-year old male patient suffering from Behcet's disease for 4 years. Diagnosis was based on oral and genital aphthosis, arthritis, skin lesions, positive HLA B51, and bilateral panuveitis. Visual acuity of his left eye was reduced to detection of hand movements, that of the right eye varied between 1/20 and 0.2. Treatment with high doses of cyclosporin and steroids resulted in an incomplete remission of the uveitis. Due to dense cellular infiltration of the left eye however, we performed a pars plana vitrectomy. Vitreous cells were collected for further investigation. Thereafter therapy was changed to interferon α whereby visual acuity of the right eye improved to 1.0 and remained at this level.

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2.2 Methods

Vitreous cells were collected during standard three port vitrectomy. Afterwards the cells were centrifuged, fixed with formalin, and embedded in paraffin. Immunohistochemistry was performed using antibodies to T-cells, CD4 and CD8, and neutrophils. Giemsa staining was also used to label the cells. In each section 1,000 cells were counted and the percentage of positive cells were calculated.

3. RESULTS

Giemsa staining and anti-neutrophil cell immunohistochemistry revealed neutrophil granulocytes as the predominent cell type in the vitreous body. Approximately 70% of the cells exhibited positive staining. In contrast, cells positive for T-cell markers were rare. Only about 3.5% of the cells carried the CD8 marker, and less than 0.1 % carried the CD4 marker on their surface.

4. DISCUSSION

Behçet's disease is a rare form of vasculitis with unknown aetiology and unclear pathogenesis. One of the main manifestations is ocular involvement. Typical is an uveitis with vitritis, retinal vasculitis and retinal vascular occlusion. Anterior uveits with hypopypn iritis is rarely observed.

In our Behçet's disease case the prominent cell type in the vitreous body was neutrophils. Some CD8 positive T-cells und only few CD4 positive T-cells were detectable.

Neutrophil infiltration is characteristic of vasculo-Behçet's disease[3,4]. Cellular infiltration, predominantly of neutrophils, has been described around the vasa vasorum. Accumulation of neutrophils was also detected in mucocutaneous lesions of Behçet's disease[5,6]. Neutrophil hyperfunction is thought to be regulated by genetic factors (HLA B51), and by immunological abnormalities in the initiating T-cell response[7]. These findings were in agreement with the observation of recruitment of both T-cells and neutrophils in the conjunctiva after stimulation in biopsies of patients with Behçet's disease[8].

In contrast to other forms of idiopathic uveitis[1,2], T-lymphocytes predominated in the vitreous body, suggesting an active T-lymphocyte-

mediated intraocular inflammation. At different stages of the uveitis a T-cell inducer or suppressor response has been reported.

Similar events seem possible in uveitis of Behçet's disease. In our case, cells found in the vitreous body may reflect the effector response rather than the initial immunological mechanism in Behcet's disease.

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Ocular Lesions Other than Behçet's Disease in Behçet's Disease-Affected Patients

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1. INTRODUCTION

Ocular involvement in Behçet's disease (BD) is usually constituted by the occurrence of a diffuse uveitis with retinal vasculitis[1,2]. A small group of patients (less than 10%) presents only with anterior uveitis. The occurrence of hypopyon, found in 16-30% of the cases, is not necessarily a distinctive feature of the ocular manifestation in BD[3,4] and may be found more frequently in children than in adults[5]. The diagnosis of BD is based on the patient's clinical findings and history. In subjects with the incomplete form of the disease according to the Japanese classification, or in those with mild or atypical presentations, it has been suggested to consider other forms of uveitis in the differential diagnosis, namely HLA-B27 uveitis for anterior uveitis and sarcoidosis for the diffuse one[1]. The aim of this study was to report the occurrence of ocular toxoplasmosis in two Italian patients with BD.

2. CASE REPORTS

2.1 Case 1

We observed a 51-year-old man with a history of oral and genital ulcers, folliculitis, transitory hemiparesis, and positive pathergy test nine years after

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the onset of vitreous haemorrhages and two subsequent episodes of vitreitis and chorioretinitis in RE. He has been treated with systemic and subtenon's steroids. He tested positive for HLA B51 antigen, while a CT and NMR of the brain was negative. A fluorescein angiography disclosed a chorioretinal scar without signs of retinal vasculitis. A diagnosis of BD was made upon the systemic symptoms. No treatment was required at our first observation, neither for systemic nor for ocular symptoms. A relapse of anterior uveitis without retinal involvement was diagnosed 3 years later and treated with topical steroids and mydriatics. During the subsequent six years of follow-up no new ocular lesions occurred. Nevertheless the recurrence of transitory hemiparesis, oral ulcers, arthralgia and the onset of gastrointestinal symptoms (diarrhea, rectorrhagia) suggested the need for immunosuppressive therapy. Therefore cyclosporine A (3 mg/kg/day) was administered for the following six months, and, after 18 months out of immunosuppressive drugs, azathioprine (100 mg/day) and colchicine for one year. One year later the patient developed a white focal retinal lesion, with overlying vitreitis at the borders of the pre-existing chorioretinal scar. The ophthalmoscopic appearance was consistent with a diagnosis of toxoplasmic retinochoroiditis, and the patient tested positive for specific IgG. A course of trimethoprim-sulfamethoxazole therapy (160 mg + 800 mg, respectively, twice daily for 30 days) in combination with systemic steroids (prednisone 25 mg/day for 7 days, then gradually reduced) was administered with a complete resolution of the chorioretinal exudate within 2 months. No recurrence of uveal inflammation was noted in the subsequent 2 years of follow-up.

2.2 Case 2

An 18-year-old man was diagnosed as having exotropia and toxoplasmic macular scar in RE at the age of 3. His mother had tested positive for toxoplasmosis during pregnancy and he presented also a positive specific IgG test. He was followed-up once a year without evidence of active ocular inflammation for 14 years, and was sent to us last year because of the occurrence of an idiopathic macular edema in LE with no associated systemic symptoms. During our follow-up we observed the onset of a diffuse bilateral uveitis with hypopyon, posterior synechiae, diffuse retinal exudates, haemorrhages and vasculitis. When the patient was carefully investigated for systemic diseases, he revealed the onset of oral ulcers at the age of 15, and genital ulcers and symptoms consistent with epididymitis at 17. He was also tested positive for HLA B51 antigen. A diagnosis of BD with bilateral ocular involvement in patient with toxoplasmic retinal scar in RE was made. The patient was first given a course of systemic steroids, but subsequent relapses

of bilateral diffuse uveitis were recorded once steroid therapy was reduced below 20 mg/day of prednisone. He is currently administered cyclosporine A (4 mg/kg/day) and systemic steroids (prednisone 17.5 mg/day) therapy, with a satisfactory control of uveitis and without recurrences of ocular toxoplasmosis.

3. DISCUSSION

The presented cases suggest the possible onset of ocular symptoms unrelated to BD in BD-affected patients. This finding arises both diagnostic and therapeutic trouble. Particularly the first patient did not present initially ocular lesions suggestive for BD, while he was diagnosed as having systemic BD with neurological involvement. In the follow-up we have diagnosed a toxoplasmic infection of the retina with subsequent recurrences. Although his ocular history seems to resemble the usual course of toxoplasmic retinochoroiditis characterized by the occurrence of relapses, we cannot disregard the possibility that immunosuppressive therapy, as it acts strongly on the immune system, may have facilitated the recurrence of toxoplasmic retinochoroiditis. The second patient presented first a diagnostic problem, as he had been already diagnosed as having a toxoplasmic retinal scar when he was 3. At the time of diffuse uveitis onset the possibility for a toxoplasmic ocular relapse was excluded because of the contemporary bilateral involvement, the presence of hypopyon, and after a careful investigation of systemic symptoms which revealed the onset of oral and genital ulcers and epididymitis. The severity of the ocular relapses needed the administration of immunosuppressive therapy. A long-term follow-up will clarify whether this therapy may facilitate a recurrence of congenital ocular toxoplasmosis or not.

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NEUROLOGICAL MANIFESTATIONS

Neuro–Behçet's Disease (Isolated Cerebral Thrombophlebitis Excluded)

Clinical pattern, prognostic factors, treatment and long term follow-up

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1. INTRODUCTION

Behçet's disease (BD) is a chronic relapsing systemic vasculitis which involves vessels of all sizes[1] and spares no organ in the body. Central nervous system (CNS) vasculitis secondary to BD is life-threatening, and may be either non-parenchymal or parenchymal[3]. In non-parenchymal CNS BD, large vein inflammation leads to dural sinus thrombosis (DST) and cranial hypertension[4]. Parenchymal form is due to a small vein inflammatory process and presents with disseminated or focal CNS dysfunction[5]. Clinical course, prognosis and management of these two major forms are different[3,6-8]. DST usually presents with limited symptoms, have a better prognosis and have been sub-classified by some authors within vasculo-Behçet[3]. On the basis of these observations we reviewed parenchymal CNS BD patients who were seen in our institution, excluding patients with isolated DST. We assessed the treatment efficacy, the prognosis, and the predictors of outcome in a large cohort of BD patients, who were followed for up to 33 years.

2. PATIENTS AND METHODS

We performed a retrospective comprehensive follow-up study of all BD patients seen in the Internal Medicine Department at Pitié Salpêtrière

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University Hospital. In this cohort of seven hundred patients with BD, we identified 161 patients who experienced neurological symptoms (NBD) from which we excluded 52 with isolated DST. The study population included a total of 109 consecutive patients who had at least one neurological attack over a 33-year study period from June 1968 to July 2001. For each patient, age, sex, dates of BD diagnosis and NBD attacks (before and after admission) were recorded from the medical history. Clinical profile (at onset and during evolution) were abstracted from the patient's medical record: neurological and systemic symptoms, results of physical examination, and cerebrospinal fluid (CSF) data. Treatment at the time of the initial diagnosis, at neurological relapse, its duration and the dates of its interruption were noted. Neuro-imaging was based on CT scan and/or cerebral angiography before 1986, and on MRI afterwards. Follow-up included review of each inpatient and outpatient medical records, therapeutic observance, neurological relapse and extra-neurological attacks, CSF data, imaging findings and causes of death. All patients fulfilled international criteria of BD[9] and had at least neurological examination abnormalities, and/or aseptic inflammation of CSF, and/or neuro-imaging abnormalities compatible with NBD. The number of neurological attacks, the achievement of complete remission, and the incidence of residual neurological disability have been assessed in relation to the initial clinical features, administration of immunosuppressive drugs, and therapeutic observance.

2.1 Statistical analysis

Data analysis was performed on SAS software. Means and standard deviation, or medians and extremes, are given for continuous variables. A chi-square test or a Fisher'exact test when required, have been used to compare dichotomous variables. An analysis of variance or a Wilcoxon rank sum test have been used for continuous variables. Odds ratio with 95% confidence intervals (95% CI) where computed. Significance level was set up at 0.05.

3. RESULTS

A total of one hundred-nine BD patients with neurological involvement (not related to isolated cerebral thrombophlebitis) were studied. There were seventy eight males and thirty-one females (M:F = 2.5) with a mean age at BD diagnosis respectively 34 years (SD 11 years) and 32 years (SD 8 years). Forty one were French, 8 of whom were from the West Indies, ten were from other European countries (Portugal, Spain, Italy, Yugoslavia), forty five

were born in Maghreb, eight in Middle east, five in Africa, and two in Far East Asia.

The first NBD attack occurred before the diagnosis of BD in 60 patients, concomitantly in 8, and after the BD diagnosis in 41. The mean age at NBD onset was 32 ± 13 years for males and 31 ± 10 years for females. The median of diagnosis delay was 14.5 months.

3.1 Systemic symptoms

Systemic symptoms were common. All cases had mouth aphthosis, 84 cases had genital ulcers and 70 cases (64%) had uveitis. Pseudofoliculitis was present in 60 cases, erythema nodosum in 24 (22%), pathergy test was positive in 45 (41%), peripheral thrombophlebitis in 41 cases (37.6%), extracranial arteries in 11 (10%) and arthralgia or arthritis in 69 (63%).

3.2 Clinical profile of neurological BD (isolated DST excluded)

The main features were: headache in 67 patients (75.8%), isolated for 6 cases; pyramidal syndrome in 61 cases (56%), out of whom: hemiparesis or hemiplegia: 39 cases (35.7%), paraplegia: 6 cases; sphincter disturbance: 15 (14%); consciousness disturbance: 30 cases (27.5%), out of whom 4 coma; cerebellar syndrome: 37 cases (34%); pseudobulbar palsy: 8 cases (7%); diplopia: 34 (31%); psychiatric symptoms: 28 cases (25.5%) with 7 dementia; meningoencephalitis: 49 cases (45%) with 37 rhombencephalitis (34%); cochleo-vestibular syndrome: 21 (19%); sensory disturbance: 14 (12%); seizure: 12 (11%); optic neuropathy: 10 (9%); and peripheral neuropathy: 3.

3.3 Cerebro-spinal fluid

Cerebro-spinal fluid was active in 73 patients out of 86 (85%): 66% showed lymphocytic predominance, whereas 34% had either neutrophilic predominance or both neutrophils and lymphocytes.

3.4 Neuro-imaging findings

Cerebral CT scan was abnormal for 16 patients of 28 (57%). MRI was normal for 11 patients (seizure: 2; isolated meningitis: 2; peripheral cranial neuropathy: 5; and isolated diplopia: 2), and abnormal for 67 patients of 78 with central involvement (86%). The brainstem was involved in 42 patients

(pons: 26; midbrain: 23; bulb: 8; and brainstem atrophy. 3), internal capsule in 20 cases, cerebral white matter in 19 and basal ganglia in 17 (thalamus: 12). Two patients presented with cerebellar atrophy and 3 with spinal cord involvement (isolated in 2).

3.5 Clinical follow-up

The median follow-up after the first NB attack was 97 months. Seventy-seven cases (70.6%) had at least one neurological relapse, 70 of them had neurological relapses before admission. Fifty patients recovered well (45.8%), 30 patients were better (27.6%), 12 patients remained stable (11%) and 6 (5.5%) had secondary progressive course. Forty-eight patients (44%) had neurological sequelae, 6 of which were severe (12.5%), 16 were important (33%), 11 were moderate (23%), and 15 were mild (31%). Fifty seven patients were dependant at admission (52%) versus 18 (19%) after admission (at the end of this study). All patients with important to severe sequelae were dependant physically and/or mentally at admission. Eleven patients died: five in bedridden invalid state secondary to NBD (29 - 56 year-old), one with intra-cranial hemorrhage (32 year-old), one with sudden death (34 year-old), 2 with cancers (pulmonary: 61 year-old and metastatic: 46 year-old), one with vascular complication (aortic arch aneurysm: 41 year-old) and one in post operative neurosurgery for meningioma.

3.6 Treatment and prognostic factors

All patients received colchicine after admission, if they had not before. One hundred and three patients received corticosteroids and 6 did not (3 of them had little-active isolated meningitis and one peripheral sensitive axonal neuropathy). Eighty-four patients received immunomodulatory drugs (50 cases received azathioprine, 45 intravenous cyclophosphamide, 12 oral cyclophosphamide, 12 chloraminophene, 6 methotrexate, 7 cyclosporine A, 3 plasma exchange and 1 interferon). Patients who had already advanced fixed neurological disability did not receive immunosupressant.

Neurological disability was associated to brainstem lesion ($p = 0.001$, OR = 5.7, 95% IC = 1.89-17.41), internal capsule lesion ($p = 0.024$, OR = 4.10, 95% IC = 1.15-14.64), and rhombencephalitis ($p = 0.002$, OR = 6.2, 95% IC = 1.9-19.9), but not to basal ganglia involvement. The neurological attacks incidence per year was lower for patients with immunosuppressive agents ($p = 0.0001$). Continuous corticosteroid treatment, on long term basis, was associated with less disability ($p = 0.006$, OR = 4.53, 95% IC = 1.5-13.5). Complete remission achievement was significantly associated with maintained oral corticosteroids ($p = 0.003$, OR = 4.9, 95% CI = 1.7-14.1)

and colchicine on a long term basis (p = 0.022, OR = 3.5, 95% CI = 1.2-10.5). Therapeutic observance (continuous versus interrupted treatment) was significantly related to complete remission (65.7% versus 36.7%, p = 0.01) and less neurological sequelae (40.5% versus 65.5%, p = 0.044).

4. DISCUSSION

In this series of neurological involvement (isolated cerebral thrombophlebitis excluded) in BD masculine predominance, previously underlined[3,6-8], was found. The frequency of parenchymal involvement was 18%. More than 60% of patients had their first NBD attack before diagnosis with a median delay of 14.5 months. Extra-neurological signs were common, but might appear after the onset of NBD. Five patients had oral aphtosis after the first NBD attack (1 to 26 months), which can make initial diagnosis difficult.

Meningoencephalitis was seen in 45% of cases and brain stem involvement in 38.6%. Hemispheres were involved in 17.4% of cases and basal ganglia in 15.6%. Most patients had active CSF (85%) with neutrophilic predominance or mixed neutrophils and lymphocytes pleocytosis in 34%.

MRI was the most sensitive neuro-imaging as underlined previously[10], but in some cases differential diagnosis may include other diseases of CNS. Five patients in our cohort study were initially admitted to the Neurosurgery Department: one for brain stem "glioma", one for brain stem "arterio-venous" malformation, one for "tumour" of midbrain-diencephalon, one for brain stem "abscess" which had been treated for one year by antibiotics before admission, and one underwent surgery for "optic chiasm tumour" and had frontal lobectomy for delirium related to BD. When white matter lesions are isolated, MRI may not discriminate from multiple sclerosis, but systemic symptoms and CSF data (neutrophilic meningitis) may be helpful.

In this large series we found one patient who developed DST 6 years after brain stem involvement, and 2 patients who had brain stem involvement respectively 6 years and 12 years after DST. To our knowledge, this association between parenchymal neurological involvement and DST has never been reported before[6-8]. These neurological complications had taken place in the 3 patients after untimely interruption of corticosteroids.

Continuous oral corticosteroids and colchicine on long term basis were the main prognostic factor for recovery following NBD relapse. Adverse prognostic factors with neurological sequelae were brain stem lesions, rhombencephalitis, and interrupted corticosteroids and/or colchicine. Of particular interest is the double-blind trial which demonstrated that colchicine reduced mucocutaneous and arthritic attacks[11].

Immunosupressants were significantly associated with a lower incidence of NBD attacks per year. 64% experienced at least one NBD relapse before admission versus 21% after admission (most of them with poor therapeutic observance). All patients with important to severe neurological sequelae (21%) were physically and / or mentally dependent at admission.

Summarising, more than half first NBD attacks occurred before diagnosis. Lesions were more commonly localized within brain stem, and chronic parenchymal NBD may result in brain stem and / or cerebellar atrophy. CSF was very often active, MRI the best imaging method, and parenchymal NBD may be rarely associated with DST. Parenchymal NBD may result in severe disability (physically and / or mentally) and may be life-threatening.

Brain stem, internal capsule involvement, rhombencephalitis, interruption of corticosteroids, and / or colchicine are adverse prognostic factors. Good therapeutic observance is associated with better clinical course, decreased neurological sequelae, and complete recovery if parenchymal involvement was not advanced and fixed at admission and the beginning of treatment.

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The Prevalence of Headache in Behçet's Syndrome

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1. INTRODUCTION

Neurological complications in Behçet's syndrome are rare; the most frequent manifestation is a subacute brainstem lesion causing ophthalmoparesis and ataxia due to the development of inflammation. Hemisphere lesions, spinal cord involvement, optic neuropathy and other neurological syndromes occur even more rarely[1].

Headache is a frequent accompaniment of meningoencephalitis and meningitis, in which escalating throbbing generalised headache occurs and worsens over several days, with or without other neurological symptoms such as photophobia and neck stiffness, vomiting and focal neurological signs. Many patients, however, complain of very frequent and recurrent headaches in the absence of associated neurological syndromes; indeed the neurological examination is normal, and MRI scans and spinal fluid examinations yield often negative results.

The purpose of this study was to define the prevalence of recurrent headache in a population of patients with Behçet's syndrome and to characterise the clinical features and severity of the headache disorder.

2. METHODS

Members of the Behçet's syndrome Society of the United Kingdom were approached and invited to fill out a questionnaire in which they were asked to note whether or not they had ever suffered a series of neurological symptoms, including headache. A further questionnaire was sent to those

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who had responded which asked them to note the characteristics of the headache, its frequency and duration, the presence of additional symptoms such as photophobia and vomiting, and whether or not there was an aura. The treatment which the patient chose to use, and its usual effect, was also noted, and a severity score, validated in migraine, the MIDAS instrument[2], was used to assess severity.

3. RESULTS

327 members returned the initial questionnaire; of these 270 (82.5%) had noted headache as a recurrent symptom. 223 returned the second questionnaire, of whom 201 (90.0%) had noted headache (Table 1).

Table 1. Characteristics and severity of the headache syndromes

Number of patients with headache	201 (90%)
Median (range) frequency of headache per month	5 (0 – 84)
Median (range) maximum duration of headache (days)	1.5 (0 – 12)
Number of patients with vascular type headaches	197 (98%)
Number of patients with visual aura	106 (52.7%)
Number of patients with sensory aura	70 (34.8%)
Number of patients with muscle tension type headaches	4 (2%)
Median (range) MIDAS score	16 (0 – 255)

4. DISCUSSION

This population of patients with Behçet's syndrome shows a very high prevalence of severe and recurrent headaches. The majority of patients displayed associated symptoms in keeping with migraine; the presence of visual and/or sensory aura, the throbbing nature of the headache, the unilateral onset and frontal situation are all highly associated with migraine.

The prevalence of migraine in the general population is 8–14%[3]. The prevalence of visual and sensory aurae is 2-30%. Thus headache in this population is hugely over-represented. Now clearly it could be argued that there is a selection bias in that only patients reporting headaches responded, but the prevalence of headache in the initial questionnaire, which did not deal specifically with headache, was still extremely high at 80%.

Headache in Behçet's syndrome, like migraine in general, is poorly treated; patients often resort to OTC medications, particularly codeine-

containing compounds which often transform recurrent migraine into the syndrome known as chronic daily headache, and medication itself can cause so-called rebound headaches (Table 2).

Table 2. Usual treatment for headache

Paracetamol	48 (23.9%)
Codeine/opiates	75 (37.3%)
NSAID/aspirin	46 (22.9%)
5HT agonist	11 (5.5%)
Migraine prophylaxis	3 (1.5%)
Nil	10 (4.9%)

Why vascular type headache is so common in Behçet's syndrome is not clear; there is no evidence that it is in any way related to neurological complications because MRI scans are usually quite normal[4]. It is, however, the author's experience that vascular headache in Behçet's syndrome responds as well to anti-migraine drugs as does the general population with similar headaches. A treatment trial to investigate this further is planned.

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Adamantiades-Behcet's Disease and Elevated Intracranial Pressure in a 12 Year Old Turkish Girl

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1. INTRODUCTION

Adamantiades-Behçet's disease (ABD) is a multisystemic inflammatory disorder of unknown origin. Diagnostic criteria are established[1]. The disease is rare in childhood, one of the largest series described 86 cases[2]. Neurological involvement has been described in adults, reports in childhood are rare. We describe a case with pseudotumor cerebri in ABD.

2. PATIENT AND CLINICAL COURSE

Since the age of three the Turkish girl suffered from recurrent oral ulcers. In these situations she refused to eat. A viral infections were diagnosed. Six years later she developed genital ulcerations. With 12 years she fell ill with severe headache and double vision.

One grandmother was reported having recurrent ulcerations.

Fundoscopy showed a prominent papilla and retinal bleeding, there was no uveitis. Angio-CT and MRI scans were normal. There was no parenchymal lesions and no evidence of sinus thrombosis. Lumbar puncture was performed with normal cell count and protein, but massive elevated pressure up to 50 cm H_2O. Perimetric examination was normal. Skin pathergy test was negative.

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We diagnosed idiopathic elevated intracranial pressure in ABD disease. Treatment was started with steroids and acetazolamide, followed by colchicine. By time headache disappeared and fundoscopy turned to normal.

3. DISCUSSION

Neurological involvement is described in ABD. The central nervous manifestations are parenchymal or non-parenchymal[3,4]. The clinical symptoms therefore can be[5]:

- Encephalomyelitis
- Aseptic meningitis
- Organic psychiatric disturbances
- Benign intracranial hypertension.

In a study with 200 patients 162 had parenchymal involvement including brainstem and spinal cord involvement[3]. Sometimes the lesions were similar to multiple sclerosis. The other 38 patients mostly had raised intracranial pressure due to dural sinus thrombosis.

We found elevated pressure without any abnormality. It is necessary to perform MRI and/or CT scans to visualise parenchymal structures and blood vessels. In this disease the manifestations of symptoms are widespread and neurological manifestation has to be considered as a possible clinical sign.

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Sensory Symptoms in Behçet's Syndrome

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1. INTRODUCTION

Behçet's disease is a heterogeneous, multisystem inflammatory disorder with vasculitis being the major pathological feature. Central nervous system involvement is common occurring in about 10% of the patients. In contrast, peripheral nervous system involvement is rare. Both may have an impact on the quality of life. Hemiparesis and brain stem signs such as ocular and bulbar paresis are frequent manifestations of the central nervous system involvement. Mental behavioral changes are also common in patients with Neuro-Behçet's disease (n-BD)[1]. Recurrent oral and genital ulcerations and uveitis usually precede the neurological symptoms for 4 to 6 years[2]. The pathological changes in n-BD are widespread. Focal softening of neural tissues, microglial proliferation, glial scarring, and perivenous infiltration are the predominant features[3].

2. CASE REPORT

In the following the case of a 34-year-old male patient with Behçet's disease who suffered painful paraesthesia is described. The sensory symptoms occurred 13 years after the onset of Behçet's disease which had been marked by recurrent uveitis of both eyes, oral and genital aphthosis and gonalgia. Generalised cerebral seizures and memory dysfunction were seen 2 years after disease onset. MRI scan revealed multiple white matter lesions of both hemispheres especially at frontal regions. The patient received orally

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chlorambucil at daily doses of 10 mg for 3 and a half years. All symptoms completely resolved soon after drug treatment had been started and the patient remained free of any neurological signs up to 6 years after medication had been stopped. Subsequently he suffered painful paraesthesia in the right V trigeminal nerve. The symptom spontaneously resolved after 4 months. Painful paraesthesia re-occurred in the right upper limb and left and right lower limb and lasted for another 8 months. Neurological examination, routine laboratory analyses, assessment of nerve conduction time and cranial MRI imaging did not reveal any pathological changes.

3. DISCUSSION

In the respective literature various types of peripheral nervous system involvement such as neuropathy, mononeuritis muliplex, and radiculitis have been reported in patients with Behçet's disease[4]. In two cases vasculitis has been demonstrated by biopsy[5]. Concomitant CNS and peripheral nervous involvement have been reported[6]. In our patient peripheral nervous system involvement was noted 6 years after complete resolution of central nervous system involvement. The sensory disturbances were asymmetrical. Careful neurological examination and assessment of nerve conduction time gave no hint of polyneuropathy or radiculitis which may favour the diagnosis of mononeuritis multiplex.

It can be concluded that sensory symptoms indicate subacute episodes of neurological dysfunction in patients with Behçet's syndrome which may reflect a low-grade background inflammation throughout the whole nervous system.

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Immunohistological Studies in Neuro-Behçet's Disease

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1. INTRODUCTION

Central nervous system (CNS) involvement in Behçet's disease, usually called neuro-Behçet's syndrome (NB), is one of the most serious complications of the disease[1]. In the present study, we carried out immunohistological examination of the brain tissue from a patient with NB.

2. PATIENT PROFILE

A 54-year-old Japanese man suffered from recurrent aphthous stomatitis and pyoderma since January 1989. In 1989, on November 20, he suddenly developed convulsion with subsequent left homonymous hemianopsia. CAT scan and cerebral angiography disclosed hypovascular mass lesion in the right occipital lobe, which reduced in size by treatment with oral corticosteroids. In 1990, he had a series of convulsions. He was admitted to the Department of Neurosurgery, Juntendo University. A diagnosis of brain tumor was strongly suspected and an open biopsy was performed on April 11. After the operation, he developed genital ulcers. He was transferred to our hospital on July 2, 1991, for further evaluation and treatment. At admission, he was taking oral prednisolone 15 mg/day. Neurological examination revealed the left homonymous hemianopsia, the left pyramidal

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tract sign, and emotional incontinence. A diagnosis of incomplete type of BD was made according to the Japanese diagnostic criteria of 1987[2].

3. RESULTS

Histopathology of the mass lesion revealed the infiltration of mononuclear cells along the arteries and veins, and of mononuclear cells and granulocytes in the parenchyma, confirming the diagnosis of NB. Immunohistological examination revealed that the infiltrated mononuclear cells consisted of mostly CD45RO+ T lymphocytes with a few CD 19+ B lymphocytes. Of further interest is the fact that tunnel staining disclosed that most neurons were undergoing apoptosis in the inflammatory lesion (Fig. 1).

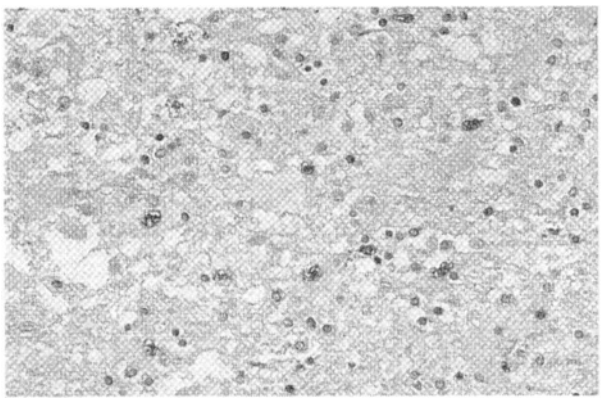

Figure 1. TUNNEL staining disclosed that most neurons are undergoing apoptosis

4. CONCLUSION

These results indicate that the infiltration of T lymphocytes and the induction of apoptosis of neurons play a pivotal role in the pathogenesis of NB. It is suggested that proinflammatory cytokines might be involved in the induction of apoptosis of neurons.

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Soluble Fas Ligand Levels in Cerebrospinal Fluid in Neuro-Behçet's Disease

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1. INTRODUCTION

Fas and Fas ligand (Fas-L) play pivotal roles in immune self-tolerance[1]. Recently, several abnormalities of Fas-mediated apoptosis have been reported in patients with Behçet's disease (BD). Fas-L, a member of the TNF family, induces apoptosis of Fas-bearing cells. To clarify the role of Fas-mediated apoptosis in the central nervous system (CNS) involvement in BD, we measured soluble Fas-L (sFas-L) concentration in cerebrospinal fluid (CSF) with neuro-BD (n-BD) using ELISA. In this study, sFas-L in CSF of BD was compared with those of disease controls (multiple sclerosis, CNS lupus etc.)[2]. Also, we measured sFas-L levels of CSF in 2 cases with n-BD in the active and inactive stage.

2. MATERIALS AND METHODS

We measured sFas-L concentration in CSF with n-BD (n=ll), CNS lupus (n=11), rheumatoid arthritis (n=4), multiple sclerosis (n=3) and Sweet's disease (n=3) by ELISA (Soluble Fas/Fas Ligand ELISA System, Mochida Pharmaceutical). Furthermore, we measured the levels of sFas-L, cell numbers and protein levels of CSF in 2 cases with n-BD in the active and inactive stage.

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3. RESULTS

Levels of CSF sFas-L in n-BD, CNS lupus, RA, MS and Sweet's disease are shown in Fig. 1. We measured sFas-L of CSF in 2 cases with n-BD in the active and inactive stage. In the first case of n-BD, the CSF levels of sFas-L was 71.5 pg/ml in the active stage and by the course of time, it was decreased to 15.1 pg/ml. In the second case of n-BD, the CSF levels of sFas-L was 15.1 pg/ml in the inactive stage and by the course of time, it was increased to 104.3 pg/ml. (Table 1).

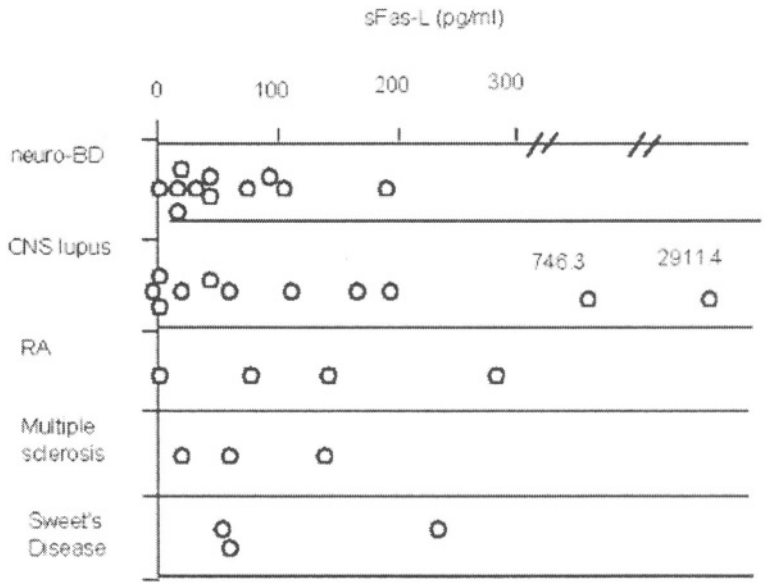

Figure 1. CSF levels of sFas-L in BD patients with neurological manifestations

4. CONCLUSION

To clarify the role of sFas-L in BD, we measured the CSF levels of sFas-L. The levels of sFas-L were increased in patients with n-BD and the levels of sFas-L in patients with CNS lupus were significantly higher than those in other patients. sFas-L levels are elevated in the active stage and sFas-L may play an important role in the pathogenesis of CNS involvement in BD.

Table 1. CSF levels of sFas-L in neurological manifestations of BD in the active and inactive stage

Case 1: I.Y., male					
Date	CSF			Stage	
	sFas-L (pg/ml)	Cell number	Protein		
12/16	71.5	93	61	active	12/16-18 steroid pulse
12/21	15.1	40	51	inactive	
12/29	41.3	36	47	inactive	

Case 2: T.T. female					
Date	CSF			Stage	
	sFas-L (pg/ml)	Cell number	Protein		
4/27	15.1	28	100	inactive	4/16-18 steroid pulse
5/13	41.4	16	175	active	
6/17	104.3	33		active	

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CARDIOVASCULAR INVOLVEMENT

Behçet's Disease with Vascular Involvement: The Contribution of Anticardiolipin Antibodies and Thrombomodulin

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1. PURPOSE

Behçet's disease (BD) has various types of vascular involvement, such as arteritis and venous thrombosis[1]. Antiphospholipid antibody syndrome (APS) has also many vascular events and is often accompanied with collagen diseases[2,3]. Some report indicated that patients with BD also present antiphospholipid antibodies[4-8].Therefore, we examined the presence of antiphospholipid antibodies in patients with BD.

2. PATIENTS AND METHODS

Patients were included by clinical records between 1998 and 2002 at our division. All patients met with international criteria of BD. Lupus anticoagulant (LA) assay was performed by Russell's viper venom time (dRVVT) and dilute APTT (dAPTT). Anticardiolipin antibodies (aCl-beta2-GP I) were assayed with specific EIA for beta2-glycoprotein I (Yamasa kit, Tokyo, Japan). "Vascular involvement" included deep venous thrombosis, subcutaneous thrombophlebitis, arteritis, and various types of venous occlusion (Table 1).

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Table 1. Background of BD with or without vascular involvement

		with vascular involvement (n=15)	without vascular involvement (n=49)	significance p value
Sex	male	80.0% (12)	38.8% (19)	NS
	female	20.0% (3)	61.2% (30)	NS
Age, years		52.5±22.5	44.6±30.6	NS
Complications	venous	73.3% (11)		
	arterial	26.7% (4)		
Major symptoms	oral aphthae	100.0% (15)	100% (49)	NS
	ocular symptom	13.3% (2)	65.3% (32)	p <0.01
	cutaneous lesions	100.0% (15)	77.6% (38)	NS
	genital ulcer	73.3% (11)	75.4% (37)	NS
	arthritis	46.7% (7)	53.0% (26)	NS

3. RESULTS

Fifteen out of 64 patients with BD (23.4%) had vascular involvement of any kind. Only two (13.3%) had LA, and one patient (6.6%) had both LA and aCl ß2-GP I Ab. All three had various forms of vascular involvement. One patient with both LA and aCl ß2-GP I positive (patient no. 13) was considered the unique clinical manifestation, as shown in Table 2. There was no significant difference in the frequency of aCl ß2-GP I Ab among BD patients irrespectively of vascular involvement. LA was detected significantly higher in BD with vascular involvement by Fisher's exact probability test ($p < 0.05$), but the positive numbers were still low as shown in Table 3. On the other hand, 14 patients (28.5%) had exceeded levels of soluble thrombomodulin of active BD phase. Soluble thrombomodulin levels were significantly higher in BD with vascular involvement compared to group without vascular involvement ($p < 0.05$) as shown in Table 4.

4. CONCLUSION

Vascular involvement in BD is not likely to be associated with aCl ß2-GP I Ab. LA may be correlated with vascular involvement in BD but the positive numbers are still low. In contrast, soluble thrombomodulin may be one of the important markers of BD with vascular involvement. Disease control, such as SLE with vascular involvement will need to clarify the mechanism of BD's vascular complications.

Table 2. Summary of clinical features in 15 BD patients with vascular involvement

Patient	Age	Gender	Clinical features	LA	Anti-ß2-glycoprotein Ab
1	30	M	Deep venous thrombosis	P	N
2	64	F	Subcutaneous thrombophlebitis	N	N
3	54	M	Deep venous thrombosis	N	N
4	57	M	Aneurysm of the aortic sinus of Valsalva	N	N
5	70	M	Aneurysm of anterior tibial artery	N	N
6	69	M	Subcutaneous thrombophlebitis	N	N
7	55	M	Subcutaneous thrombophlebitis	N	N
8	62	M	Subcutaneous thrombophlebitis	N	N
9	44	M	Deep venous thrombosis	P	N
10	48	F	Subcutaneous thrombophlebitis	N	N
11	32	M	Budd-Chiari syndrome	N	N
12	58	M	SVC occlusion	N	N
13	48	M	Deep venous thrombosis	P	P
14	39	M	Subcutaneous thrombophlebitis	N	N
15	58	F	Aortitis	N	N

P = present, N = not present

Table 3. Correlation of LA and Anti-ß2-GP Ab in BD patients with and without vascular involvement

	with vascular involvement (n=15)	without vascular involvement (n=49)	Significance
Lupus anticoagulant (%)	20.0%(3/15)	2.0%(1/49)	$p < 0.05$*
Anti-ß2-glycoprotein Ab (%)	6.7%(1)	2.0%(1/49)	NS

NS = not significant, * Fisher's exact probability test

Table 4. Thrombomodulin (TM) levels in BD disease patients with and without vascular involvement

TM	with vascular involvement (n=7)	without vascular involvement (n=42)
	Mean (±SD)	
Values (ng/mL±SD)	42.01±35.12*	18.45±8.96

* P = 0.05, All values of TM were assayed in active phase of BD

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Arterial Wall Characteristics in Patients with Adamantiades-Behçet's Disease

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1. INTRODUCTION

Adamantiades-Behçet's disease (ABD) is a chronic, relapsing, multisystemic inflammatory disease with vascular involvement in approximately one third of the patients[1].Manifestations may include arterial aneurysm formation, arterial or venous occlusive disease, and varices. The pathophysiological mechanisms underlying vascular complications in ABD are not well understood. Vasculitis, affecting the vessel wall and perivascular tissues, has been proposed as the predominant cause[2,3] .The impact of ABD on arterial wall characteristics such as arterial stiffness and wall thickness has not been studied yet. Aim of the present study was to examine both functional and structural properties of peripheral arteries in patients with active and inactive stage of ABD attempting to elucidate more of the pathophysiological mechanisms underlying vascular complications.

2. METHODS

In order to investigate the characteristics of the arterial wall in ABD we examined 48 patients (age: 38.9±11 years, male: 12) who fulfiled the inclusion criteria[5]. All patients had history of recurrent oral aphthae, 34 had

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genital ulcer, 22 had positive pathergy test, 32 had skin lesions, 30 had eye lesions, 28 had arthritis, 6 had vascular complications (deep vein thrombosis), and 14 had neurological disease. No sign of active vascular disease was present at any patients. Twelve patients had active ABD defined as having at least two symptoms from international study group criteria of ABD during the time of vascular tests. Patients with ABD were compared to 18 control subjects of similar age. Sex, severe smoking, hypertension, hyperlipidemia, and diabetes mellitus, were comparable within two groups.

Endothelial vasomotor function was studied by means of brachial artery flow-mediated dilation (FMD)[6]. A high resolution ultrasonic vessel wall tracking system (7.0 MHz, Accuson 128XP/10, Mountain View, CA) was used. In brief, brachial artery was scanned longitudinally and diameter was measured at rest. A pneumatic cuff was placed at the level of the wrist and inflated to suprasystolic pressure for 4 min. Sixty seconds after cuff deflation, during reactive hyperemia, a second scan was performed and brachial artery diameter was measured. Nitroglycerin was then administered (400 μg subl.) and after 4 min a third scan and measurement of brachial artery diameter was taken. Brachial artery dilation during reactive hyperemia, as expressed by percentage increase from baseline, is used as an index of endothelial vasomotor function. Arterial dilation is induced by increased shear stress which causes production of endothelium-derived vasodilating factors; mainly nitric oxide (NO)[7]. Nitrate-induced dilation (NID) is used as an index of endothelium-independent vasodilatation.

Radial artery tonometry and pulse wave analysis (PWA) technique, using Sphygmocor apparatus (PWV Medical, Sidney, Australia)[8] were performed for estimation of arterial stiffness. Being compared to invasive methods, this method estimates non-invasively and successfully the central pressure wave at the aorta by the use of mathematical transformation of radial pressure wave[9]. Antergrade arterial pressure waves are reflected back from the periphery, arriving in the central arteries normally after the central peak systolic pressure. As arteries stiffen profound changes occur in the arterial pressure waveform. Pulse wave velocity increases, and this results in the reflected wave arriving earlier, thus adding to the central pressure wave to produce an augmented central systolic pressure. Computerized PWA of the central waveform obtained by Sphygmocor allows the determination of central systolic blood pressure augmentation. Augmentation of central arterial, expressed as augmentation index (AI), serves as an indirect index of arterial stiffness.

Assessment of intima-media thickness (IMT) of peripheral arteries has evolved as a promising technique for non-invasive evaluation of atherosclerosis. IMT of the carotid artery has been shown to be related to the extent of coronary atherosclerosis and it seems to have prognostic value for

cardiovascular events[10,11]. B-mode ultra-sonographic examination was performed using a 7.0 MHz linear array transducer (Accuson 128XP/10, Mountain View, California). Carotid IMT measurement was taken from the far wall at two different sites: at the distal one cm of the common carotid artery proximal to the bifurcation (IMTcc) and at the carotid bulb (IMTcb).

All variables are expressed as mean ± SD. SPSS (10.0 version) was used for statistical analysis. T-test was applied in order to evaluate differences in demographic traits, FMD, NID, IMT, and AI among patients and control subjects. ROC analysis was applied for the evaluation of sensitivity and specificity of AI. A $p<0.05$ was considered the level of statistical significance.

3. RESULTS

FMD in patients with ABD was significantly lower compared to control subjects (3.9±3.8% vs 6.6±2.7%, p=0.01) indicating abnormal endothelial function in ABD patients. NID was similar between the two groups (14.3±6.5% vs 17.4±6.7%, ns) indicating normal smooth muscle vasodilating response to exogenous NO. IMT at the common carotid artery and at the carotid bulb was similar in both groups (IMTcc: 0.59±0.11 mm vs 0.55±0.09 mm, ns, IMTcb: 0.71±0.16 mm vs 0.72±0.22 mm, ns), and below 1 mm which is considered to be the upper normal limit. Arterial stiffness as assessed by AI was similar in ABD patients and control subjects (123.9±21% vs 123.8±34%, ns).

Patients with active ABD (n=12) had comparable FMD (active: 4.9±4.5% vs inactive: 3.6±3.6%, ns) when compared to those patients with inactive ABD (n=36). Mean value of FMD in both groups was below 5%, (far below normal value in our laboratory for FMD[13]) indicating abnormal endothelial function. Difference of FMD in patients with active ABD when compared to control group did not reach statistical significance (4.9±4.5% vs 6.6±2.7%, p=0.1) probably due to the low number of patients; patients with inactive ABD had significantly lower FMD compared to control subjects (3.6±3.6% vs 6.6±2.7%, p=0.01). NID was similar with both groups (active: 16.3±4.3% vs inactive: 13.6±3.6%, ns). IMT in common carotid and carotid bulb was similar in group with active ABD when compared to group with inactive ABD (IMTcc: 0.57±0.05 mm vs 0.59±0.1 mm, ns, IMTcb: 0.57±0.08 mm vs 0.60±0.1 mm, ns). Arterial stiffness as assessed by AI, was significantly lower in the group of active ABD compared to inactive group (112.15±8% vs 129.0±21%, p=0.03). In ROC analysis AI less than 111% had 80% sensitivity and 67% specificity for detecting active disease.

In the subgroup of patients with history of vascular complications (n=6) no differences were found in FMD (4.8±4.5% vs 3.8±3.3%, ns), NID (14.8±7.4% vs 14.0±6.1%, ns), IMTcc (0.57±0.1 mm vs 0.59±0.1 mm, ns), IMTcb (0.62±0.1 mm vs 0.57±0.09 mm, ns) or AI (121.4±23% vs 125.9±24%, ns), when compared to patients without vascular complications.

4. DISCUSSION

The role of vascular endothelium in vascular complications in ABD has been examined during the past decade with indirect methods and inconsistant results[18,19]. Since 1992[6], brachial artery flow-mediated dilation test, a simple reproducible and noninvasive tecnhique, has been widely used in order to study directly endothelial vasomotor function in patients at high risk for vascular disease. Increased oxidative stress seems to be one of the main underlying causes for endothelial vasomotor dysfunction in patients with risk factors for coronary artery disease[17]. Furthermore, endothelium-dependent dilation was found impaired in patients with systemic sclerosis and Raynaud's phenomenon[13]. Recently, endothelial vasomotor function has been reported to be impaired in patients with active ABD[4]. Chambers et al.[4] found in their study that endothelial dysfunction was reversed after intravenous infusion of ascorbic acid, which is the most potent antioxidant in human plasma. Thus, it seems reasonable that endothelial dysfunction is a result of increased oxidative stress in patients with ABD. This observation is consistent with previous studies in which reduced plasma concentration of nitrates and nitrites - the main metabolites of NO - were found in active ABD[12]. However, it should be mentioned that other authors found increased NO metabolite's levels in active ABD[15] and proposed it as a potential marker of the activity of the disease[14]. Inducible NO synthase, but not endothelial NO synthase, can produce substantial amounts of NO when expressed during inflammatory processes; in such concentrations NO antagonizes superoxide dismutase and becomes highly reactive with superoxide radicals to form peroxynitrite which has a quite harmful direct toxic effect on tissues[16].

The study of Chambers et al.[4] raised the question whether brachial artery flow-mediated dilation test might recognize patients with increased susceptibility to future vascular complications. In our study, patients with ABD had low FMD independently of the activity or inactivity of the disease at the time of vascular tests. Therefore, it does not seem possible that endothelial vasomotor dysfunction could be used as a marker of the activity of the disease. Furthermore, no difference in FMD was found in patients with a positive history of vascular complications when compared to those

patients without vascular complications. In our study group only six patients had a history of vascular complication and none had peripheral arterial disease (all had deep venous thrombosis). So, it is not safe yet to conclude whether brachial artery flow-mediated dilation test has any value in predicting present or future vascular complications.

Arterial complications in patients with ABD have been considered to occur mainly due to vasculitis leading to aneurysm formation at the aorta or other large vessels; acute myocardial infraction and Takayasu's-like pulseless disease can occur due to vasculitis but no coronary artery disease has ever been reported[1]. IMT is a well established index of extracoronary atherosclerosis in patients with risk factors or coronary artery disease[10,11] and increased values have been observed in other diseases, such as systemic sclerosis and Raynaud's phenomenon[13]. Our study is the only one in which IMT was estimated in patients with ABD; it shows no sign of peripheral artery atherosclerosis in these patients, since IMT is within normal levels.

Arterial stiffness as assessed by radial applanation tonometry and AI, shows no difference between patients with ABD and control group. Surprisingly, it seems that patients with active ABD had lower AI than patients with inactive ABD. Furthermore, AI value lower than 111% could detect activity of the disease. These findings indicate abnormal arterial wall characteristics and reduced arterial stiffness in patients with active ABD, probably due to subclinical vasculitis, since no sign of clinical arterial vascular disease was present at any patient.

5. CONCLUSIONS

Endothelial vasomotor dysfunction was observed in patients with ABD in both active and inactive stages of the disease, thus brachial artery flow-mediated dilation test seems unable to detect activity of the disease. No sign of peripheral atherosclerosis as assessed by IMT was observed in our study group. Arterial stiffness was reduced in patients with active disease, and AI could be useful in the future for detecting ABD activity; more studies are needed on this issue. Our study is being continued in order to increase the number of our study objects and validate the use of AI and FMD for the control of the activity of ABD, and prediction of vascular complications, respectively.

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The Influence of Plasma Fibrinogen and Serum Ferrum on Blood Viscosity in Adamantiades-Behçet's Disease

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1. INTRODUCTION

Adamantiades-Behçet's disease (ABD) was described about sixty years ago by the Greek Ophthalmologist Adamantiades and the Turkish Dermatologist Behçet. It presents various clinical features, such as recurrent oral aphthae, genital ulcers, skin manifestations and relapsing iritis. Human leukocyte antigen (HLA) B51, herpes simplex virus and *Streptococcus sanguis* have been reported to be strongly involved in the development of this disease. More recently, heat shock proteins, immunoglobulins, increased pro-inflammatory cytokines, and thrombosis have been identified as additional risk factors[1-5].

In ABD, a marked increase of vascular complications is seen: approximately 40% of patients with ABD suffer from thrombosis of arteries and veins, varices, aneurisms, and thrombophlebitis of superficial or deep veins and it is recognized as a systemic disease of unknown cause.

Many studies on lipids, lipoprotein metabolism, plasma protein C, protein S and antithrombin III[6-8] in connection with ABD have been reported, but there are very few studies on blood viscosity of ABD[9]. It was reported that the levels of various lipoproteins, as well as total cholesterol were increased in ABD, and viscosity of blood was significantly higher in

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patients than in control subjects. However, the roles of these proteins which work as risk factors in ABD have not yet been clarified.

The aim of the present study was to investigate the involvement of blood viscosity and biorheological factors in patients with ABD.

2. PATIENTS AND METHODS

2.1 Patients

Thirty-five (16 male and 19 female) Japanese patients with ABD participated at this study after written consent. The patients were recruited during their regular attendance at the ABD Clinic at the Teikyo University Hospital in Tokyo. Of the 35 patients, 22 were classified as complete, and 13 as incomplete ABD, according to the diagnostic criteria proposed by the Behçet's Disease Research Committee of Japan[10]. Their ages ranged from 40 to 78 (mean 59.7±8.1) years, the average duration of the disease from the time of diagnosis was between 12 and 34 (mean 22.8±6.5) years.

The patients were divided into two groups, active ABD group and inactive ABD group. Patients with active ABD presented three worsening clinical symptoms at the time of the study, ESR exceeded 15 mm/hr, and c-reactive protein (CRP) titre more than 0.3 mg/dl. Sixteen patients were with active ABD, and 19 patients with inactive ABD. 10 patients among them were taking low dose anti-hypertensive treatment, e.g., captopril (10-20 mg/day) or alacepril (25 mg/day). Their blood pressure varied from 124 to 150 mmHg (systolic pressure), and 70-96 mmHg (diastolic pressure). The blood pressure of the other patients showed 100-150/62-96 mmHg (mean 124/74). The control group consisted of 53 (23 male and 30 female) healthy subjects, matching with the patients in respect to age (40-71 years, mean: 59.2±7.2), gender and lifestyle.

2.2 Measuring methods

Blood viscosity was measured in a Low Shear 40 Couette viscometer (Contravers, Switzerland) at 37°C. Whole blood viscosity (WBV) and plasma viscosity (PV) were analysed at a shear rate of 25 1/s (shear rate from 0 to 50 1/s). The red blood corpuscle (RBC), hemoglobin (Hb), hematocrit (Ht), platelet (PL), serum ferrum (Fe), total cholesterol (T-cho), HDL cholesterol (HDL-cho), LDL cholesterol (LDL-cho), total proteins (Tp), albumin (ALB), globulin (GLB), triglycerides (TG), erythrocyte sedimentation rate (ESR), and CRP of 35 patients with ABD and 53 healthy

subjects (except ESR and CRP) were measured by the laboratory of Teikyo University Hospital and other laboratories. Plasma fibrinogen (Fib) was determined using the Fibrinogen Kit (Coagrex-100, International Reagents Corporation, Japan).

ABD and 53 healthy subjects (except ESR and CRP) were measured by the laboratory of Teikyo University Hospital and other laboratories. Plasma fibrinogen (Fib) was determined using the Fibrinogen Kit (Coagrex-100, International Reagents Corporation, Japan).

2.3 Statistical analysis

All assays were performed simultaneously for each experiment and the results were expressed as mean±SD. Statistical significance was ascertained using Student's unpaired *t* test. Coefficients of correlation between WBV or PV and all factors were determined by linear regression analysis "StatView" computer program. The results were considered significant when $P < 0.05$.

3. RESULTS

Table 1 shows the differences of blood viscosity and correlative blood factors between the patient and control groups. The results showed a significant decrease in Hb, Ht, Fe, and a significant increase in Fib, T-cho in all patients with ABD compared to controls. There were tendencies that the WBV was lower and the PV was higher than those of the controls, but these differences were not statistically significant. The levels of Hb, Ht, and Fe were significantly lower, and the level of Fib was significantly higher in patients with active ABD compared to controls. There were trends of decreased WBV and increased PV in the active group compared to the control group. ESR and CRP were increased in the active group (Table 1).

Correlation coefficients between rheological parameters and major risk factor for all patients, active patients with ABD and control groups revealed significant associations (Table 2).

The results showed an obvious positive correlation between WBV and Hb, Ht and Fe, and a negative correlation between WBV and ESR. Table 3 displays results which show an obvious positive correlation between PV and Fib, CRP, ESR in all patients and active patients with ABD.

It was shown that there were significant positive correlations between PV and Fib in all patients and active patients with ABD. Those correlations were strongly positive in active patients with ABD. Moreover, the positive correlations were shown between PV and T-cho in all patients and active

patients with ABD. The obvious positive correlation between PV and Fib was stronger than between PV and T-cho in the two groups.

Table 1. Comparison of mean values (±SD) of blood viscosity and correlative blood constituents in patient and control groups

Groups	Age (years)	WBV (mPa x s)	Hb (g/dl)	Ht (%)	Fe (μg/dl)	PV (mPa x s)	Fib (mg/dl)	T-cho (mg/dl)	ESR (mm/h)	CRP (mg/dl)
all ABD	59.7±8.1	6.51±0.9	13.3±1.1**	40.1±2.9*	79.0±32.7**	1.66±0.13	274±41.9*	215±30.9***	25.7±19	0.47±0.74
active ABD	62.2±6.5	6.33±1.7	12.9±3.3***	39.2±9.8***	63.0±31.8*	1.70±0.43	289±80.6**	214±63.0	38.7±20†	0.84±0.96
inactive ABD	57.6±9.0	6.66±1.0	13.7±1.2	40.8±3.1***	92.4±30.9	1.63±0.12	261±40.4*	216±25.9***	14.8±11‡	0.15±0.16‡
Control	59.2±7.2	6.62±0.8	14.0±1.1	43.1±4.3	102.7±31.0	1.65±0.22	213±39.3	200±28.1		

all ABD: all patients with ABD group, active ABD: active ABD group, inactive ABD: inactive ABD group.
WBV: whole blood viscosity, Ht: hematocritFe: serum ferrum, PV: plasma viscosity, Fib: plasma fibrinogen, T-cho: total cholesterol, ESR: erythrocype sedimentation rate, CRP: c-reactive protein
*P<0.001, **P<0.01, ***P<0.05 vs. control group; † P<0.01, ‡ P<0.05 vs. all patients with ABD group

Table 2. Correlation coefficient between whole blood viscosity and risk factors in all patients and active patient groups with ABD

	Hb (g/dl)		Ht (%)		Fe (μg/dl)		ESR (mm/h)	
	all	active	all	active	all	active	all	active
WBV	0.882*	0.834*	0.895*	0.788*	0,205	0,342	0.353***	0.39
Hb			0.969*	0.949*	0.432**	0.629**	0.524*	0.553***
Ht					0,278	0,482	0.506**	0.696**
Fe							0.474**	0.26

all: all patients with ABD group, active: active patients with ABD group,WBV: whole blood viscosity, Hb: hemoglobin, Ht: hematocrit, Fe: serum ferrum, ESR: erythrocyte sedimentation rate. *P<0.001, **P<0.01, ***P<0.05

4. DISCUSSION

The major finding in this study was that the levels of Hb, Ht, Fe and Fib, T-cho were associated with the changes of blood viscosity in both patients and controls groups. The levels of Hb, Ht and Fe were lower, and the levels of Fib, T-cho were higher in patients with ABD than those of the control group. The patients group displayed a non-statistical decrease in WBV and increase in PV.

Table 3. Correlation coefficient between plasma viscosity and risk factors in all patients and active patient with ABD

	Fib (mg/dl)		CRP (mg/dl)		ESR (mm/h)	
	all	active	all	active	all	active
PV	0.555*	0.683**	0.576*	0.659**	0.523*	0,406
Fib			0.4***	0,442	0.424***	0,413
CRP					0.616*	0.547***

all: all patients with ABD group, active: active patients with ABD group, PV: plasma viscosity (mPa x s), Fib: fibrinogen, CRP: c-reactive protein, ESR: erythrocyte sedimentation rate. * P<0.001, ** P<0.01, ***P<0.05

The WBV showed a positive correlation with Hb, and Fe in the control and patients groups, but in the patients group there were no significant differences between WBV and Fe. In both patients and active patients groups, the levels of Hb and Fe were lower, and WBV was decreased compared to the control group, and in comparison with the active patients group, and all patients group, these values were lower in the active patients group. This result indicates lower Fe leading to Hb reduction in ABD, particularly in the active group.

On the other hand, in the active patients group the levels of Fe were lower and ESR was higher than in the all patients group. Moreover, Fe showed a negative correlation with ESR (r=-0.474, P<0.01); the lower Fe possibly being the cause of the increased ESR. In addition, we noticed that the level of Fe was decreased in those patients who were in the active inflammatory stage and it was increased after substitution treatment. Those data implicate that ferrotherapy can prevent inflammatory activity of patients with ABD. Those results suggested that low Fe levels are one of the risk factors for the development of ABD.

Many studies have reported that increased PV is a risk factor for cardiovascular disease and that the correlation between PV and some proteins, such as Fib and T-cho, are also risk factors for inflammatory diseases[11-15]. Similarly, the present study reported the fact that in the patients with ABD there was a significant positive correlation of PV with Fib compared to age and sex-matching controls.

Fib is not only a major determinant of blood viscosity but also an acute phase-reactant of inflammation and it is considered to be a risk factor in some inflammatory diseases, such as vasculitis. In epidemological studies, Fib has been shown to carry independent prognostic information[16,17]. In this study, we showed that in all patients and active patients the level of Fib was higher than in the control group, and also in the active patient group the level of Fib was higher than that of the all patients group. However, this study showed that the level of T-cho was increased in all patients and active

patients groups but the differences were significant only between all patients group and control group. Although T-cho and Fib showed positive correlation with PV, the difference was not significant and the correlation coefficient of Fib was stronger than that of T-cho. These results suggested that increased PV in ABD was caused by higher Fib, not by T-cho, and that the change of PV as a risk factor can distinguish between some inflammatory diseases or cardiovascular disease and ABD.

In healthy persons, ESR and CRP are very low but can increase gradually in response to the grade of inflammation, and are good indicators for activity of ABD. This investigation found ESR and CRP to be higher in the active group compared to all patients group, and also showed a positive correlation with PV. The correlation coefficient of CRP was stronger than that of ESR (Table 3), These results suggest that high CRP and Fib levels are not only inflammatory markers but also contribute to PV increase in the active stage of ABD.

Although of different pathogenesis, ABD and systemic lupus erythematosus (SLE) may belong to the same category of collagen diseases. Reid et al.[18] observed that in the SLE group the mean Ht value was significantly lower than in the control group. Therefore, Ht downregulation would result to lower WBV in the SLE despite the elevated PV. However, our study showed that WBV was decreased in the ABD group, particularly in the active group. The decreased WBV may have been caused by low Ht, low Fe and Hb.

Several studies have shown that some anti-hypertensive drugs such as Ca antagonists reduce blood viscosity in hypertensive patients[19]. Beta-blockers, such as propranolol, also reduce Fib and PV levels in cardiovascular diseases as previously reported[20]. However, in this study the captopril treated group showed increased PV, the levels of Fib and T-cho were higher than those of controls and all patient groups. The investigation shows that among anti-hypertensive drugs ACE cause changes on blood viscosity of ABD patients by affecting blood proteins.

Many recent studies reported that inflammatory response in ABD may be induced by increased cytokine production, which confirms the previous reports on elevated cytokine levels (TNF-α, IL-6, IL-8, IL-10, and IL-12)[3,4,21]. These elevated values can be a good marker for assessment of the activity of ABD, although the specific factors which change blood viscosity in ABD are not yet clear. In this study, the levels of CRP, ESR and Fib were found significantly increased, and correlated with blood viscosity. How these factors may correlate with cytokines and change blood viscosity has not yet been found being a target of future studies.

In conclusion, this study found that WBV of patients with ABD is affected by the low Fe and Hb, and PV is affected by high Fib and CRP levels.

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Does Hyperhomocysteinemia Increase the Risk of Thrombosis in Behçet's Disease?

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1. INTRODUCTION

Deep vein thrombosis (DVT) constitutes the most frequent vascular manifestation in Behçet's disease (BD), seen in up to 40% of cases with this disease[1]. This complication may be life-threatening, and although several studies were conducted to determine association of specific coagulation abnormalities with BD, the mechanism of DVT in BD remains unknown[2]. Homocysteine (hcys) is a non-protein forming, sulfer amino acid formed by demethylation of methionine. It is metabolised by remethylation to methionine or by transulfuration to cysteine. Hyperhomocysteinemia (Hhcys) may occur as a result of inherited disorders, which alter enzyme activity in the transulfuration and remethylation pathways. Alternatively nutritional deficiencies of essential cofactors or enzymes substrates, including cobalamin (vitamin B_{12}), folate, or pyridoxine (Vitamin B_6) can result in blockade of hcys metabolic pathways. Several other disease states, medications and physiological factors also cause elevation in plasma hcys[3]. Hhcys has recently been established as an independent risk factor for thrombosis and vascular disease including coronary heart disease[4], cerebro-vascular disease[5], and peripheral arterial occlusive disease[6], and more recently for deep vein thrombosis[7] and non-arteritic anterior ischaemia optic neuropathy[8]. Fortification of food with folate and nutritional supplementation with vitamins (B_6 and B_{12}) lower plasma hcys. Thus, Hhcys could be a treatable

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risk factor for arteriosclerosis and thrombosis. In this study we aim to evaluate whether Hhcys is a contributive risk factor for DVT in BD.

2. PATIENTS AND METHODS

Patients with BD according to the criteria of the International Study Group (ISG) for BD, who visited our Department of Internal Medicine from October 10, 2001 to March 30, 2002, were consecutively enrolled in this study. The exclusion criteria were diseases' states and medications that cause elevation in plasma hcys[3]. Patients were divided into two groups according to the occurrence of DVT in the past. The diagnosis of DVT was made using conventional venous angiography, venous ultrasonography, thoracic or abdominal computed tomography (for vena cava thrombosis), and/or cerebral angio-MRI (for cerebral venous thrombosis). The disease was considered active in all patients with more than one criteria of the ISG at the time of clinical assessment. Fifty-nine healthy subjects matching the patients group in terms of age and sex were included as control group. Smoking status was determined in all patients and control group subjects. Total plasma homocystein (thcys) was measured by fluorescent-polarizing immunoassay on an Abbott axsym analyser. Hyperhomocysteinemia was defined as a homocysteine level above the 95^{th} percentile in the control group (15.7 μmol/l). Plasma vitamine B_{12} and folate were determined by microparticular enzyme immunoassay and ion capture respectively. All blood samples were collected in EDTA containing tubes after 12 h of fasting and were centrifuged immediately; then plasma was stored at 80°C until analysis (within one month).

2.1 Statistical analysis

The statistical analysis of data has been done with the help of the software STATA (version 6). The quantitative variables have been expressed in mean ± SD and the qualitative variables in percent for different categories. The Student test was used for the comparison of the thcys means levels. The test of variance analysis while using the general linear model (GLM) has been applied to adjust this comparison to age, sex and tobacco consumption. For the analysis of the relation between thcys and DVT complication, the Log Rank test has been used for univariate analysis, while taking the delay of apparition of the venous thrombosis in relation to the beginning of the illness as "survival time", and while distinguishing two classes depending on whether values were normal or elevated. The model of Cox has been used for adjustment to age, sex and the tobacco consumption.

For all tests the two tail situation has been chosen and the significance level has been fixed at 0.05.

3. RESULTS

Fifty-nine patients with BD were included in this study. There were 40 males and 19 females with a mean age of 39.9 years (range 17 to 67). Thirty-five patients had DVT in their past; there was no significant difference in mean age and sex ratio between patients with and without DVT. The control group included 36 males and 23 females with a mean age 35.8 years (range 20 to 61). The median thcys and the prevalence of Hhcys in the patients group (13.3 µmol/l and 16.9% respectively) were significantly higher than in the control group (10.9µmol/l and 5% respectively). Plasma Vit B12 and folate concentrations were not significantly different in these 2 groups as shown in Table 1.

Table 1. Comparison between patients and control groups

	Patients	Controls	P value
M/F	40/19	36/23	NS
Age	35.9	35.8	NS
Thcys µmol/l	13.31 ± 6.8	10.96 ± 2.4	0.01
% Hhcys	16.9 %	5 %	0.04 (OR: 3.8)
Vit B12	316	614	NS
Folate	5.96	6.67	NS

The median thcys and the prevalence of Hhcys in the patients group with DVT (15.3 µmol/l and 15.8% respectively) were also significantly higher than in the control group. The same results were found when comparing men in patients and in control group. But no significant differences were noted when we compared women in these 2 groups.

Comparison between patients with and without DVT showed no significant differences in the median thcys and the prevalence of Hhcys even after adjustment to age, sex, disease duration, disease activity, tobacco consumption, and body mass index (BMI) as seen in Table 2.

Then we looked at any relation between thcys and DVT recurrence. No significant difference was observed between the median thcys and the prevalence of Hhcys in patients with and without DVT recurrence. At last, we compared BD patients according to sex. We found that DVT frequency was significantly higher in men than in women but independently from thcys; and that the median thcys and the prevalence of Hhcys were also

significantly higher in men but independently from DVT occurrence, age, sex, disease duration, disease activity, tobacco consumption and BMI.

Table 2. Comparison between patients with (+) and without (-) DVT

	BD DVT+	BD DVT-	P
M/F	21/3	19/16	0.01
Age	35.4	36.3	NS
Thcys µmol/l	14.36	12.41	NS*
% Hhcys	20.8 %	14.2 %	NS OR = 1.58
Vit B12	337	302	NS
Folate	5.82	6.05	NS

4. DISCUSSION

In this study, thcys and prevalence of Hhcys were significantly higher in BD patients than in controls. Three others studies reported these same results[9-11] Thcys and prevalence of Hhcys in BD were also significantly higher in male patients with DVT than in controls but not in women. As opposed to Lee[9] and Aksu[11], we found no significant differences in the median thcys and the prevalence of Hhcys between patients with and without DVT. Thcys and prevalence of Hhcys were significantly higher in male patients than in female but independently from occurrence of DVT. Lee et al.[11] showed that thcys in the thrombosis patients were positively correlated with plasma of Willbrand factor levels; and Er et al.[10] found that the mean thcys levels were significantly increased and correlated with endothelin–1 and nitric oxid among patients with ocular BD when compared with non ocular disease and control subjects. These relationships suggest injury of the vascular endothelium induced by high levels of homocysteine.

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Pulmonary Artery Involvement in Behçet's Disease

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1. BACKGROUND

Behçet's disease (BD) is chronic inflammatory disorder characterised mainly by recurrent attacks of oral and genital aphthous ulcers, skin lesions, uveitis, arthritis, thrombophlebitis, arterial aneurysms, central nervous system and gastrointestinal system involvement[1].

Aortic, pulmonary, and peripheral arterial inflammation is one of the major causes of death due to BD[2].

2. AIM

To analyse 13 patients with pulmonary artery (PA) involvement, co-existence and relation with other vascular involvements, clinical course and management retrospectively.

3. MATERIALS AND METHODS

Patients were diagnosed as BD according to the ISG criteria[3]. Thirteen patients (Table 1) who had recurrent haemoptysis and chest pain were diagnosed to have pulmonary artery aneurysm (PAA) by radiological methods (i.v. contrast enhanced MR angiography, i.v. contrast enhanced dynamic CT scan, i.v. contrast enhanced CT angiography).

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Nine patients were male, 4 were female. The mean age was 42.8 years (27-71 years). The mean age of onset BD was 30.1 years (13-50 years). The mean age of the onset of the symptoms of PA involvement was 47.19 years (13-55 years). The mean duration between the onset of BD and PA involvement was 6 years (0-12 years). Two patients had PA involvement within the first year of onset of BD. One of them (21 years old) died 3 months after the diagnosis of PAA with an unknown cause despite treatment. Four patients had no venous involvement other than PAA. Nine patients had superficial vein thrombophlebitis and PAA occurred afterwards (Table 2).

Table 1. Clinical findings of the 13 PAA patients

Clinical findings	Number of Patients	Percentage (%)
AU	13	100
GU	12	92.30
OI	6	46.15
EN	7	53.8
PF	8	61.5
Arthritis	5	38.5
SVT	9	69.2
DVT	6	46
Arterial involvement other than PA	1	7.7
Pathergy	10	77
HLA B5	3 of 4	
Family history		

AU: Aphthous ulcers, GU: Genital ulcers, OI: Ocular involvement, EN: Erythema nodosum, PF: Pseudofolliculitis, SVT: Superficial vein thrombosis, DVT: Deep vein thrombosis

One of the patients had DVT during treatment for PAA with cyclophosphamide. Meanwhile, cyclosphospamide had to be withdrawn because of pulmonary tuberculosis. Two months later, cavernous sinus thrombosis occurred and methyl prednisolone has been administered (80 mg/day).

Table 2. Accompanying venous involvements of the 9 patients with superficial vein thrombophlebitis (SVT) before PAA

Venous involvement	Number of patients	Involvement
Arterial	1	Arteria femoralis aneurysm
DVT	6	1 VCI+VCS
		1VCI
		3 calf vein thrombosis

Once the PA involvement was diagnosed, patients received cyclophosphamide 1gr i.v. infused monthly, and 40 mg methyl prednisolone/day for 6 months. Then the treatment continued with azathioprine 150 mg/day (Table 3).

Table 3. The treatments of the 13 patients before PAA involvement

Medication*	**Number of patients**
Colchicum dispert 0.50 mg 3x/d	13
Azathioprine 50mg 2x/d	3 patients (2 for DVT, 1 for arteria femoralis aneurysm)
Cyclophosphamide 50mg 2x/d	1 for recurrent DVT
Cyclophosphamide 50mg 2x/d, corticosteroid 40mg daily	1 for recurrent DVT and ocular involvement

*The patients developed PAA while under these medications.

4. DISCUSSION

Aortic, pulmonary artery and peripheral arterial inflammation are severe complications of BD and responsible for the majority of deaths due to BD[2,4].

Arterial involvement is less common than venous disease. There is marked male predominance. Out of our 13 patients 9 were male, 4 female.

No association between pulmonary embolism and deep vein thrombosis is found in BD perhaps due to sticky thrombi, tightly adherent to inflamed veins, which may eventually organize into fibrous cords[5].

SVT developed in 9 of the 13 patients during clinical course. All were male. Five of them had DVT before PAA. We think that the presence of SVT (9 patients), and DVT (5 patients) before PAA is not related to each other but demonstrates that especially men are predisposed to vascular involvement in BD.

In 5 of the 13 patients PAA developed while under treatment with cytostatics (3 azathoprine, one cyclophosphamide and one corticosteroid + cyclophoshamide). In one patient DVT occurred when the treatment for PAA with corticosteroid +azathioprine was stopped after a long period. Later the treatment started again, but pulmonary tuberculosis occurred so the treatment had to last. Two months later cavernous sinus thrombosis occurred. When we look at the treatment schedules and the clinical course we see that cytostatics alone or with corticosteroids can help to regress venous involvement but does not prevent new involvement in some patients.

In conclusion various medications, untilised in BD treatment do suppress the general activation of the disease, but pulmonary artery involvement follows its natural course. However, since this involvement is life threatening, medications can not be ruled out. Thus, it is quite difficult to determine the effects of medications on pulmonary involvement.

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Cardiac Manifestation in Four Patients with Adamantiades-Behçet's Disease

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1. INTRODUCTION

Cardiovascular involvement is a rare but serious complication in patients with Adamantiades-Behçet's disease (ABD). The reported incidences vary between 7-31%[1-7].

Cardiac manifestation in ABD essentially includes intracardiac thrombus[8], myocarditis[9], pericarditis[10], endocarditis[11], myocardial fibrosis[12], myocardial infarction[13], dilated cardiomyopathy[13], left ventricular aneurysma[14] and ventricular arrhythmia[15].

We report on four male patients with different forms of cardiac manifestation in ABD.

2. PATIENTS

1) A 47-year-old German patient suffered from oral aphthosis, ocular manifestation, recurring erythema nodosum, and pulmonary embolism, and he had orchitis and venous thrombosis. A factor V Leiden mutation was diagnosed. In 2000, he developed fever and cough. The laboratory investigations showed signs of inflammation. A thrombus in the right ventricle was detected which reduced in size after initiation of anticoagulative and antibiotic therapy.

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2) A 44-year-old German patient suffered from bipolar aphthosis, recurrent epididymitis, arthritis, and thrombophlebitis, and had a factor V Leiden mutation. In 1994 he presented with acute myocardial infarction without provable coronary stenosis.

3) A 33-year-old Libanese patient with bipolar aphthosis, ocular manifestation, and arthritis developed exercise-induced dyspnea in 1999. Coronary insufficiency could be excluded but dilated left ventricle and diastolic dysfunction were detected leading to the diagnosis of dilated cardiomyopathy.

4) A 29-year-old Turkish patient suffered from bipolar aphthosis, arthralgia, and epididymitis. One sister suffers from ABD. In 1999, he developed acute thoracic pain due to an exercise-induced inferolateral ischemia caused by a small vessel disease. Acute myocardial infarction could be excluded.

3. DISCUSSION

Cardiac manifestation in ABD is a rare but often fatal complication. The underlying pathologic process is considered a vasculitis of the vasa vasorum. Resected vascular specimens have been shown to have fragmentation and splitting of the elastic fibers in the media with perivascular mononuclear cell infiltration[1].

We reported four patients with different forms of cardiac manifestation in ABD. Intracardiac thrombus formation is very uncommon. The thrombus is usually located in the right ventricle[16], probably due to the lower blood pressure or caused by endomyocardial fibrosis in the right heart. The exact reason for the predominance of the right ventricle is not known. The histological appearances were usually those of an organized thrombus[16].

Coronary artery vasculitis and microvasculitis have been postulated as the underlying pathologic features in ischemic heart disease in ABD. An interesting type of coronary artery involvement is the “silent myocardial ischemia” defined as objective evidence of myocardial ischemia in the absence of symptoms in patients with ABD[17]. In our patients we diagnosed a microvascular disease of the myocardium in the absence of major coronary involvement. Two patients had no signs of ischemic heart disease and were patients with a silent ischemic heart disease before. Myocardial scintigraphy could have detected the ischemic heart disease before the patients become symptomatic.

ABD strongly predisposes to arterial and venous thrombosis. Coagulopathies should therefore always be a matter of investigations[8,18]. In two of our patients we found a factor V Leiden mutation.

According to the recent data of the German Registry of Adamantiades-Behçet's Disease cardiac manifestation occurs in 2.2% of all patients and in 4.8% of male ones. There is no difference concerning nationality. These data are confirmed by other groups indicating that in severe ABD, such as arterial involvement, women are far outnumbered[19]. Also in cases with intracardiac thrombus a predominance of men is reported[16].

4. CONCLUSION

Cardiac manifestations represent a serious complication of ABD and occur almost exclusively in male patients.

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Quantitative Evaluation of Microvessels in Behçet's Disease

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1. INTRODUCTION

Behçet's disease (BD) is a chronic polysymptomatic systemic vasculitis clinically characterised by recurrent oral and genital ulcers, skin manifestations, ocular lesions, arthritis, and intestinal and neurological involvement. Micro- and macro-vascular involvement is always seen and seems to be correlated to the clinical pattern[1-4].

Videocapillaroscopy is safe, reliable, not invasive, and able to explore both skin and mucosal microcirculation. It explores capillary network and the arteriole-venule compartment which represents the so-called para-microcirculation[5-6]. Capillaries may undergo changes of their disposition, of their morphology, of length and diameter of afferent-intermediate-efferent branch. The morphologic modifications include alterations of wall profile and of whole vessel (incisures, mega-capillaries, micro-aneurysms, twisting vessels) and are often associated with neoangiogenesis, but also with avascularised areas (desertification)[6,7]. Alterations of arterioles and venules are represented by similar morphological changes and by modifications of arteriole-venule diameter[8]. Both micro- and paramicro-circulation show the alterations of blood flow which is detected as granular appearance (sludging).

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All these changes can be considered as a "steady state" modification: a micro-vascular system undergoes local modifications for homeostasis restoring. This long-lasting process induces vessel structure alterations and chronic disruptions which are the morphological expression of the disease and may produce clinical symptoms as a consequence[9].

Videocapillaroscopy is largely employed to study microcirculation in autoimmune diseases where specific morphologic patterns may be detected[10]. Our previous observation already showed micro-vascular alterations in BD[11]; in this work we wanted to further define the severe involvement of microcirculation in BD, giving qualitative and quantitative indices of vessel damage.

2. PATIENTS AND METHODS

2.1 Subjects

16 patients (7 males and 9 females, mean age 35±7) affected by BD according to International Study Group of BD criteria[4] entered the study. All of them gave informed consent to the study. None of them received any treatment, or was being a smoker. A similar group of 16 normal subjects of matching age and sex was considered.

2.2 Intravital videocapillaroscopy

We used a videocapillaroscopy of last generation Video Cap, MDS group, software release[8,7]. The images were taken by a videoprobe connected with the instrument by an optical fiber cave which allows a 200-fold magnification. The body of instrument is composed of an epi-illuminated microscope containing a 100 Watt mercury vapor lamp light source providing an automatic and continuous regulation of light intensity and of the filter selection. Several objectives can be applied to the camera with a range of 50- to 1500-fold final magnification. The microscope is connected with a computer composed of a video-tape, a video-colour, and a colour printer; the system allows the data storage and analysis.

The sites of observation were: peripheral microcirculation represented by hand and foot nail-fold, gingival edge and labial mucosae, and conjunctival microcirculation. The first one allows the analysis of capillary network and of terminal loop; the second one explores the arteriole-venule compartment. To minimize rhythmical variation in individual capillaries, the patients were examined under the same environmental conditions: in the morning between

9 and 11 a.m. after an overnight fast, in a temperature-controlled laboratory (21°C to 24°C), the study subjects having rested for at least 20 minutes in supine position. If necessary, room temperature was adjusted by using fan heaters or air conditioning. For conjunctival analysis the subject must focus an area opposite to the examiner. The upper lid is lifted to increase the evaluation area avoiding straining and capillary stasis. No contact between probe and explored surface must be taken. Peripheral microcirculation is examined in both hands and feet nail-fold, in gingival edge and labial mucosa keeping immobility. The probe must not press but only touch the skin.

Videocapillaroscopy observation permits to detect various qualitative-morphological alterations which may be analysed and quantified.

Peripherally, we can find the following morphological aspects: capillary dystrophies, mega-capillaries, micro-aneurysms, and saccules (singles and multiple) avascularised zone (desertification), petechiae, pericapillary oedema, haemorrhage. Quantitatively: capillary density (number of capillaries/mm^2); length and diameter of afferent, intermediate, efferent branch (μm) and the ratio (a/e ratio); vessel wall incisures; instantaneous flow (sludging) can be measured. Conjunctival observation shows similar morphological aspects; quantitatively, we measure arteriole and venule diameter (μm) and a/v ratio, incisures and sludging. Qualitative changes are quantified as percent of subjects affected. Capillary density is evaluated in frozen images by using an automatic grid (averaged four square 0.5 mm^2) supported by the utility program of the computer; the linear measurements (diameter of arteriole-venule as well as length and diameter of capillary branches) are being executed by using a misuration utility annexed to the capillaroscopy: almost three measures are accomplished, and the average value is recorded. Dynamic flow changes are seen as presence of granular flow and are pointed out as presence of microgranules, coated erythrocytes and aggregated erythrocytes; a semiquantitative scale is performed as 0=normal flow, 1=slight sludging, 2=mild sludging, 3=severe sludging. Similarly, the presence of wall incisures is quantified as score from 0 to 3 = absence, slight, mild, severe incisuring.

2.3 Statistical analysis

The data were processed by Mann-Whitney Test.

3. RESULTS

3.1 Conjunctival videocapillaroscopy

As indicated in Table 1, conjunctival observation shows morphological and quantitative changes: the first ones were detectable in a high percentage of patients and the second ones were significant compared to the controls. In particular, we found a significant difference of sludging and incisures score.

Table 1. Conjunctival alterations

Morphological Aspects	**Patients**	**Controls**
Capillary dystrophies	75%	6%
Capillary distribution alterations	65%	8%
Microaneurysms, microsacculi	60%	3%
Coiling	50%	16%
Kinking	40%	7%
Saw-thooted aspect of vessel wall	70%	30%
Sludging	85%	9%
Quantitative Alterations		
Vessel diameter (μm)		
- arteriolar	16.7±4.6	18.0±6.4
- venular	33.3±6.1	38.2±11.2
a/v ratio	0.51±0.14	0.47±0.06
Incisures (score)	2.07±0.75*	0.06±0.24
Sludging (score)	1.84±0.98*	0.33±0.61

Statistical analysis: Mann Whitney test; *p<0.0001

3.2 Peripheral videocapillaroscopy (nail-fold hand and foot, gingival edge, labial mucosa)

Table 2 resumes the observed findings: high percent of BD patients showed morphological alterations of microvessels with various patterns; the same changes were found in healthy subjects at a very low percentage. Quantitative evaluation showed significant difference of capillary density, of sludging and incisures score and of intermediate branch length in comparison with the control group.

Table 2. Peripheral alterations

Morphological aspects	**Patients**	**Controls**
Capillary dystrophies	70%	10%
Capillary distribution alterations	30%	2%
Numeric abnormalities of capillaries	75%	7%
Avascularized zones	30%	-
Megacapillaries	20%	1%
Saw-thooted aspect of microvessel wall	40%	1%
Sludging	80%	15%
Petechiae	60%	-
Pericapillary oedema	50%	5%
Background pallor	40%	1%
Quantitative alterations		
Capillary density (n/mm^2)	13.4±1.2*	31.1±9.1
Branch length (μm)		
- afferent	157.0±62.2	150.8±52.1
- efferent	221.4±74.3	218.9±78.7
- intermediate	31.6±11.7*	25.4±18.6
Branch diameter (μm)		
- afferent	11.2±3.2	12.5±2.9
- efferent	18.6±6.7	19.3±3.2
- intermediate	24.4±11.6	21.1±6.5
a/e ratio	0.57±0.21	0.13±0.35
Incisures (score)	1.67±0.78*	0.06±0.25
Sludging (score)	1.17±0.77*	0.13±0.35

Statistical analysis: Mann Whitney test; *p<0.0001

4. DISCUSSION

In our BD patients, videocapillaroscopy showed several morphological and quantitative alterations of peripheral and conjunctival microvessels. Vessel alteration included changes both of the number and of the whole structure with an important rearrangement of microvascular disposition; the presence of microaneurisms, desertification areas and flow change represent a severe vascular impairment which may be detected both in peripheral and conjunctival observation, exploring the two different vascular beds of microcirculation and paramicrocirculation. Furthermore, our BD patients showed severe vascular lesion which involved different aereas and induced whole vascular structure damage. Clinically, patients showed an active phase of disease with diffuse cutaneous and mucous lesions, arthritis, uveitis, but

no neurological damage; each one was firstly diagnosed and did not receive any treatment.

Diagnostic criteria of BD are mainly clinical but they presume the vessel inflammatory lesion as histologic and pathogenetic substrate of BD as responsible for the clinical pattern[4]. Thus, videocapillaroscopy may represent an important tool to investigate the presence and severity of vascular damage.

There are a few data concerning videocapillaroscopy in BD. Weschler et al.[12] described the capillaroscopic aspects in BD; microvascular involvement was described, and direct and indirect signs were defined, in the first ones including the vessel wall alteration and in the second ones their consequences (edema, haemorrhage). Weschler et al. also underlined the role of systemic involvement of microcirculation in BD[12]; however, his observations were limited to the peripheral nail-fold areas. Our data showed the association of peripheral and conjunctival involvement. In conjunctiva is also possible to observe the arteriole-venule compartment which may add further evidence to systemic vascular damage.

Microcirculation involvement is a diagnostic tool in systemic autoimmune disease to distinguish them from districtual syndromes with microcirculatory damage limited to the area of clinical evidence[10]. In our patients videocapillaroscopic study confirms BD characteristics of systemic vasculitis.

We conclude that videocapillaroscopy, especially in peripheral and conjunctival districts, represents an important diagnostic tool of BD defining the systemic extension of vascular inflammatory process and the alteration of vessel structure. Videocapillaroscopy can be useful in both the follow-up and the response to the treatment.

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^{18}F-FDG-Positron Emission Tomography for Diagnosis of Large Vessel Arteritis in Behçet's Disease

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1. INTRODUCTION

^{18}F-Fluorodeoxyglucose-positron emission tomography (^{18}F-FDG-PET) has already been successfully used in patients with various large vessel diseases. Behçet's disease (BD) is another autoimmune disease possibly involving the arterial walls. Here we describe a BD patient with suspected large vessel involvement who underwent ^{18}F-FDG-PET during diagnostic assessment.

2. ^{18}F-FDG-PET IMAGING

^{18}F-FDG-PET studies were performed according to the standard protocol of the Society of Nuclear Medicine (SNM)[1] using a GE Advance PET scanner with an axial field of view of 15.2 cm (General Electric Medical Systems, Milwaukee, Wisconsin, USA).

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3. CASE REPORT

At presentation this female BD patient had fever of unknown origin and an elevated erythrocyte sedimentation rate (ESR), which could not be explained by a neoplasm or an infectious process. As shown in Fig. 1, PET studies revealed an increased ^{18}F-FDG uptake in the left femoral artery and hyperactive inguinal lymph nodes on the right side. After corticosteroid treatment clinical symptoms improved and ESR decreased.

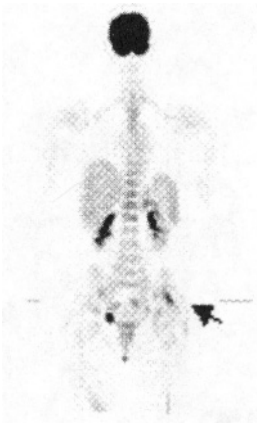

Figure 1. ^{18}F-FDG PET showing increased metabolic activity in the left femoral artery

4. DISCUSSION

Histological diagnosis is the gold standard for diagnosing arteritis including BD arteritis. At present, sonography, computerized tomography, magnetic resonance imaging, and angiography are considered the noninvasive imaging techniques showing structural changes as surrogates of inflammation. ^{18}F-FDG-PET is a new, minimally invasive technique which may help to diagnose active inflammatory processes of large vessel arteritis in BD patients.

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Spontaneous Platelet Aggregation in Patients with Behçet's Disease by Using Laser-Light Scattering Aggregometer

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1. PURPOSE

Patients with Behçet's disease (BD) have a variety of platelet and coagulation system abnormalities[1-5]. Recently, a new laser-light scattering aggregometer can detect even small-size platelet aggregation[6-8]. Therefore, we examined various sizes of spontaneous platelet aggregation in patients with BD compared with normal individuals.

2. MATERIALS AND METHODS

2.1 Patients

All BD patients (n=18) fulfilled the International Study Group criteria for BD. Control group (n=10) was free of any risk factors such as arterial sclerosis, and use of anti-platelet drugs / anti-coagulant drugs (Table 1).

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Table 1. Demographic data of the patients with BD and the healthy controls

		Behçet's disease	Healthy controls
		(n=18)	(n=10)
Gender:	male	44.0% (8)	40.0% (4)
	female	56.0% (10)	60.0% (6)
Age, years (mean±SD)		44.9 (±14.6)	27.8 (±5.2)
Range		27 - 75	21 - 34

* The healthy control group was younger than BD group. The control group was free of any risk factors of vascular events.

2.2 Blood sampling

Venous blood was collected into glass tubes containing sodium citrate by a 21-gauge needle, and then centrifuged at 150 g at room temperature for 10 min to obtain platelet-rich plasma (PRP).

2.3 Measurement of platelet aggregation

PRP aggregation was assayed by evaluating maximum percent decrease in optical density (OD), and by assessing laser-light scattering (LS) intensity using aggregometer (PA-200, Kowa, Tokyo, Japan; Fig. 1). Platelet aggregation was assessed in the spontaneous state. The total light intensities of small, medium and large aggregates were assayed. Particles with an intensity of 25 to 400 mV represented small aggregates (9-25 μm), those with an intensity of 400 to 1000 mV represented medium aggregates (25-50 μm), and those with an intensity of 1000 to 2048 mV represented large aggregates (50-70 μm). Small aggregates contained approximately 70-1400 platelets, medium aggregates with 1000-11,000 platelets, and large aggregates with 11,000-31,000 platelets, as shown in Fig. 2, respectively[6]. To determine the peak intensity of the laser-light scattering produced, we performed a quantitative estimation of platelet aggregation. Hyper-coagulation of small platelet aggregation was more than 3.0×10^4 (V) in the spontaneous state without coagulation drugs.

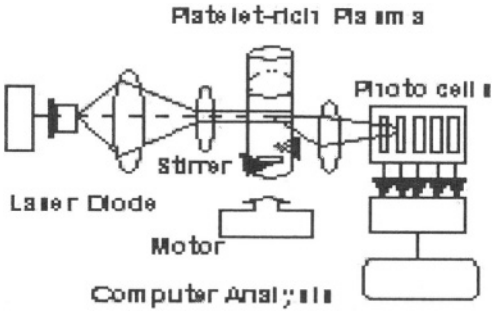

Figure 1. Aggregometer with laser-light scattering. A laser beam measuring 40 μm in diameter was generated using a 20-mW diode laser (675 nm), which was passed through platelet-rich plasma (300 μl) stirred in a cylindrical glass cuvette with a 5-mm internal diameter. The light scattered from the observation volume (48×140 ×20 μm) was detected via a photocell array. The signal frequency was recorded at 10-s intervals. Measurements are expressed as the change over time (seconds) in the number of aggregates (counts per second) of individual sizes (determined by light intensity, expressed in volts).

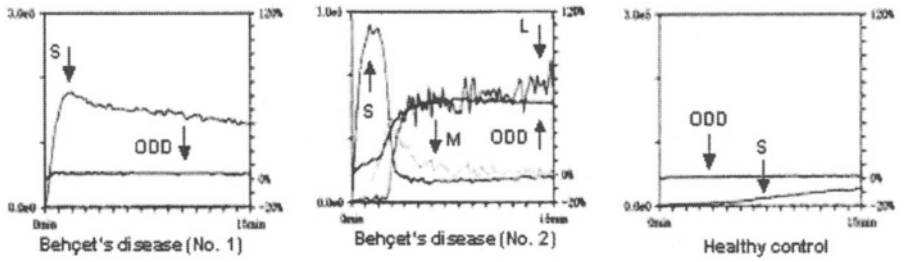

Figure 2. A representative pattern of platelet aggregation measured with the laser-light scattering method between BD patients and healthy control. S line a indicates small aggregates with the left scale labelled. M line, medium aggregates; L line, large aggregates. OD line indicates optical density (%) with the right scale labelled. The number of small-sized platelet aggregates was significantly higher in BD patients (No. 1) than in healthy controls. Especially, the second patient with BD patients (No. 2) had developed platelet clots (a large-size platelet aggregation).

3. RESULTS

Fifteen patients with BD (83.3%) had small-size spontaneous platelet aggregation compared with 2 (20%) of normal individuals. The values in BD patients were significantly higher than those in normal individuals ($p<0.01$) as shown in Figure 3A. Two patients with BD (11.1 %) had developed

platelet clots (a large-size platelet aggregation) after incubation of small-size aggregation. On the other hand, no platelet clots were detected in normal individuals. Although the values of large-size platelet aggregation were not significant compared with normal individuals, the OD values in BD patients were significantly higher than those in normal group ($p<0.05$) as shown in Figure 3D.

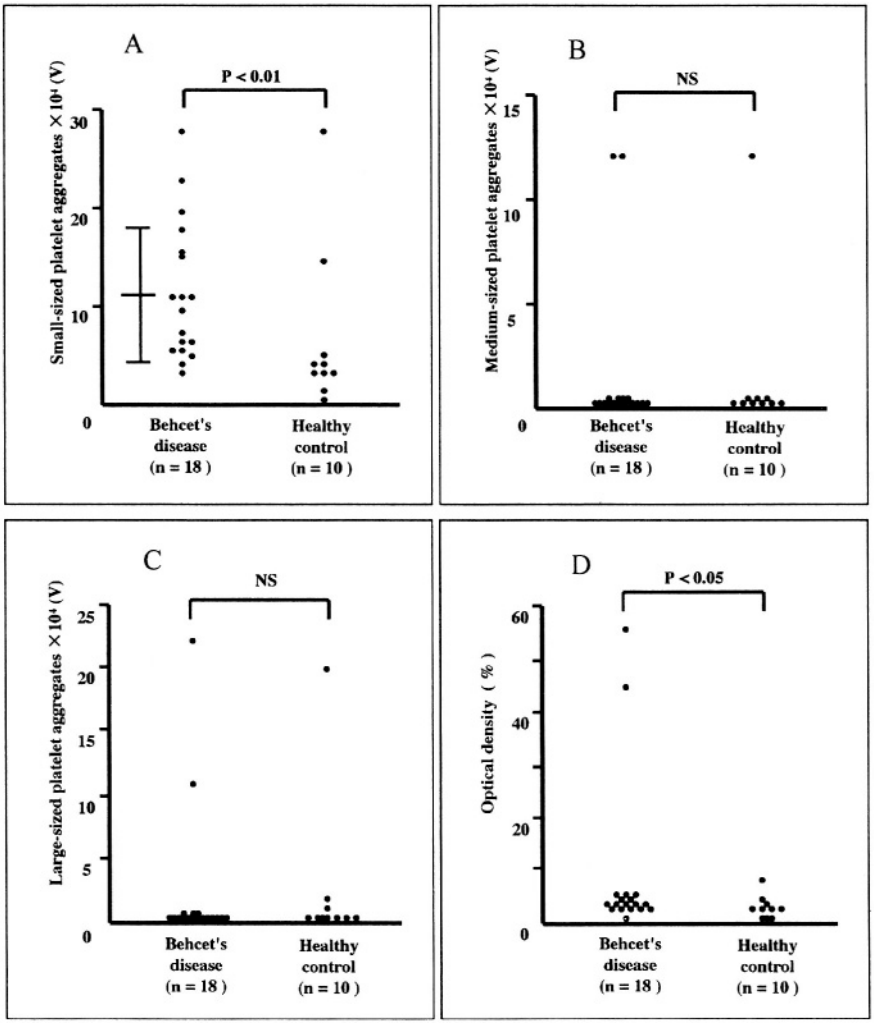

Figure 3. The number of small-, medium- and large-size platelet aggeregates (panel A, B, C,) in the BD group and healthy control group measured by the laser-light scattering method. *Panel D*, Values on both groups are given by the optical density method.

4. CONCLUSION

Most of patients with BD had spontaneous small-size aggregation in this system. Moreover, platelet clots (a large-size platelet aggregation) were only detected in patients with BD compared with normal individuals. These date suggest that BD may exhibit spontaneous hyper-coagulation of platelet function.

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VARIOUS CLINICAL MANIFESTATIONS

New Perspectives of Imaging Techniques for Diagnosis of Organ Manifestations in Behçet's Disease

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1. INTRODUCTION

Early and rapid detection of organ involvement may be crucial for the outcome of Behçet's disease (BD), but can also be important for fulfillment of diagnostic criteria for this disease. Vital organ involvement like large vessel involvement including myocardial infarction have been described even as the presenting manifestation of BD before fulfillment of BD criteria[1,2]. We anticipate that most organs may be involved even before diagnosis of BD. Thus, improved detection of organ manifestations may help to find the diagnosis, especially in patients presenting with single vital organ involvement and a rapid need for adequate therapy.

New diagnostic techniques have been presented at the 10th International Conference on Behçet's disease in Berlin, 2002, including assessment of minimal erythema doses of UVB exposure, videocapillaroscopy, radial artery tonometry with pulse wave analysis, and electron beam computed tomography (EBCT). We want to focus on our recent experiences with functional diagnostic approaches including color Doppler ultrasound (CDUS) of the joints and ^{18}F-fluorodeoxyglucose positron-emission tomography (^{18}F-FDG-PET) as a nuclear medicine technique.

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2. STRUCTURAL IMAGING TECHNIQUES

At present, structural imaging techniques like ultrasound (US), X-rays, computerized tomography, magnetic resonance (MR) imaging, and conventional angiography predominate the diagnostic armamentarium in BD. These techniques, however, are able to document structural changes only in limited areas of interest.

Recently, more sophisticated techniques were introduced for diagnosing cardiovascular diseases. Thus, performance of EBCT or MR imaging will provide more detailed insight into the occurrence and development of cardiovascular abnormalities. At this meeting a pilot study with EBCT to detect coronary artery involvement in BD patients was presented, with detection of abnormalities in 12.5% of BD patients with severe vascular disease elsewhere[3]. Cardiovascular MR was recently shown to detect occult myocardial changes in symptomatic patients with syndrome X[4]. In these patients, MR showed ischemia during the first pass of gadolinium, whereas other techniques had failed to detect any pathological changes.

3. FUNCTIONAL IMAGING TECHNIQUES

It has been proposed that in systemic inflammatory diseases like BD, screening for functional changes including hypervascularity and areas of hyperactive metabolism as markers for inflammation may be even more sensitive to diagnose organ involvement.

3.1 Color Doppler ultrasound

In BD patients, CDUS has already been used to determine the vascular resistance of orbital vessels. This parameter was increased in BD patients with ocular involvement, which may predict onset of ocular involvement[5,6]. Besides, frequency of microembolic signals detected by transcranial color Doppler US appears to be higher in BD patients with CNS involvement than in those without, or healthy controls.[7]

We present here our first experiences with contrast-enhanced Doppler US in BD patients to detect inflamed pannus and erosive joint disease (Fig. 1). A microbubble-based US contrast agent (Levovist; Schering, Berlin, Germany) is intravenously infused. The results are comparable to those from patients with rheumatoid arthritis, but erosions are less frequent[8].

3.2 ^{18}Fluorodeoxyglucose positron emission tomography

^{18}Fluorodeoxyglucose positron emission tomography (^{18}F-FDG-PET) as a nuclear medicine scintigraphic technique has been described for functional imaging of neoplastic and inflammatory processes in major organs like the central nervous system, heart, lung, gastrointestinum, and large vessels excluding the urinary bladder and the kidneys. Our experiences with ^{18}F-FDG-PET for detection of hypermetabolism as a marker for inflammation in large arterial vessels are pointed out elsewhere in this book[9].

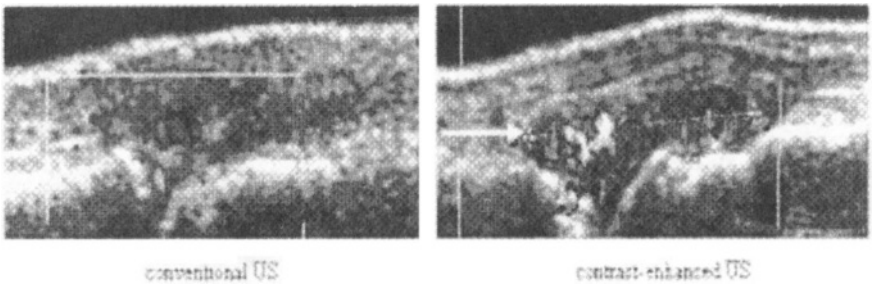

Figure 1. Improved detection of arthritic manifestations by application of a microbubble-based US contrast agent in a BD patient (arrow marking white area of increased vascularity)

Here we show an example of myocardial deficit of ^{18}F-FDG uptake in a symptomatic patient with systemic lupus erythematosus (SLE) but normal ^{201}Thallium scintigraphy (reversed mismatch, Fig. 2)[10]. The lack of correlation with acute elevation of cardiac enzymes or with ECG changes in SLE patients suggests an underlying chronic process. Such a sensitive technique as ^{18}F-FDG-PET may also help to detect myocardial changes in symptomatic BD patients.

4. CONCLUSION

CDUS and scintigraphic techniques like ^{18}F-FDG-PET may be helpful to detect unexpected functional deficits without major structural changes. Future studies are warranted to compare sensitivity and specificity of structural and functional imaging techniques in BD patients. Improved imaging techniques may help to shorten the delay until diagnosis of BD manifestations.

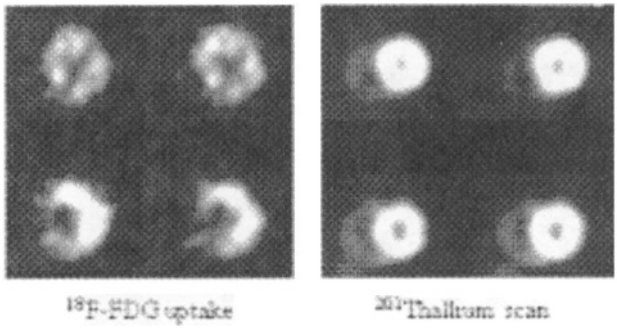

Figure 2. Pathological ^{18}F-FDG uptake but normal ^{201}Thallium myocardial perfusion scans in a symptomatic patient with systemic lupus erythematosus

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The Comparison of Ankylosing Spondylitis in Behçet's Disease and Normal Population

A control study

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1. INTRODUCTION

The association of Behçet's disease (BD) and ankylosing spondylitis (AS) has been reported in many cases[1-7]. We previously reported that AS was associated with BD in Iranian patients[8]. We found AS in our BD patients to be 8 to 9 times more prevalent than in the general population of Iran[9].

It seems that AS is found especially in BD patients who carry the HLA-B27 antigen. The prevalence of HLA-B27 is extremely high among patients with both BD and spondyloarthropathy[3-10]. In our patients, the prevalence of HLA-B27 in BD patients with AS was extremely high in comparison with BD patients without AS (44.5% versus 9.5%, $p< 0.00001$). On the other hand, in the study of Ben Taarit et al, HLA-B27 was not found in their two BD patients who had AS[11].

It seems that the features of AS in BD patients differ from those in the general population. This study was designed to evaluate the features of AS in BD (ASBD) and compare them with AS in the general population (ASGP) of Iran.

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2. MATERIALS AND METHODS

We found 73 ASBD patients in a cohort of 4700 patients with BD. Among them, 70 met the European Spondyloarthropathy Study Group (ESSG) criteria and 44 met the modified New York Criteria for AS.

We compared them with 180 ASGP patients (control) from our AS database. Among them, 145 met the ESSG criteria and 100 met the modified New York criteria.

Different manifestations of the disease including: sex, age, pattern of presentation, various symptoms and signs, and laboratory findings were compared by the chi square test between the two groups.

3. RESULTS

The prevalence of ASBD among BD patients was 1.55 (CI=0.4). Compared with the prevalence of ASGP in the general population of Iran (reported to be 0.18%), it showed a significant difference ($p<0.0001$).

The mean age of the patients at the onset of joint involvement was 26.4 years (SD=8.3) for the ASBD group, and 27.3 years (SD=11) for the ASGP group. The difference was not statistically significant.

Males included 67% (CI=13.2) of patients in ASBD group and 80% (CI=5.8) in ASGP group. The difference was significant ($p<0.03$).

The most frequent pattern of presentation of joint involvement was the insidious type. It was 93% (CI=6.1) in ASBD versus (vs) 92% (CI=4.2) in ASGP. The difference was not statistically significant ($p=0.8$).

The comparison of symptoms and signs between the two groups showed a higher prevalence of peripheral joint involvement in ASBD group, 55% (CI=15.4) vs. 27% (CI=4) in ASGP group. The difference was statistically significant ($p< 0.0001$).

However, the prevalence of constitutional signs (2.7%, CI=23.6 vs. 24%, CI=6.8): morning stiffness lasting more than one hour (42.5%, CI=17.4 vs. 66%, CI=6.9), limitation of motion in lumbar spine (41%, CI=17.6 vs. 62%, CI=7.1), abnormal schober test (58%, CI=16.1 vs. 74%, CI=8.4), dorsal involvement (19%, CI=20 vs. 54%, CI=7.3), and cervical involvement (4%, CI=22 vs. 56%, CI=7.3) were less in ASBD group than in ASGP group, all with significant difference (Table 1).

The inflammatory low back pain (57.5%, CI=14.9 vs. 67%, CI=6.9) and enthesopathy (61.6%, CI=14.2 vs. 63.3%, CI=7) showed no significant difference in both groups (Table 1).

Table 1. Comparison (symptoms and signs)

Symptoms	ASBD*	ASGP˜	P
Peripheral joint involvement	55	27	<0.001
Constitutional	2.7	24	<0.001
Morning stiffness > 1 hr.	42.5	66	<0.0001
Limitation of motion in lumbar spine	41	62	<0.002
Abnormal Schober test	58	74	<0.02
Dorsal involvement	19	54	<0.00001
Cervical involvement	4	56	<0.00001
Inflammatory low back pain	57.5	67	NS
Enthesopathy	61.6	63.3	NS

*ASBD: Ankylosing spondylitis in Behçet's disease
˜ASGP: Ankylosing spondylitis in general population

Among the extra articular manifestations, eye involvement was much more frequent in ASBD group (55%, CI=15.4 vs. 11%, CI=4.5). According to expectation, all layers of the eye were involved in ASBD group: anterior uveitis in 49% (CI=16), posterior uveitis in 36% (CI=18), and retinitis in 22% (CI=20), while only anterior uveitis was seen in ASGP group. The comparison of anterior uveitis in both groups showed significant difference (P<0.000001). Diarrhea was seen in 9.6% (CI=22) of ASBD group vs. 8.3% (CI=4) of ASGP group. The difference was not significant (p=0.9). We did not find any significant difference in the prevalence of cardiovascular and pulmonary systems involvement between the two groups. There were two cases of renal amyloidosis in ASGP group while no cases were encountered in the ASBD group.

The comparison of laboratory findings between the two groups showed no significant difference in the prevalence of HLA-B27 (44.5%, CI=17.2 vs. 46.5%, CI=12). The frequency of high ESR (60%, CI=14.5 vs. 73%, CI=7.5), positive CRP (58%, CI=19.3 vs. 75.5%, CI=7.9), and radiographic changes of sacroiliac joints (89.4%, CI=10.4 vs. 96.4%, CI=5) was higher in ASGP group with significant difference (Table 2).

Table 2. Comparison (laboratory findings)

Tests	ASBD*	ASGP˜	p
HLA-B27	44.5	46.5	NS
High ESR	60	73	<0.02
Positive CRP	58	75.5	<0.02
Radiographic changes of sacroiliac joints	89.4	96.4	<0.02

*ASBD: Ankylosing spondylitis in Behçet's disease
˜ASGP: Ankylosing spondylitis in general population

Radiographic changes (unilateral or bilateral) were graded from I to IV according to the modified New York criteria in both groups (Table 3).

Table 3. Radiographic changes of sacroiliac joints

Radiographic changes	ASBD*	ASGP~
Normal	10.6	3.6
Grade I	8.7	4.2
Grade II	28.8	24.7
Grade III	37.4	36.6
Grade IV	14.5	30.9
Unilateral involvement	13.5	7.5
Bilateral involvement	86.5	92.5

*ASBD: Ankylosing spondylitis in Behçet's disease
~ASGP: Ankylosing spondylitis in general population

4. DISCUSSION

We have previously demonstrated that AS is more frequent in BD patients than in the general population of Iran.

In this study we found that the features of AS in BD patients also differ from AS in general population. Male involvement, constitutional signs, morning stiffness lasting more than one hour, limitation of motion in lumbar spine, abnormal Schober test, dorsal and cervical involvement, high ESR, positive CRP, and radiographic changes of sacroiliac joints were all less frequent in AS of BD patients than in AS in general population of Iran.

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Gastro-Intestinal Manifestations of Behçet's Disease

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1. INTRODUCTION

Gastro-Intestinal (GI) involvement is considered a minor criterion of Behçet's disease (BD). The frequency of GI involvement varies widely in different ethnic groups; while one third of the Japanese patients (pts) have GI involvement[1], reports from the Mediterranean countries show that these manifestations are rare[2]. The aim of this study was to analyse the frequency of GI manifestations of BD in Jordan.

2. MATERIALS AND METHODS

The records of 295 pts fulfilling the ISG criteria for BD were reviewed for GI involvement. Pts were followed-up between 1994-2001. They were all of Arab descent. Mean age of pts was 31.2±9.3 years (y), mean age of disease onset was 25.8±8.9 y, and mean disease duration was 5.5±5.7 y. Male: female ratio was 2.8:1.

3. RESULTS

The frequency of the ISG criteria in 295 pts was as follows: *Major criteria* - ROU was present in 99%, genital ulcers in 83.4%, eye involvement in 43.7%, skin lesions in 90.8%, and positive pathergy test in 50.8%.

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Minor criteria - Arterial involvement in 2%, superficial thrombophlebitis in 10.2%, DVT in 19%, arthralgia in 41%, arthritis in 27%, CNS in 16%, pleuropulmonary lesions in 2%, epididymitis in 18.3%, and positive family history in 58.6%.

Among the 295 pts, 37(12.5%) experienced various GI manifestations; abdominal pain alone was seen in 7(2.4%), diarrhea in 4(1.4%), abdominal pain and diarrhea in 11(3.7%), upper GI bleeding in one, rectal bleeding in one, diarrhea mixed with blood in 7 patients (2.4%), melena in one, and occult bleeding in 2 pts (1.5%). Upper and lower endoscopy was performed in 14 pts, while 2 pts having diarrhea mixed with blood refused the procedure. Endoscopy results revealed negative findings in 6 pts, while the rest had the following: Gastric ulcers were found in 2 pts, duodenal ulcers in 2 pts, one pt had ulcers in the rectum while one pt had ulcers in esophagus and total colonic involvement, the remaining 2 pts had total colonic and terminal iliac involvement. Two pts had colonic perforation and required emergency colectomy. Biopsies from intestinal lesions showed non-specific inflammation. All pts with intestinal involvement were male.

4. DISCUSSION

GI involvement is regarded as one of the minor criteria of BD, the prevalence of this involvement varies among different ethnic groups.

12.5% of our pts experienced various GI symptoms and only 4 of them had intestinal involvement verified by endoscopy, which corresponds to reports from Iraq[2] and contrasts reports from Asia. In the latter, Japanese and Korean pts have more frequent GI involvement (30 and 41.3% respectively). Intestinal ulcers in BD tend to perforate[3]; 2 of our pts had intestinal perforation. The involvement of the ileocecal region could have been mistaken for Crohn's disease but the absence of the granulomatous changes and the presence of genital ulcers should favor BD. All pts who had intestinal involvement were male, reflecting a more severe course of the disease among males.

5. CONCLUSION

Symptomatic inflammatory bowel disease is not common in pts with BD from Jordan. Endoscopy must be performed in BD pts with GI symptoms.

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Gastrointestinal Disease in Behcet's Disease

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1. BACKGROUND

Behcet's disease (BD) is a systemic vasculitis of unknown etiology. Blood vessels of any size may be affected, including the aorta and its branches as well as veins of all caliber. Although a number of clinical manifestations of BD have been described, mucocutaneous lesions are seen most often, reflecting involvement of small vessels. The disease follows a highly variable clinical course, characterized by periods of remission juxtaposed between periods of active disease.

The highest incidence of the disease occurs in countries of the Eastern Mediterranean region, the Middle East, and the eastern Asian rim. The prevalence ranges from 300 per 100,000 in Turkey[1] to 0.5-3 per 100,000 in Europe[2,3], depending on the population. A prevalence rate of 6.6 per 100,000 has been reported in the United States[4]. The average age of onset of the disease is between 25 and 30 years[5,7]. The male:female ratio of patients in Eastern countries appears to be nearly 1:1, despite earlier reports of a male predominance. In Western countries, including the United States, the disease occurs more commonly in females[8]. There is a strong association with the HLA-B51 genotype in Eastern countries, but there does not appear to be such an association in western patients'.

A variety of clinical manifestations of BD have been described. Oral aphthous ulcers are the most common finding, and are usually the earliest manifestation of the disease. Similar lesions are commonly seen on the genitalia. Skin lesions associated with BD include erythema nodosum, pyoderma gangrenosum, and erythema multiforme, among others. Pathergy,

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or skin hyperreactivity, is strongly associated with the disease, especially in Eastern countries. Ocular disease, including uveitis and optic neuritis, occurs in up to 90% of patients, and can lead to blindness. Synovitis occurs in more than 40% of patients, and may affect the knees, ankles, hands, and wrists. Neurologic manifestations of BD include aseptic meningitis, stroke, and cerebrospinal fluid pleocytosis[7].

The gastrointestinal (GI) tract is not infrequently involved in patients with BD. The highest rate of GI involvement is seen in Japan, with a reported frequency between 50 and 60 percent[8-9]. A significantly lower rate, less than 5%, is seen in Turkish patients[10]. GI involvement has been reported in 8% of patients in the United States[11]. Any portion of the GI tract may be involved. The upper gastrointestinal tract is less frequently affected, but ulcerations in the esophagus and stomach have been reported[12]. These patients commonly present with symptoms of hematemesis, melena, and epigastric pain. However, the terminal ileum, cecum, and ascending colon are the sites more frequently affected, resulting in abdominal pain, diarrhea, and hematemesis. Kim et al. have described the colonscopic findings of 94 Korean patients with BD who presented with lower gastrointestinal complaints[13]. All of the patients had ulcerative lesions, with ileo-cecal involvement in all but three. The average ulcer size was 2.9 cm, and approximately two-thirds of the patients had single ulcerations. Complications of GI ulceration in BD may include perforation and enterocutaneous fistula. Surgical treatment is often necessary, but post-operative recurrence is as high as 68%[14]. In many cases it is difficult to distinguish intestinal BD from Crohn's disease. Lee et al. report that intestinal ulcerations due to BD tend to be more focally distributed and are more commonly round or oval compared to the more diffuse and irregular ulceration of Crohn's disease[15]. Rectal involvement may also occur, in which case the possibility of ulcerative colitis may be considered in the differential diagnosis[12]. Despite a growing recognition of GI involvement in BD, the natural history of patients with GI lesions, especially in the United States, is not well known.

2. METHODS

One hundred and sixty-four consecutive patients seen at the Mayo Clinic over a 13-year period (1985-1997) were clinically diagnosed with BD. The clinical records of these patients were reviewed, and those with GI involvement were identified. The clinical features of this subgroup of patients were examined. Patients were not prospectively evaluated.

3. RESULTS

Thirteen patients with both BD and GI disease were identified, representing 8% of the total 164 patients. Two additional patients were infected with the hepatitis C virus but did not have involvement of the GI mucosa. Ten of these thirteen patients were evaluated with gastrointestinal endoscopy or surgical exploration. Seven of these were female, and the average age of disease onset was 26 years. Clinical features of the thirteen patients are listed in Table 1.

Table 1. Clinical features of patients with BD and gastrointestinal disease

No	Sex	Age of Onset of BD	Age of Onset of GID	First Manife-station	GI signs	Anatomic Location of GID	Endoscopic or surgical findings	Pathologic findings	Surgical Treat-ment
1	M	20	40	Oral ulcers	Abdom-inal pain	Terminal ileum Cecum	4 cm mass in cecum	Inflammatory tissue without granulomas	Right hemico-lectomy
2	M	18	18	Chronic diarrhea age 18 Oral ulcers age 25	Diarrhea	Entire colon	Granular mucosa entire colon	Chronic active colitis without granulomas	None
3	F	42	54	Oral ulcers	Abdom-inal pain	Stomach Pancreas	Erosive gastritis		None
4	F	14	14	Hematochezia, diarrhea age 14 Arthritis age 40 Genital ulcers age 43	Hemato-chezia, abdom-inal pain	Entire colon			Total cole-ctomy
5	F	33	49	Oral ulcers	Hemato-chezia	Stomach Duodenum Terminal ileum Entire colon	Antral erosions Single 5 mm ulcer terminal ileum Multiple panco-lonic ulcers <1 cm	Apthous-type ulcers without granulomas	Procto-col-ectomy
6	F	40	41	Oral ulcers	Abdom-inal pain	Stomach	Gastric ulcers		None
7	F	15	15	Oral ulcers, hematochezia	Hemato-chezia Diarrhea	Esophagus Stomach Ileocecal	Esophageal ulcers Antral erosions Multiple ileo-cecal ulcers	Apthous-type ulcers without granulomas	Ileal re-section
8	F	16	30	Oral ulcers	Diarrhea Abdom-inal pain	Descending colon	Multiple ulcers descending colon		None
9	F	27	32	Oral ulcers	Abdom-inal pain	Stomach	Three gastric ulcers		None
10	M	36	38	Oral ulcers	Abdom-inal pain	Ileo-cecal	Terminal ileitis	Acute and chronic ileitis without granulomas	None
11	M	10	39	Oral ulcers	Diarrhea				None
12	M	13	25	Oral ulcers	Diarrhea				None
13	M	33	43	Oral ulcers	Diarrhea				None

GID=Gastrointestinal disease; GI=Gastrointestinal

Only one patient had involvement of the esophagus, with upper gastrointestinal endoscopy demonstrating multiple esophageal ulcerations. This patient, as well as four others, had gastric involvement as well, with erosions or ulcers seen on endoscopy. The antrum was the site of gastric involvement in three patients. The average age at the time of onset of these upper GI manifestations was 38 years. In four of these five patients the GI manifestations occurred after other manifestations of BD. The patient with esophageal ulcerations was the only patient of these five with the onset of GI

symptoms within one year of other symptoms of BD. All of the patients with gastric involvement were women. None of these patients required esophagectomy or gastrectomy.

Excepting the terminal ileum, the small bowel was involved in only one patient. In this case a small bowel X-ray demonstrated irregularity in the duodenum and polypoid changes in the middle portion of the small intestine. This patient presented with hematochezia at the age of 49 years, after developing oral ulcers at the age of 33 years. She was also found to have pancolonic involvement and eventually underwent proctocolectomy.

The ileo-cecal region and colon were the sites most frequently involved, with seven of the ten patients investigated being affected in these areas. All patients with colonic involvement also had oral ulcerations at some point in the course of their disease. The average age at the time of onset of ileo-cecal or colonic disease was 29 years. Within this subgroup, GI disease occurred at the same time or within a year of other manifestations of BD in three patients, all of whom were teenagers. Four of these seven patients were women. Hematochezia occurred commonly but was not a universal symptom among patients with colonic involvement. Four patients required colectomy or ileal resection.

Involvement of the esophagus and/or stomach as well as the colon occurred in two patients. One patient who had gastric erosions also experienced recurrent pancreatitis. Endoscopic retrograde pancreatography performed in this patient was unrevealing, and the cause of the patient's pancreatitis was not identified.

4. DISCUSSION

Eight percent of patients seen at the Mayo Clinic over a thirteen-year period with BD had GI involvement. Our findings are consistent with previous reports of a low rate of GI involvement in non-Japanese patients[10]. The ileo-cecal region and colon were the anatomic sites most frequently involved, which is also consistent with past reports. Seven of the thirteen identified patients were women, reflecting the slight female predominance among Western patients. Interestingly, all of the patients with esophageal or gastric involvement were women. The male:female ratio among patients with colonic involvement was 3:4. A previous report of five patients with upper GI involvement describes a nearly equal male:female ratio[12]. Further studies with larger sample sizes will be necessary to determine if female patients truly develop upper GI disease more commonly than men.

The average age of disease onset in these thirteen patients was 24 years, and the average age of onset for the patients who underwent endoscopy or

surgical evaluation was 26 years. These findings are consistent with previous reports[5-7]. The average age of onset for any manifestations of BD was 31 years in the patients with upper GI involvement, and 21 in the patients with ileo-cecal or colonic involvement. The average age of onset for GI symptoms was 39 for patients with upper GI involvement and 29 years for the patients with lower GI involvement. This suggests that patients who will eventually have ileo-cecal or colonic disease tend to develop both BD and GI manifestations at a younger age than those who will have esophageal or gastric disease. Of the patients with upper GI involvement, two experienced GI symptoms within a year of onset of other manifestations of BD, and one of these patients also had ileo-cecal involvement. Including this patient, three out of seven patients with lower GI involvement experienced GI symptoms within a year of onset of BD.

Regarding the distribution of colonic lesions, our findings were consistent with those of Lee et al. in that most of the colonic ulcers were confined to one section of the colon in any given patient[15]. One patient had an inflammatory mass in the ileo-cecal region, a finding that has not been frequently reported. Pathologic data, when available, demonstrated ulceration or inflammation without granulomas. The presence of granulomas on biopsy specimens can aid in identifying those patients with Crohn's disease, but in many cases it is difficult to distinguish BD from idiopathic inflammatory bowel disease. Indeed, many of the extraintestinal manifestations of Crohn's disease, including erythema nodosum, inflammatory arthritis, and ocular lesions are also seen in BD.

One of the patients experienced recurrent pancreatitis as well as gastric erosions. A case of acute pancreatitis occurring in a patient with BD was described by O'Duffy and colleagues in 1971[16]. This patient developed acute pancreatitis one week after the onset of bilateral iridocyclitis. She subsequently developed other manifestations of BD, including oral and genital ulcerations. She did not have any further episodes of pancreatitis, in contrast to the patient in our study, who experienced recurrent bouts of acute pancreatitis. Pancreatitis has not been a commonly reported manifestation of Bd, and it is impossible to say whether pancreatitis occurring in either one of these patients was truly due to BD. An alternative cause for the pancreatitis was not determined in either case.

Two patients with BD were infected with the hepatitis C virus without involvement of the GI mucosa. There has not been a previously reported association between hepatitis C infection and BD. These findings most likely reflect random coincidence.

Therefore, based on these findings, it may be suggested that women with BD are more likely to develop involvement of the upper GI tract than men. Also, it appears that patients with ileo-cecal and/or colonic involvement tend

to exhibit signs and symptoms of BD as well as GI manifestations earlier than those with esophageal or gastric involvement. All examined patients with colonic involvement also had oral ulcers at some point during the course of their disease. Larger studies will be required to determine if these trends truly reflect the natural history of GI disease in patients with BD.

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Clinical Features of Behçet's Disease Patients with Epididymitis

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1. INTRODUCTION

Behçet's disease is a chronic idiopathic syndrome involving multi-organs, mainly vasculitic in nature, of which mechanisms or causes still remain to be proven. Main symptoms of the disease include recurrent oral aphthous ulcers, recurrent genital ulcers, ocular inflammation commonly presenting as uveitis, and cutaneous manifestations such as erythema nodosum. Minor symptoms vary from arthritis, vasculitis, ulceration of gastrointestinal tract, and epididymitis, to cardiovascular and CNS involvement such as meningoencephalitis. Although many clinical branches of modern medicine have stated in textbooks and described the individual features of the disease, we could hardly find a description of epididymitis related to Behçet's disease even in the fields of dermatology or urology. The authors have diagnosed 780 male patients with Behçet's disease at the Behçet's Disease Specialty Clinic, Yonsei University College of Medicine and Ajou University School of Medicine; 36 of them turned out to have either medical history of epididymitis in the past or clinical symptoms of epididymitis examined by a physician.

The authors intended to establish a clinical significance of epididymitis, based on thorough investigation into the clinical features of this group of patients.

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2. PATIENTS AND METHODS

About 7,000 patients have visited the Behçet's Disease Specialty Clinic, Yonsei University College of Medicine and Ajou University School of Medicine from 1985 to 2002. We have included a total of 36 patients with epididymitis in the study, 16 showing clinical symptoms of epididymitis examined by physicians at our clinic, and 20 having either given reliable self-reports on the symptoms or been diagnosed and treated at other clinics. The group of patients with epididymitis was selected from the population of the 780 male patients who fulfilled the criteria set by International Study Group for Behçet's disease, or met the Revised Criteria of Behçet's Disease Research Committee of Japan as complete or incomplete forms of Behçet's disease. As control, 744 Behçet's disease patients without epididymitis were selected. Each and every patient who had reports of symptoms suggesting epididymitis, such as pain and edema on the epididymis, resolving within a few days regardless of treatment with exclusion of other possible causes including infection by urologic evaluation, was analyzed of his clinical features obtained by a review of the medical records. Chi-square tests and Student's t test were used for statistical analysis. The results were considered significant when the p-value was less than 0.05.

3. RESULTS

The mean age of onset of any manifestation indicating Behçet's disease was 27.4 years (range 15-51), and the prevalence of epididymitis was 4.6%, i.e., 36 out of 780.

Thirty patients (83.3%) had oral ulcers as an initial presenting symptom, and genital ulcer, ocular involvement, and cutaneous involvement presented as initial manifestation in 2 patients each (5.6%). By the Revised Criteria of Behçet's Disease Research Committee of Japan, 13 were classified as complete form (36.1%), and 23 as incomplete (63.9%). In descending order of frequency, oral ulcer and cutaneous symptoms were observed in all 36 patients, genital ulcers in 32 (88.9%), arthritis in 18 (50.0%), ocular involvement in 17 (47.2%), neurologic symptoms in 2 (5.6%), gastrointestinal ulceration in 1 (2.8%), and pathergy test, depicting the cutaneous hypersensitivity, was positive in 4 patients (11.1%) (Table 1).

Table 1. Comparison between the characteristics of Behçet's disease patients with epididymitis and without epididymitis

		With epididymitis (n=36) Number (%)	Without epididymitis (n=744) Number (%)	P-value
Classification	Complete	13 (36.1)	197 (26.5)	
	Incomplete	23 (63.9)	542 (72.8)	
Frequency of involvement (major symptom)	Oral ulcer	36 (100.0)	736 (98.9)	
	Genital ulcer	32 (88.9)	548 (73.7)	< 0.05
	Eye lesion	17 (47.2)	485 (65.2)	< 0.05
	Skin lesion	36 (100.0)	540 (72.6)	< 0.01
Frequency of involvement (minor symptom)	Joint lesion	18 (50.0)	230 (30.9)	< 0.05
	CNS lesion	2 (5.6)	7 (0.9)	< 0.05
	GI lesion	1 (2.8)	35 (4.7)	
	Vascular lesion	0 (0)	25 (3.4)	
	Pathergy test	4 (11.1)	23 (3.1)	< 0.05

4. DISCUSSION

Epididymitis is a clinical syndrome consisting of various inflammatory symptoms such as edema and pain at the site of epididymis which can result in abscess formation, testicular infarction, chronic pain, and even infertility in severe cases. Epididymitis as a manifestation of Behçet's disease had not come to much attention yet because of its rarity and infrequent complications.

Previously reported prevalence of epididymitis in Behçet's disease is 6%[1], 12.3%[2], 19.2%[3], and 31%[4] respectively, with lowest at 2%[5,6], and highest at 44%[7] in literature. One report[2] claims a higher incidence in adolescents (25%) than in adults (8.9%), though most of the other reports show no significant differences. In this study, 36 patients were diagnosed of epididymitis out of 780 male patients with resultant prevalence of 4.6%.

Kaklamani et al.[2] have put into analysis the clinical features of seven patients with epididymitis; all 7 patients had symptoms of ocular and cutaneous involvement, and neurologic involvement was observed in 4, and positive pathergy test in 4. All seven patients were carriers of HLA B5(51). In our study, the patients presenting with epididymitis had higher prevalence of cutaneous involvement (100.0% vs. 72.6%), genital ulcer (88.9% vs. 73.7%), and arthritis (50.0% vs. 30.9%) than 744 Behçet's disease patients without epididymitis. Thirteen patients out of the group of 36 have satisfied the criteria set by Behçet's Disease Research Committee of Japan as complete forms. The percentage of complete form in the population of complete and incomplete forms was 36.1%, which is higher than 25.0%

previously reported by Bang et al.[8]. It can be concluded that Behçet's disease with epididymitis tends to involve more organs than otherwise.

In addition, we found that epididymitis is more likely to present in advanced disease rather than as initial symptom. In contrast to the finding that 36 out of 780 patients with incomplete or complete forms of Behçet's disease had epididymitis, only 1 patient out of about 1500 patients who had been diagnosed of the suspected forms or meeting only one criterion of Behçet's disease such as oral aphthous ulcer, had epididymitis. Epididymitis as an initial presenting manifestation is known to be rare[9], and our study agreed on that point. Most common initial symptom seems to be oral ulcer (83.3%), genital ulcers, ocular disease, and cutaneous symptoms fill the rest.

Up to now, no possible mechanism for the development of epididymitis in Behçet's disease was confirmed. A vasculitic nature has been postulated but no histological confirmation was made in the literature. Epididymitis as a manifestation of Behçet's disease seldom progresses to cause urologic complications or sequelae, and most patients can do without therapeutic intervention, experiencing improvement of symptoms in a matter of few days whereas some require anti-inflammatory medications.

However, the cases with frequent recurrences cannot be controlled by these conservative measures, and no standard regimens for relapses are given. A few minor successes have been reported using cytotoxic drugs such as azathioprine, cyclosporine, dapsone, and colchicine[10] for such cases.

In conclusion, epididymitis, although its incidence is not that high, must be considered one of the important diagnostic and prognostic factors in Behçet's disease, especially in the prevalent countries.

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Tonsillectomy and Behçet's Disease

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1. INTRODUCTION

Increasing development of several autoimmune and malignant diseases (such as rheumatoid arthritis[1], multiple sclerosis[2], Hodgkin lymphoma[3]) was reported after tonsillectomy. Cooper et al have reported increased risk of Behçet's disease (BD) associated with tonsillectomy[4].

This study was designed to investigate the association between tonsillectomy and/or adenoidectomy (T/A) and subsequent development of BD, and its possible effects on disease expression.

2. MATERIALS AND METHODS

In a prospective case control study, 128 consecutive patients with BD (according to Iran or Japan criteria) and 139 non-BD controls from the orthopedic clinic (age and sex matched) were studied regarding previous T/A. A complete ENT clinical examination was performed for all of them. A computerized form, including 68 clinical and paraclinical data, was used to collect data about this examination and different manifestations of the disease. Comparison was made between 2 groups by chi square test and Fisher test. A confidence interval at 95% (CI) was calculated for each item.

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3. RESULTS

T/A was found in 6 patients with BD (4.7%, CI:3.8) and 10 controls (7.2%, CI:4.2) with no significant difference (p=0.26). Positive history of recurrent throat infections was significantly higher (p=0.005) in BD patients (27%, CI:7.7 vs. 13%, CI:5.6). This was true for herpes simplex infection (39%, CI:8.4 vs. 30%, CI:8.1), but not statistically significant (p=0.93). No difference was found in the prevalence of tonsils (17%, CI:6.5 vs. 17%, CI:6.2, p=0.99) or adenoids hypertrophy (4%, CI:3.4 vs. 1%, CI:1.7, p=0.18). Different manifestations of the disease did not show any significant difference between BD patients with or without antecedent T/A (Table 1).

Table 1. Comparison of clinical manifestations between patients with or without antecedent tonsillectomy/adenoidectomy (T/A)

	T/A+	T/A-	P
Oral aphthosis	100	98	0.12
Genital aphthosis	67	70	0.99
Skin lesions	67	61	0.70
Ocular lesions	67	52	0.89
Joint involvement	33	20	0.54
Vascular lesions	17	7	0.85
CNS involvement	0	8	0.13
GI involvement	0	3	0.09

4. DISCUSSION

In conclusion, these data suggest that T/A was neither causally related to the development of BD, nor to the manifestations of the disease. The higher rate of recurrent throat infections in BD patients is due to the pathogenic role of infections, acting as a trigger, in this disease.

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Destructive Sinusopathy and Middle Ear Involvement in Behçet's Syndrome

A case report

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1. INTRODUCTION

Behçet's syndrome is a multisystemic disease with internationally agreed diagnostic criteria[1]. Vascular, central nervous system (CNS) and gastrointestinal (GI) involvement have been documented[2]. We report a first case with destructive sinusopathy and middle ear involvement on a 47-year-old Caucasian male, studied for recurrent oral ulcerations. Previously healthy, he suffered from recurrent oral ulcerations and dysphagia from 1995 to 1998. In 1998, nasal discharge and facial "congestion" started together with right side ear pain without hearing loss. During 1999 right-side repletion and hearing loss sensation began. In 2000-2001 the patient suffered a crisis with ear, nose and throat (ENT) symptoms, dysphonia, strength-loss and worsening of muco-cutaneus lesions; recurrent genital ulcerations and subcutaneous nodules. There was never eye, CNS or GI involvement.

2. DIAGNOSIS, INVESTIGATION AND DISCUSSION

Behçet's syndrome was diagnosed on the basis of the negative biochemical and immunological results, positive pathergy test, recurrent oral ulcerations, and the presence of both recurrent genital ulcerations and skin lesions. Additional periodontal disease and dysphonic laryngitis were

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diagnosed (laryngoscopy showed glottis oedema and a 1-2 mm white lesion on the right ventricular band). On the basis of synchronous symptoms we investigated two associations: ear involvement with hearing loss and rhinitis with sinusopathy.

Rhinitis with sinusopathy was diagnosed based on rhinoscopy showing hyperaemia with no focal lesion and perinasal sinuses CT-scan images compatible with chronic pansinusopathy of ethmoido-maxilar predomination, with destructive characteristics (amputation of the conchae and sept perforation). ENT findings were: normal otoscopy; positive hearing tests for mixed conductive and sensory hearing loss of light degree on the left side and of moderate degree on the right side. CT scan of the temporal bones showed bilateral middle ear involvement; right side occlusion of the oval and round windows by liquid collection; no signs of cholesteatoma or of ossicle destruction, compatible with bilateral medial chronic otitis, particularly on the right ear. It is known that sensory hearing loss is common in Behçet's syndrome as part of neural involvement[1,3] and that in several diseases, external, middle or inner ear structures are subject to immunological injury[4]. What seems strikingly new in this case is the existence of a chronic bilateral middle ear otitis, especially on the right ear, which could explain the conductive component of the patient hearing loss. CT-scan images, hearing tests and clinic show a good correlation, especially on the right side. We cannot yet prove that Behçet's syndrome is the cause but it seems acceptable that part of these complaints can be attributed to this non-sensory hearing loss component. To our knowledge there is no published material about destructive sinusopathy in Behçet's syndrome, and one of the few diseases that show these features is Wegener's granulomatosis which is excluded in this patient. Without a reliable histological marker for the nasal sinus biopsy histological evaluation, a clear documentation of the association between Behçet's syndrome and this entity will have to be postponed.

3. CONCLUSIONS

Dysphonic laryngitis caused by a documented lesion and correlated with a typical clinical picture was present in this case. Rhinitis with destructive sinusopathy caused by Behçet's syndrome is most probably present in this patient; given a reliable histological marker a nasal sinus biopsy may come to support this conclusion. Though middle ear involvement with chronic bilateral middle ear otitis is undoubtedly proved in this patient, no histological confirmation can be presented.

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Urine Abnormalities in Behçet's Disease

Study of 4704 cases

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1. INTRODUCTION

Despite the multi-systemic nature of Behçet's disease (BD), renal involvement is rare in this disease[1]. However, various involvements including amyloidosis[2], focal and diffuse proliferative glomerulonephritis (with or without sclerosis)[3], crescentic glomerulonephritis[3], and IgA nephropathy[4] have been reported in association with BD. As with other manifestations of the disease, prevalence varies widely due to ethnic variations or environmental factors[1].

The aim of this study was to investigate the prevalence and characteristics of urine abnormalities and renal involvement of BD in Iran, and their effects on disease expression.

2. MATERIALS AND METHODS

In a cohort of 4704 patients with BD, urinalysis was performed to screen patients with urine abnormalities. Different manifestations of the disease, including 100 clinical and paraclinical parameters, were determined in these patients and compared with the remaining group of patients (with normal urin alysis) by chi square test, and corrected by Fisher exact test. A confidence interval at 95% (CI) was calculated for each item.

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3. RESULTS

Urinalysis has been performed at least once in the course of disease in 4386 patients. Abnormal urine was found in 475 (10.8%, CI:0.9) patients.

Proteinuria was seen in 101 patients (2.3%, CI:0.4). It was present as pure proteinuria in 46, with hematuria in 42, and with renal casts in 8 cases. It was related to joint ($p=0.00001$), GI ($p<0.0008$), and vascular lesions ($p<0.02$), high ESR ($p<0.00003$), and false positive VDRL ($p=0.000001$).

Hematuria was seen in 222 cases (5.1%, CI:0.6). It was associated with renal casts in 8 cases. Pure hematuria was seen in 148 patients. It was related to genital aphthosis ($p<0.02$), retinitis ($p<0.03$), joint ($p<0.0002$), GI ($p<0.004$), CNS ($p<0.02$) lesions, high ESR, and positive VDRL ($p<0.02$).

Renal casts were seen in 13 cases (0.3%, CI:0.2). It remained as the sole urine abnormality only in 2 cases, and was associated with other abnormalities in the remaining cases. It was related to arthralgia ($p<0.007$) and high ESR ($p<0.02$).

Urine abnormalities were transient in most cases. Kidney biopsy was performed in only 14 of such cases, showing mesangial glomerulonephritis (GN) in 3, focal and diffuse proliferative GN each in 5, and amyloidosis in 2 cases.

4. DISCUSSION

Renal involvement is rare in BD patients, although transient urine abnormalities are not uncommon. These abnormalities are related to joint and GI involvement, high ESR, and false positive VDRL. Amyloidosis is rare in Iranian patients.

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TREATMENT

Behçet's Syndrome: From Aetiology to Treatment

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1. AETIOPATHOGENESIS

Behçet's syndrome is an inflammatory disorder of unknown cause, pathologically characterised by a vasculitis, with a predilection for small venules. A T-helper 1 polarisation of the immune response has been suggested[1]. Hulûsi Behçet himself suggested herpes simplex as an aetiological agent and more recently, superantigens, heat shock proteins and streptococcal antigens have been suggested as putative aetiological factors.

Clustering of cases along the ancient Silk Road is often cited to support a genetic basis for Behçet's syndrome, but that is clearly not the whole story. The prevalence of Behçet's syndrome amongst Turks in Germany is lower than that of Turks in Turkey, but is higher than that in the indigenous German population[2], suggesting a strong environmental influence. Similarly, the rarity of Behçet's syndrome in Japanese migrants in Hawaii and California contrasts with the higher incidence in mainland Japan. A family history is present in up to 12% of non-caucasoid cases, and a sibling risk ratio of 11.2 is reported[3, 4].

The genetic susceptibility to Behçet's syndrome has been linked with HLA-B51[5], MIC-A6[6] and more recently, 6p22-23[7]. However, HLA-B51 is not the elusive "Behçet's gene", as there is a marked geographical variation in relative risk, up to 75% of patients worldwide are HLA-B51 negative, and HLA-B51 transgenic mice do not develop the Behçet's phenotype. Nevertheless, these disease markers are all adjacent to TNFα, suggesting a key role for this cytokine. This is supported by the efficacy of anti-TNF

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agents including thalidomide and infliximab, and the increased production of TNFα by Behçet's syndrome monocytes and γδ T-cells[8].

2. TREATMENTS

2.1 Corticosteroids

These are indicated for the treatment of active Behçet's syndrome; however, no controlled studies have been performed. Long term, low dose steroid use is often a necessary compromise, but should be avoided where possible.

2.2 Colchicine

In a randomised, double-blind, placebo controlled trial (RBDPCT) involving 84 patients, 72% completed the 24 month study comparing colchicine l-2mg per day with placebo. Colchicine treatment revealed benefit in genital ulcers, erythema nodosum, arthritis for women more than men, and was deemed best for mild disease[9].

2.3 Penicillin

In a RDBPCT, the influence of adding prophylactic penicillin treatment to colchicine therapy on the course of arthritic episodes was investigated[10]. The number of episodes of arthritis was reduced in the penicillin arm, however, the duration, severity and pattern of arthritis was similar.

2.4 Azathioprine

A RDBPCT of azathioprine in Behçet's Syndrome using 2.5mg/kg body weight/day for two years, recruited 48 males with eye involvement and 25 without. Corticosteroid treatment remained available to all of the patients during the trial. Azathioprine was shown to be superior to placebo in the prevention of the onset of new eye disease, and there were fewer episodes of hypopyon uveitis amongst those with established eye disease[11]. Furthermore, patients taking azathioprine had less frequent oral and genital ulcers and arthritis. An eight year follow up of this cohort demonstrated that early treatment with azathioprine favourably affected the long-term prognosis, especially in those with recent onset eye disease[12]. It would be interesting to

audit how many Behçet's patients currently receive azathioprine to prevent the onset of new eye disease!

2.5 Chlorambucil

Chlorambucil is indicated for the treatment of active Behçet's syndrome; however, no controlled studies have been performed. Long term, low dose steroid use is often a necessary compromise, but should be avoided where possible.

2.6 Cyclosporin and Tacrolimus

Cyclosporin has been shown to be effective in open studies in small numbers of patients[14], however flares do occur when the drug is withdrawn.

Tacrolimus (FK506) has been reported to be effective in a small case series of Behçet's posterior uveitis refractory to Cyclosporin[15]. Sirolimus, a non-calcineurin dependent cytokine antagonist, has theoretical possibilities for the treatment of Behçet's syndrome.

2.7 Interferon α-2a

Fifty patients were treated in a DBRPCT using 6×10^6 i.u. three times per week for three months versus a placebo. There was decreased pain and duration of oral ulcers, frequency of genital ulcers and papulopustular lesions[16].

2.8 Methotrexate, Levamisole and Pentoxifylline

Methotrexate[17], pentoxifylline[18] and levamisole[19] have been suggested to be effective agents in the treatment of Behçet's syndrome, but only in a small number of cases. No formal RDBPCTs have been undertaken.

2.9 Thalidomide

Thalidomide has many anti-inflammatory/immunological effects, and most recently has been shown to be a co-stimulator of human T-cells in vitro, CD8>CD4, and also to inhibit NFκB through suppression of IκB kinase activity, a key regulator of TNFα and IL-8[20]. A RDBPCT of 96 males, showed significant improvement in oro-genital ulcers and follicular lesions in both the 100mg and 300mg per day treatment groups[21]. Unfortunately, polyneuropathy was a problem even on the lower dose, and

there was an increase in the frequency of attacks of erythema nodosum. A recent prospective study of 135 dermatological patients showed that the risk of neuropathy was related to the daily dose regardless of treatment duration, however, this risk was negligible at doses below 25mgs per day[22]. There are published guidelines for the U.K.[23] and the U.S.A. for the use of thalidomide[24].

3. ANTI-TNFα AGENTS

3.1 Infliximab

Infliximab is a chimeric anti-TNFα monoclonal antibody that binds soluble and trans-membrane forms of TNFα. This agent is highly effective in the treatment of rheumatoid arthritis and Crohn's disease[27-29], but is contraindicated in cases with infection, particularly *mycobacterium tuberculosis*[30,31].

In the last year, the beneficial effects of infliximab in five patients with sight threatening panuveitis in Behçet's syndrome have been described[32]. In this report, following a single infusion of 5mg/kg, there was an improvement at 24 hours with complete suppression at seven days. All eyes were satisfactory at four-week follow up. In a single case report, infliximab has been shown to benefit colonic ulcers, again with a satisfactory follow-up at four weeks[33]. A further report of a 40 year old male with severe oro-genital ulceration and retinal vasculitis who received l0mg/kg at four weekly intervals was in remission at twelve months having been greatly improved two weeks following the first injection[34]. Finally, infliximab in the treatment of soft tissue inflammation in Behçet's syndrome has shown benefit for ankle synovitis and panniculitis[35]. Our own clinical experience in five patients supports the beneficial effects of infliximab therapy. A RBDCT of infliximab in Behçet's syndrome is urgently required.

3.2 Etanercept

A RDBPCT trial of etanercept 25 mg subcutaneously two to three times a week showed benefit for mucocutaneous lesions and treatment resistant uveitis[36].

4. THE FUTURE

Agents such as infliximab, etanercept and even sirolimus, a functional cytokine antagonist hold much promise. Hence, the prospect of controlling the severe complications of Behçet's syndrome is now a reality. Optimal treatment regimes need to be established to define whether biological agents such as infliximab should be given on a regular basis or whether they can indeed be given as single infusions when required.

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Treatment of Ocular Manifestations of Behçet's Disease

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1. INTRODUCTION

Ocular manifestations are one of the main features of Behcet's Disease (BD). They occur in more than half of the patients. In our series they were seen in 58% of cases[1]. The natural course of ocular manifestations, like other manifestations of BD, is a progression by repetitive attacks followed by remission. However, in contrast to other lesions (mucocutaneous), the remission is much slower in posterior uveitis, and practically absent in retinal vasculitis. Usually the healing process is not completed before the next attack. Therefore, lesions accumulate during successive attacks and lead to severe loss of vision, or blindness[2].

A treatment will have 2 goals: 1) Combat the inflammation. Prednisolone is the best choice, 1/2 to 1 mg/kg/daily is the necessary dose. 2) Control the autoimmune disorder and prevent from further attacks. There is a large choice of drugs on this purpose. Immunomodulators such as cyclosporine A, many cytotoxic drugs (cyclophosphamide, chlorambucil, azathioprine, methotrexate), and recently biologic agents (Interferon-α, anti-TNF drugs) can meet these requirements.

The treatment must start as early as possible. Any delay of the treatment will cause a drop of visual acuity which will not recover completely under the treatment. However, it is important to note that the treatment will always remain efficient even in those patients with a visual acuity as low as finger count, or even hand movement[3-4].

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We have shown in several studies that cytotoxic drugs have approximately the same efficacy in ophthalmological lesions of BD[5-17]. Biologic agents[18-20] seem to work well with ocular lesions, however, their data are not standardised, and it is impossible to compare them with cytotoxic drugs.

2. DISEASE ACTIVITY INDEX

A disease activity index (DAI) has to be calculated for each section of each eye, at each visit. The calculation is based upon the inflammatory state of the eye. The severity of the inflammatory indices is graded from 0 to 4. These indices are determined as follows: Anterior uveitis (AU): cells, flare, keratic precipitate and hypopyon in the anterior chamber. Posterior uveitis (PU): cells, snow ball and snow banking in the posterior chamber. Retinal vasculitis (RV): periarteritis, periphlebitis, edema of disk and macula and retina, papillitis, and active peripheral lesions in retina. The visual acuity (VA) as an overall index of the eye function was determined by the Snellen chart. VA is influenced by the inflammatory state of the eye, and complications such as synechia, cataracts, vitreous organization, and retinal scars.

For an overall patient's inflammatory index (*Total Inflammatory Activity Index*) a coefficient of gravity was given to each section of the eye. For AU it was 1, for PU it was 2, and for RV it was 3. The index was calculated as follows: *TIAI= Right [(AU×1) + (PU×2) + (RV×3)] + Left [(AU×1) + (PU×2) + (RV×3)].* Also, an overall patient's evaluation (Total Adjusted Disease Activity Index) was calculated by giving an appropriate coefficient of gravity to VA, and adding it to the TIAI as follows: *TADAI= TIAI + [(10 - right VA) ×2] + [(10 – left VA) ×2.*

3. CYTOTOXIC DRUGS

Cyclophosphamide can be used in 3 different forms: 1) The classical way is by oral administration (OCP)[11]. The dose is 2 to 3 mg per kg body weight, to be taken every day. Blood count must be performed regularly due to suppression of bone marrow. Hemorrhagic cystitis is one of the major side-effects encountered with this mode of administration. 2) Pulse cyclophosphamide (PCP)[5-6]. It is used by perfusion as 1 g cyclophosphamide per m^2 body surface. Cyclophosphamide is mixed with 1000 ml serum saline and infused slowly upon one hour. The main side-effect is nausea and vomiting, which can be avoided by keeping the stomach empty and giving

repeated perfusions over the first 24 hours. Tranquilizers may be of help. Hemorrhagic cystitis and dangerous suppression of bone marrow is exceptional. The pulse is repeated once a month until a good result is achieved. The gap between pulses is increased to 2 months, and then to 3 months, and finally stopped when the disease seems to be in remission. 3) Low dose pulse cyclophosphamide (LDP)[7]. The method is the same as for PCP, only the dosage of cyclophosphamide is half of it (0.5 gr per m^2 body surface).

Methotrexate (MTX) is used as low dose weekly pulses of 7.5 mg orally[9-10]. MTX is given as tablets of 2.5 mg per day, in one intake or 3 separated intakes. MTX is well tolerated. Laboratory tests for liver function (SGOT, SGPT) and blood count may be performed every 2 to 3 months. One of the advantages of MTX is that it can be continued over a long period of time without any serious side-effect.

Chlorambucil (CHL)[15-17] is used by oral administration. It is one of the oldest cytotoxic therapies for ocular lesions of BD. It is used as 0.2 mg per kg body weight per day. As with oral cyclophosphamide, close monitoring of blood cell counts is necessary.

Azathioprine (AZA)[13,17] is a well known cytotoxic drug, easy to use and manage. It is given as 2 to 3 mg per kg per day by oral root. It needs blood cell count monitoring, but less than cyclophosphamide. It is an easy cytotoxic drug to manipulate.

Cyclosporine A (CyA)[12,15-17,21,22] is an immunomodulator in use for tissue transplant and many autoimmune diseases like rheumatoid arthritis. In ocular lesions of BD it is used as 5 mg per kg daily. As soon as a therapeutic response is obtained the drug must be reduced to the minimum dose, keeping the patient in remission. There are major side-effects, especially nephrotoxicity which leads to renal insufficiency.

In severe cases, combination therapy with 2 cytotoxic drugs may be more efficient. The two combinations we have used are LDP-MTX and LDP-AZA. The doses are the same as when being used in single therapy.

4. TREATMENT STRATEGY

Before the treatment, a complete ophthalmological evaluation is mandatory. Different inflammatory indices and visual acuity (VA) have to be calculated.

As stated before, all 8 methods we evaluated in a same standardised protocol (OCP, PCP, LDP, MTX, CHL, AZA, CyA, LDP-MTX) have approximately the same efficacy. The important factor of which to choose is mainly the availability of the product and how easy it is to handle. Next

come costs of the drug and of regular controls regarding their side-effects. Another consideration is the presence of retinal vasculitis, which responds better to PCP or LDP than to the other methods.

Once the treatment protocol is selected, the patient will have prednisolone as 0.5 mg per kg daily by oral route at the same time. Repeated and regular ophthalmologic evaluation is mandatory. The first evaluation is scheduled in a month, or two months. When the inflammation is subsided prednisolone has to be tapered gradually. When prednisolone arrives at low doses (7.5 mg daily), the cytotoxic drug can be tapered to the minimum efficient dose. In case of relapse, an increase of the dosage may suffice, otherwise the protocol is started anew from the beginning.

Usually 70 to 75% of patients will respond favorably to the selected protocol. If after 3 to 6 months of treatment the patient's eye (eyes) does not respond to the treatment (non-responder), their treatment protocol has to be changed. The percentage of a favorable therapeutic response for the second protocol is the same as for the first protocol[23]. Non-responders have to change to a third protocol. Their chance to respond favorably is about 50%. Some of the non-responders may still profit from another change, although chances then reduce to not more than 30%.

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The Influence of the Delay of Aggressive Treatment on the Vision and its Outcome in Behçet's Disease

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1. INTRODUCTION

The natural course of eye lesions is well known in Behçet's disease (BD), leading to severe loss of vision or blindness. Eye lesions progress by attacks and remissions. The remission is usually incomplete. Therefore, from one attack to another, lesions accumulate and lead gradually to severe loss of vision, or blindness. Moreover, the healing process usually leaves sequelae.

The aim of this study was to evaluate the effect of the delay of aggressive treatment on the visual acuity (VA) and its long-term outcome.

2. MATERIALS AND METHODS

The study design was a cohort study. All Behcet's patients of the Ocular Treatment Registry (1009 on January 20, 2002) entered the study. All patients fulfilled the Classification Tree criteria for BD. All of them had active posterior uveitis (PU), and/or retinal vasculitis (RV) before treatment.

Patients were divided into subgroups according to the delay of aggressive treatment. Group 1: delay of less than one year; group 2: delay of 1 to 2 years; group 3: delay of 2 to 3 years; group 4: delay of 3 to 4 years.

The mean VA was calculated at the entry and after the last evaluation in each group by the Snellen chart on a scale of 10/10. The mean VA before and after the treatment were compared with the Student paired t test.

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3. RESULTS

Group 1 (429 impaired eyes): The mean follow-up was 20.8 months. The mean VA improved from 4.4/10 before the treatment to 5.6/10 after the treatment (t: 6.56, p <0.000001), 60% of the eyes improved (CI: 4.7), 12% remained stable (CI: 3.1), and 28% were aggravated (CI: 4.3).

Group 2 (340 impaired eyes): The mean follow-up was 16.8 months. The mean VA improved from 3.9/10 before the treatment to 4.5/10 after the treatment (t: 3.308, p: 0.00104), 49% of the eyes improved (CI: 5.3), 21% remained stable (CI: 4.4), and 30% were aggravated (CI: 4.9).

Group 3 (209 impaired eyes): The mean follow-up was 18.1 months. The mean VA improved from 3.4/10 before the treatment to 4.1/10 after the treatment (t: 2.999, p: 0.00304), 45% of the eyes improved (CI: 6.8), 22% remained stable (CI: 5.6), and 33% were aggravated (CI: 6.4).

Group 4 (115 impaired eyes): The mean follow-up was 20.6 months. The mean VA improved from 3.4/10 before the treatment to 4.2/10 after the treatment (t: 2.550, p: 0.0121), 55% of the eyes improved (CI: 9.2), 13% remained stable (CI: 6.2), and 32% were aggravated (CI: 8.6).

4. DISCUSSION

Although there was a delay of aggressive treatment in nearly all patients, they all received a treatment mainly under the category of intermittent steroids. The delay of aggressive treatment led to a decrease in mean VA as seen by the results in groups G1, G2, and G3. From that point, the mean entry value did not decrease any further. The delay of aggressive treatment did not impede the improvement course of the therapy, except for G6 where the improvement was not significant.

5. CONCLUSION

When ocular lesions occur in BD, an aggressive treatment must start as soon as possible. Any delay will cause a decrease of the visual acuity. The treatment will improve the VA in any case, but for best results in the final VA the treatment must be started immediately.

The Clinical Outcome and Treatment in Behçet's Disease with Deep Vein Thrombosis

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1. INTRODUCTION

In this retrospective study we evaluated 42 patients of our Behçet's Disease Clinic, all fulfilling the International Study Group (ISG) criteria. Seven patients were diagnosed as deep vein thrombosis both clinically and radiologically. We also evaluated their response to treatment both clinically and radiologically.

2. MATERIALS AND METHODS

42 patients with Behçet's disease fulfilling the ISG criteria were grouped in three groups, namely those presenting deep vein thrombosis (DVT), those with DVT + superficial vein thrombosis (SVT), and those with chronic venous insufficiency (CVI), and were assessed according to their demographic data, clinical prognosis and treatment. We also used radiological imaging techniques (Doppler US) during follow-up.

Treatment given to these patients was classified in three groups:

a) Colchicine + anticoagulants-antiaggregants
b) Corticosteroids + anticoagulants-antiaggregants
c) Immunosuppressives + anticoagulants-antiaggregants

Their response to treatment was summoned in three groups:

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a) Full response to treatment and no activation: Clinical full remission and no recurrence

b) Persistence of existing vascular involvement: Persistence and chronicity of the present clinical state

c) Addition of new vascular involvements to the present one and CVI.

Their response to treatment was evaluated both clinically and radiologically. The response to treatment assessed by Doppler US was defined in three categories:

a) Full response: Total recanalisation and near to normal Doppler US

b) Partial response: Partial recanalization with collateral formation

c) No response: Addition of new thromboses to the existing ones.

Doppler US was applied to 40 of 42 patients before treatment. The procedure was applied to only 22 of them after treatment due to socioeconomical incapabilities. This includes 2 patients who were not radiologically evaluated before the treatment.

The treatment was classified according to drug given, dosing of the drug, and duration of treatment. The response to treatment was assessed according to clinical criteria and the Doppler US criteria. The remission period for each drug given was defined separately. While considering the remission period, the remission periods for the patients with full clinical response and no activation were taken into account, while the remission periods for the patients with no clinical response and with clinical activation were excluded.

3. RESULTS

Four of 42 patients studied retrospectively were females, 38 of them were males. Their ages were between 15-50 with mean value 35.4. The mean duration of Behçet's disease was 9.3 years (1-24 years). The mean follow-up period was 4.9 years (3 months-20 years).

The onset of DVT, DVT-SVT, and CVI ranged from 14 to 44 years with a mean age of 28.6 years. The follow-up for vascular involvement in our Behçet's disease patients ranged from 3 months to 14 years with a mean value of 3.6 years. The demographic data of the cases are shown in Table 1.

All patients had deep vein thrombosis in distal extremities. Twenty-four of them (57.1%) had deep vein thrombosis only, nine had both deep vein thrombosis and superficial vein thrombosis (21.4%), and nine had chronic venous insufficiency (21.4%). Thirteen out of 42 patients (31%) were diagnosed as having SVT before the onset of DVT. Deep vein thrombosis included the right leg in 16 patients, left leg in 15 patients and both legs in 9 patients. The localization was not cited for 2 patients.

The evaluation on the basis of treatment given can be summarized as such: Colchicine 1.5 mg/day +anticoagulants (heparin and/or Fraxiparine®) -antiaggregants (i.e. acetyl salicylic acid, etc.) were used in 20 patients. Forteen of them gave full response to treatment according to clinical assessment criteria (Table 2). The Doppler US was applied only to 8 of them and 3 out of 8 proved to give full response to treatment (Table 3). One patient did not show for follow-up and was excluded from the study.

Table 1. Demographic data of the 42 cases studied

Oral apthous ulcerations	42 (100%)
Genital ulcerations	38 (90%)
Erythema nodosum	26 (62%)
Pseudofolliculitis	25 (59.5%)
Arthritis	14 (33%)
Arthralgia	14 (33%)
Eye involvement	17 (40.4%)
Epididymoorchitis	6 (14.2%)
Neurological involvement	2 (4.7%)
Gastrointestinal involvement	1 (2.35%)
Urinary tract involvement	1 (2.35%)
Pathergy positivity	27 (71%)
Pathergy negativity	11 (28.9%)
Pathergy test undone	4 (9.4%)
HLA B51 positivity	4 (9.4%)
HLA B51 negativity	3 (7.1%)
No HLA 51 typing	33 (78%)

Ten of the patients were treated with corticosteroids, either prednisolone or methyl prednisolone, 20-100 mg/day and anticoagulants and/or antiaggregants. Three of them gave full response to treatment. In 5 of them clinical activation or CVI occurred (Table 2). One patient did not show up for follow-up and was excluded from the study.

Table 2. Clinical assessment according to treatment

Clinical assessment	Full remission	No change	Activation and CVI
Treatment administered (n=40)	23	8	9
Colchicine 1.5 mg/day + anticoagulants and/or antiaggregants (n=19)	14	3	2
Corticosteroids (CS) 20-100mg/day + anticoagulants and/or antiaggregants (n=9)	3	1	5
Azathioprine (AZT) 100-150 mg/day Cyclophosphamide 100-150 mg/day and/or 1 gr/month i.v. for 6 months anticoagulants-antiaggregants (n=12)	6	4	2

In the third group we used in 12 patients azathioprine (AZT) 100-150 mg/day and cyclophosphamide 100-150 mg/day or 1 gr pulse i.v./month for 6 months. In this group, 6 patients had full clinical response, 4 had partial response, and 2 had clinical activation.

The duration of treatment, remission and activation of the three groups treated is given in Table 4.

Table 3. Doppler US results according to drug group

Drug groups (n=40)	Radiological results			
	Full response	Partial response	No radiological response	Total number of cases
Colchicine treated group (n=19)	3	5	0	8
Corticosteroid treated group (n=9)	0	4	0	4
Immunusuppressive and corticosteroid treated group (n=12)	4	4	2	10

Table 4. Drug group, treatment duration, full remission period and activation period

Drug groups (n=40)	Median duration of treatment	treatment	activation
Colchicine treated group (n=19)	3.35 years (3 mo-10 years) (n=19)	1.6 years (3 mo-6 years) (n=14)	5.1 years (2-8 years) (n=5)
Corticosteroids treated group (n=9)	2.5 years (3 mo-7 years) (n=9)	0.5 years (3 mo-12 mo) (n=3)	3.9 years (1-7 years) (n=6)
Immunosuppressives treated group (n=12)	1.74 years (n=12)	1.6 years (3 mo-3 years) (n=8)	3.4years (9 mo-12 years) (n=6)

All three groups were given anticoagulants (heparin-Fraxiparine®) for 3 months and antiaggregants afterwards.

Two out of 42 patients did not show up for routine clinical follow-up, therefore they were not included in this study. Twenty-three out of 40 patients showed full response to treatment and no activation during follow-up. In Doppler US study of 22 patients out of 23, 7 had full radiological response while 13 had incomplete radiological response. In 8 of 40 patients, the present vascular involvement persisted. In 9 of 40 patients new vascular thrombotic attacks and CVI were observed (Tables 2, 3).

4. DISCUSSION

Great vessel involvement due to Behçet's disease is a big challenge to the clinician[1-9]. Controlled studies can hardly be planned due to the fact that vascular involvement can be lethal[9] and threaten the life quality of the patient[10]. Therefore treatment given is based on several clinical studies, experience of the clinician and the severity of vascular involvement of the patient.

In our retrospective study, 42 patients treated for deep vein thrombosis (DVT) of the distal extremities, DVT and superficial vein thrombosis (SVT), and chronic venous insufficiency (CVI) were assessed after treatment both clinically and radiologically by Doppler US. They were treated with three groups of drugs with dosing and duration given in Table 2.

The use of anticoagulants, which have been included in all three groups, is still debatable[9]. In this study, we routinely treated the patients with anticoagulants for the first 3 months in the acute phase, followed by antiaggregant treatment. As a consequence of our clinical observations, we apply anticoagulant-antiaggregant treatment in all venous involvements excluding arterial involvement.

The role of colchicine in great vessel involvement is not mentioned in the controlled studies in the literature. This drug has been potent in controlling mucocutaneous and eye involvement, arthritis and erythema nodosum[11]. In our study the clinical remission rate of 73.6% with colchicine treatment is supported by the 42% total or partial remission rate with Doppler US (Tables 2 and 3). The remission duration was 1.6 years. This result implies that colchicine should be used in the treatment of distal extremity deep vein thrombosis; yet this data should be supported with controlled clinical studies.

The second group of drugs used as monotherapy is corticosteroids (CS). Corticosteroids are already used in the treatment of systemic involvement in Behçet's disease as well as in great vessel involvement[9,11,12]. Ten patients have been treated with 20-100 mg/day prednisolone or methyl prednisolone only. One patient was missed to follow-up. In the remaining 3 patients, full remission rate was 33%. In 55% of the patients, clinical activation and/or CVI intervened. In this group, Doppler US was applied to only 4 patients after treatment and all 4 had partial remission (44%). Remission duration was 0.5 years and activation duration was 3.9 years (1-7 years). In this group the rate of unresponsiveness to the corticosteroid treatment (no clinical change or clinical activation) was 66.6% (n=6).

The rate of success in the colchicine-treated group (73.6%) is near to the rate of activation in the corticosteroid-treated group (66.6%). The result does not imply directly that corticosteroids are unsuccessful in the treatment of

distal extremity deep vein thrombosis still the clinician must be careful in using corticosteroids for such an indication.

Azathioprine has been found effective in the treatment of arterial and venous involvement[13-15]. Cyclophosphamide has been effective in reducing the diameter of arterial aneurysms and/or treating them[9]. In our study, 12 patients have been treated with cychlophosphomide and azathioprine (AZT), either as a monotherapy or in combination with each other or with corticosteroids. Cyclophosphamide had been given 1 gr/i.v./month as pulse therapy for 6 months and/or 100-150 mg/day p.o. AZT had been given as 100-150 mg/day p.o. In this group of 12 patients 6 patients had full remission (50%) and 6 showed either no change or clinical activation (50%; Table 2). When we add up the group with no clinical change and the full remission group, the rate of success can be accepted as 66.6%. In this group radiological full + partial remission rate was 66.6% (Table 3). These 12 patients used the drugs for 1.7 years and the remission duration was 1.6 years.

The results of the immunosuppressive-treated group are close to the colchicine-treated group, yet the former group was treated for half the duration of the latter group. The rate of clinical remission (73.6%) is not concordant with the rate of radiological remission (42%) in the colchicine-treated group; yet the group treated with immunosuppressives showed the same rate of clinical (66.6%) and radiological remission (66.6%). The clinical activation rate was nearly the same in both groups, immunosuppressives, however, are more expensive and toxic than colchicine. Since this is an uncontrolled and retrospective study, we cannot claim that one drug is superior to the other. Another finding of our study is that clinical remission may not be supported by Doppler US. Therefore Doppler US seems to be superior to clinical examination in the follow-up of patients with DVT of distal extremities.

In our study group, there have been no complications or adverse affects due to treatment. Forty patients out of 42 have been evaluated. 23 of them (57.5%) had clinical remission while 17 (42.5%) showed no clinical change. Doppler US has been applied only to 22 patients after treatment. The rate of full remission was 31.8% (n=7). The rate of partial remission was 59%. In 9% of the patients there was no radiological change.

The rate of superficial vein thrombosis before the onset of DVT is 33% (n=14). This does not support the fact that deep vein thrombosis is more frequent in patients with superficial vein thrombosis[16]. In our study, the rate of deep vein thrombosis in patients with superficial vein thrombosis is 1/3. This supports the result of our former study which claims that Behçet's disease showed benign prognosis in 2/3 of the 17 cases.

As a result of this retrospective study, colchicine can also be an alternative treatment modality in the treatment of deep vein thrombosis

besides immunosuppressive therapy, although the mechanism of the effect of colchicine is not yet clear. Corticosteroids are of better use in combination with other drugs instead of monotherapy. We also believe that the use of Doppler US is preferable in the follow-up of Behçet's disease patients with deep vein thrombosis.

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The Use of Interferon-α in Behçet's Disease – Review of the Literature and Possible Mechanisms of Action

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1. INTRODUCTION

Standard treatment of Behçet's Disease (BD) comprises immunosuppressive agents such as azathioprine and cyclosporin A in combination with glucocorticosteroids for the more serious manifestations of BD as for example ocular vasculitis, or colchicine for mucocutaneous lesions[1]. Prognosis of BD is mainly influenced by ocular, neurological or vascular manifestations. Although prognosis has improved by early and aggressive immunosuppressive treatment[2], many patients have refractory diseases and require treatment with combinations of various immunosuppressants or cytotoxic agents such as chlorambucil or cyclophosphamide, which may lead to serious infections or secondary malignancy in the long run. Furthermore, the neurological disease has been described to occur in spite of treatment with cyclosporin A, making this treatment inappropriate for patients with CNS manifestations[3]. Consequently, new therapeutic options are required.

Interferon (IFN) is a cytokine which was discovered more than 40 years ago by Isaacs and Lindenmann[4], who observed that virus-infected cell cultures produced a protein that rendered cells resistant to infection by many viruses. IFN-α belongs to the so-called type-1-IFNs and can be produced virtually by all somatic cells after viral infection. By inducing the release of intracellular enzymes such as 2`5`-oligoadenylate synthetase and double-stranded RNA-dependent protein kinase, it causes degradation of viral

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messenger RNA and inhibits protein synthesis. There are two different human recombinant α-IFN's in use and approved for the treatment of viral hepatitis and myeloproliferative syndromes, as well as for certain solid tumours and lymphomas (IFN-α2a and IFN-α2b). They differ in one amino acid only[5].

IFN-α therapy was introduced to the treatment of BD by Tsambaos et al.[6] in 1986 because of its antiviral and antiproliferative properties. The objective of the present analysis was to summarise what has been achieved with respect to IFN-α treatment of BD by July 2002 and to discuss possible mechanisms of action.

2. METHODS

Reports in all languages published by July 2002 were identified by the PubMed database, the BD conference proceedings, and abstract booklets. The indexing terms used were Behçet and interferon.

3. RESULTS

Thirty-two original reports and four selected abstracts were included in the analysis. Systemic IFNα was administered to 405 patients (441 including those redundantly reported). In the majority of publications, acute ocular manifestations were excluded. Thirty studies were designed to evaluate efficacy for mucocutaneous and articular symptoms, 6 evaluated efficacy for ocular disease. Two studies were randomised[7,8], one of which unfortunately later had to be retracted due to fabrications with respect to authorship and possibly also the reported data and ethical transgressions[7,9]. Thus, the patients included in this study were considered for calculations of the absolute numbers of patients treated, but not for the more detailed subanalyses on efficacy of IFNα. Two hundred and sixteen patients with acute ocular disease were treated with IFNα. Two hundred and ninety eight patients received IFNα2a, 141 IFNα2b. The dosages were between 5×10^6 international units (IU) 1 x weekly and 18×10^6 IU per day for 11 days to 64 months. 85.6% of the patients with mucocutaneous symptoms, 95.8% with arthritis, and 95.6% with uveitis exhibited a partial or complete response. The efficacy for mucocutaneous symptoms seemed to be inferior to that for the other manifestations of the disease, as partial remissions were achieved to the same extent as complete remissions (42.8%). Higher IFN doses were more effective than low dose regimens and led to up to 56% long-term remissions after discontinuation of IFNα. Response (50% improvement of

the lesions) was achieved as early as 2 weeks after initiation of IFN treatment, complete remissions were achieved after 4-6 weeks. IFNα2a was apparently superior to IFNα2b with more complete remissions (IFNα2a 52% for mucocutaneous symptoms, 73% for arthritis, and 91.7% for uveitis IFNα2b 42.8%, 60.4% and 78.1%, respectively), but this was probably due to a bias caused by the larger number of patients treated with IFNα2a, and by different dosages, endpoints, and outcome measures between the studies for both types of IFN. Side effects were dose dependent and similar to those reported for patients with hepatitis or haematological diseases, mostly flu-like syndromes, leukopenia, and alopecia[6-42].

4. DISCUSSION AND CONCLUSIONS

IFNα is effective for the treatment of BD. The most impressive results have been achieved for severe and/or refractory ocular manifestations.

A more detailed statistical analysis is not possible since the comparability of the studies is hampered by the different IFN dosages, study designs, inclusion and exclusion criteria, additional medications, and outcome measures. Another problem is the documentation of "improvement" in studies on BD as already stated by Zouboulis[23] in his review on IFNα treatment for BD in 1997. Most of the lesions of BD are recurrent with spontaneous improvements or even remissions. Thus, in 4 of the studies reviewed here, pre- and post-treatment observation periods were included[8,32,35,39].

The combination of IFNα with immunosuppressants, as performed in some of the studies and case reports, remains a matter of debate. From an immunological point of view, immunosuppressive agents might antagonise the effects of IFNα.

The efficacy of IFNα2a was apparently better than that of IFNα2b. This may be due to a real difference between the two forms, or merely to the difference in the number of patients treated with the subtypes, which were higher for IFNα2a, or to a bias caused by the study designs differing between the studies for IFNα2a and 2b. As for other disorders being treated with IFNα, substantial differences in efficacy or side effects could not be proven (although often debated)[5], it would be surprising if this was the case in BD.

The mechanism of action of IFNα in BD remains to be elucidated. IFNα has antiviral, antiproliferative and immunostimulatory properties. It diverts the immune response towards Th1, activates NK cells, and enhances the expression of HLA class I antigens on lymphocytes and antigen presenting cells[43,44]. All these mechanisms might help eliminating foreign antigens such

as a persisting (yet not proven) virus. Furthermore, IFNα also exerts immunosuppressive actions, such as a reduction of γδ T cells[45], inhibition of adhesion of T-cells to endothelial cells[46], dose-dependent reduction of neutrophil phagocytosis and free radical production[47], and reduction of IL-8 secretion by endothelial cells[48]. Especially the latter are probably relevant for the activity of IFNα in BD because neutrophil hyperactivity[49], impaired NK-cell cytotoxicity[50], elevation of IL-8[51-53], and an increased number of γδ T cells[54] have been described in active BD.

In conclusion, IFNα is a very promising treatment option for BD. Although the ideal dose of IFNα for the treatment of BD still remains to be determined, the preliminary recommendation would be to start with a higher dosage, as for example 6 or even 9×10^6 IU per day, to be reduced to 4.5×10^6 IU after 4 weeks, and to 3×10^6 IU after another 4 weeks, and to the maintenance dosage of 3×10^6 IU 3 x per week after complete remission was achieved. It is still unclear when IFNα can be discontinued. We would recommend continuation for at least 8 weeks after complete remission has been achieved. IFNα may be superior to conventional immunosuppressive agents with respect to its rapid action and the possibility of long-term remissions without further treatment. In particular, severe and refractory ocular manifestations respond to IFNα, whereas mucocutaneous lesions and arthritis improve but often do not completely disappear and tend to relapse. As there are other cheaper therapeutic agents than IFNα for these indications, IFNα should be reserved for more serious manifestations of the disease. Here, it should be used in early disease, for example after one standard immunosuppressive agent has been found to be ineffective. IFNα is also indicated in patients with concomitant infectious diseases, malignancies or immunodeficiencies where it is clearly superior to immunosuppressants as it does not impair the immune response but may actually augment it.

In times of evidence-based medicine there is now an urgent need for controlled randomised studies with IFNα2a using standardised outcome measures and, if possible, pre- and post-treatment observation periods against standard immunosuppressants such as cyclosporin A in order to determine its place in the treatment of BD.

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Interferon alfa-2a in the Treatment of Ocular Adamantiades-Behçet's Disease

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1. INTRODUCTION

Adamantiades-Behçet's disease (ABD) is a chronic relapsing multisystemic vasculitis involving small and large vessels. The etiology of the disease still remains unknown. Relapsing ocular involvement is one of the major manifestations of Adamantiades-Behçet's disease, characterized by iritis (Fig. 1), uveitis, retinal occlusive vasculitis (Fig. 2), and optic nerve neuropathy that often lead to blindness if untreated. Retinal detachment and secondary glaucoma are severe complications of occlusive retinal vasculitis. Surgical intervention of these complications often leads to a recurrence of the inflammation limiting the surgical results. In the case of Adamantiades-Behçet's disease with eye involvement, a combination of systemic corticosteroids and cyclosporine A is currently the treatment of choice, although severe side effects, such as Cushing syndrome, osteoporosis or renal failure and hypertension frequently occur[1]. Interferon alfa (IFNα) has been shown to be an effective treatment in mucocutaneous ABD[2,3]. It has also been suggested to improve ocular lesions[2,4-7]. We examined the effects of interferon alfa-2a (6-9 Mio IU 3x/week) in a case series of 24 patients with ocular involvement.

Adamantiades–Behçet's Disease, Edited by Christos C. Zouboulis
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2. PATIENTS AND METHODS

Since 1999 we included 24 consecutive ABD patients with ocular involvement who provided informed consent in an IFNα treatment protocol. The mean follow-up since initiation of the treatment was 18 months (6-36 months). The mean age at the onset of the disease was 28 years (18-38 years), whereas the beginning of ocular symptoms 30 years (23-40 years). At the time of initiation of the treatment all 24 patients showed oral ulcerations, whereas some patients presented genital ulcerations (n=7), arthritis (n=9), skin lesions (n=16), or cerebral involvement (n=1). All patients underwent a complete ophthalmologic examination including visual acuity measurement, slitlamp examination of the anterior segment, and indirect ophthalmoscopy of the vitreous body and fundus. We detected iritis in 20 of the 24 patients, 21 had ocular vasculitis, and 12 patients had a neuropathy of the optic nerve.

Patients received IFNα-2a (6-9 Mio IU 3x/week) subcutaneously as long-term therapy. Treatment was initiated at relapse of the eye disease. Corticosteroids (prednisolone 100 mg/day/p.o.) were administered additionally, and were tapered within 2 weeks to a maintenance dose of 10 mg/day. IFNα-2a was lowered to 6 and then to 3 Mio IU 3x/week, provided a 4-month period without ocular inflammation. After a 6-month period without ocular inflammation corticosteroids were discontinued.

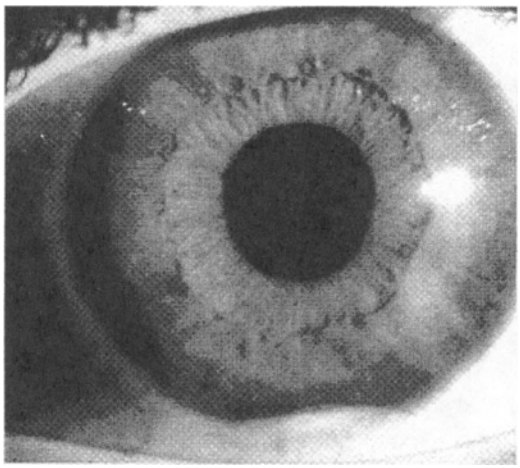

Figure 1. Hypopyon iritis

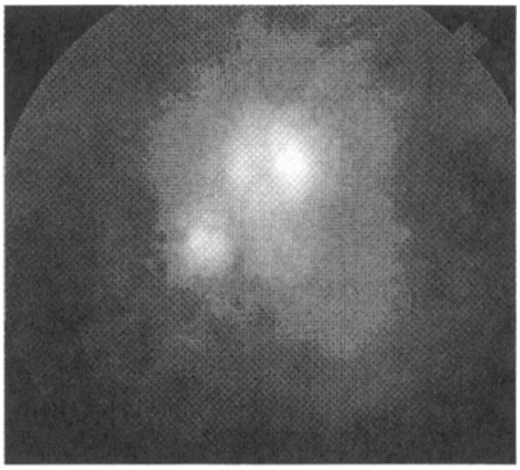

Figure 2. Retinal occlusive vasculitis, peripapillary located with retinal bleeding as first ocular symptoms

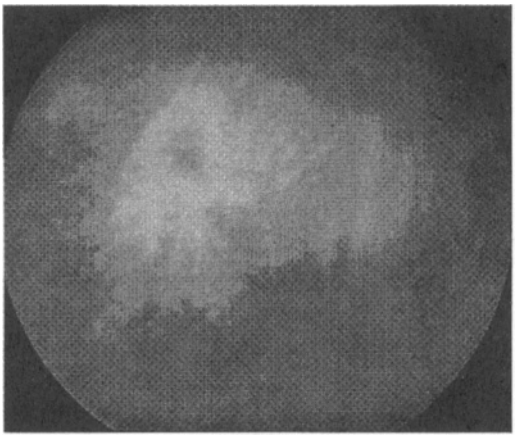

Figure 3. Acute retinal vasculitis of small vessels in the lower periphery, one week after the patient stopped IFNα-treatment on her own

3. RESULTS

In all 24 patients acute manifestations of ocular involvement resolved within one week (Figs. 4 and 5). The treatment was effective against vasculitis (n=21), iritis (n=20) and neuropathy of the optic nerve (n=12). Five patients showed a recurrence. Among them, one patient stopped

treatment on her own (Fig. 3), another one because of pregnancy. One patient had seven recurrences under IFNα-2a treatment, however he also presented recurrences under cyclosporine A, chlorambucil, and tumour necrosis factor-α antagonist etanercept. In four patients we were able to discontinue IFNα treatment without any recurrence for a year.

Constant visual acuity was reached in 10 patients (42%), 11 patients (46%) showed a better (2-3 lines) visual acuity as compared to the acute period of inflammation. In three patients (13%) visual acuity decreased because of the development of cataract and neuropathy. Altogether, a constant or improved vision was achieved in 88% of all patients (21 out of 24 patients).

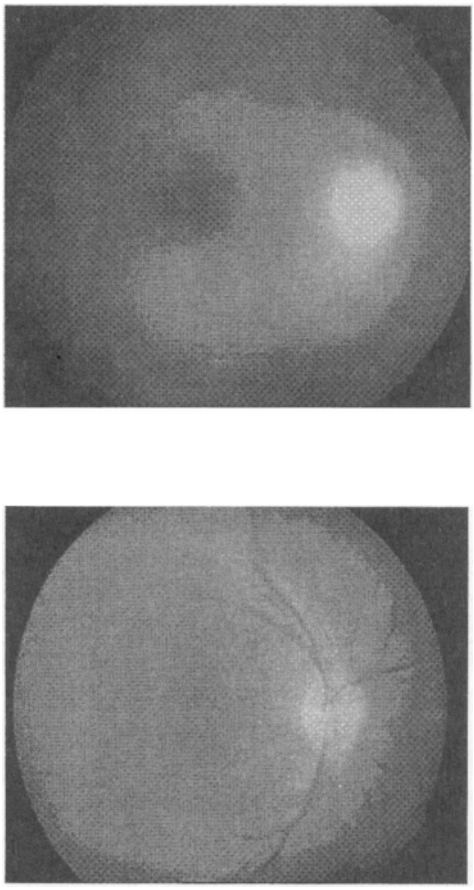

Figure 5. The patient in Fig. 4 with intraocular findings following two weeks of IFNα-treatment

3.1 Case report

One of the patients underwent repeated intraocular surgery because of complications due to his Behçet's disease. He was 24 years old when he was introduced to us in 1999. He was of Turkish origin and had a visual loss in his right eye. Adamantiades-Behçet's disease has been diagnosed two years before. Arthritis of the knees, oral aphtosis, and skin lesions, manifesting as folliculitis were his first symptoms. At that time the treatment was a monotherapy with 10 mg/d prednisolone per os. Three months before he was sent to us he noticed a loss of vision in his right eye. He had a hypopyon-iritis, and a retinal detachment was suspected.

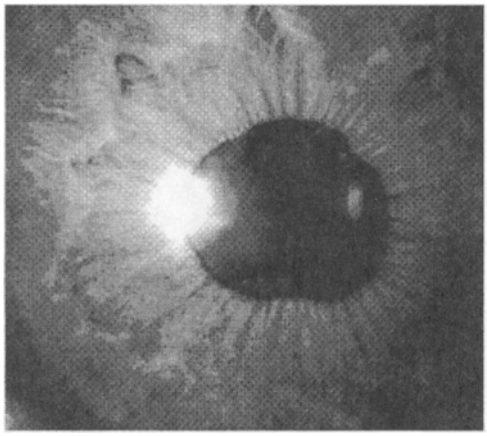

Figure 6. Anterior chamber findings with rubeosis and posterior synechias before therapy

At the initial presentation visual acuity was light perception on his right eye and 20/20 on his left eye. On his right eye the cornea was clear, anterior chamber deep with cells and tyndall positive. The iris showed a rubeosis and posterior synechiae (Fig. 6). Funduscopy and ultrasound examination showed a total retinal detachment (Fig. 7). On his left eye the anterior chamber showed no signs of acute inflammation, but funduscopy showed inflamed and occluded vessels in the nasal periphery and snowballs in the lower periphery. The macular and the optic disks were without pathology. Intraocular pressure was 5 mmHg on the right and 35 mmHg on the left side.

General symptoms were oral aphthosis, arthralgia in his right knee, folliculitis, and a positive pathergy test. Family history was negative.

After the diagnosis has been confirmed a therapy as described before was started. After two weeks the acute inflammation disappeared. Quite remarkably, the rubeosis also decreased on his right eye (Fig. 8).

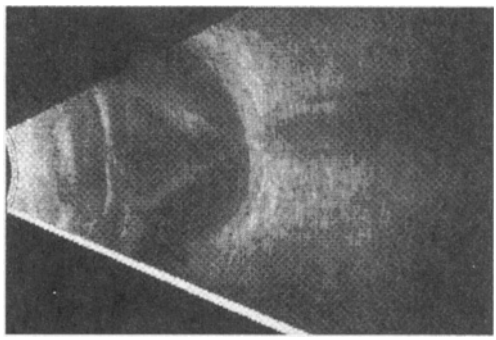

Figure 7. Ultrasound examination shows a total retinal detachment of the right eye

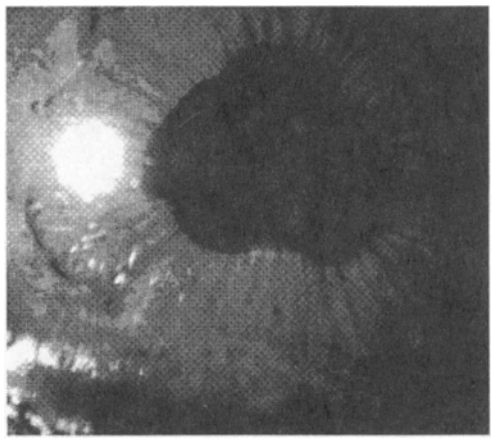

Figure 8. Decreased rubeosis following IFNα-treatment, right eye

A vitrectomy was performed to save the right eye. Intraoperatively a total retinal detachment with an anterior traction, just behind the lens adhesive to the posterior lens capsule was detected and a 360° retinectomy and a lentectomy were performed. After removal of several epiretinal membranes, the central retina was reattached under a silicone oil tamponade (Fig. 9).

Following the operative treatment, a monotherapy with interferon-alpha-2a (3x9 Mill. IU/3xweek) was administered, and no recurrence of the disease occurred. The visual acuity was 20/200 on the right eye at that time.

On the left eye there was no possibility to treat the glaucoma conservatively. Gonioscopy showed neovascularisation in the anterior chamber angle. A panretinal laser photocoagulation of the left retina was performed. The neovascularisation decreased but the intraocular pressure was still increased, therefore a trabeculectomy was performed.

Postoperatively the tension is now under control without any additional medical treatment since three years (Fig. 10).

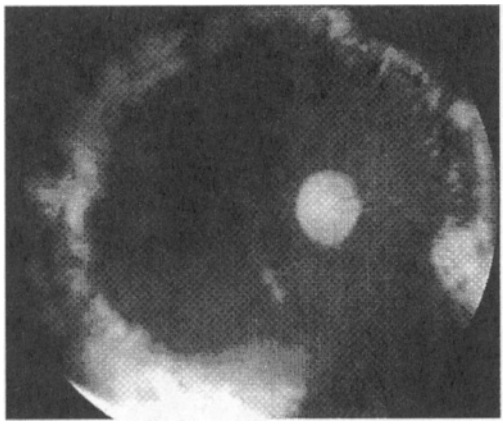

Figure 9. Retinal findings two years after operation on the right eye: reattached retina with optic nerve atrophy and visual acuity of still 20/200

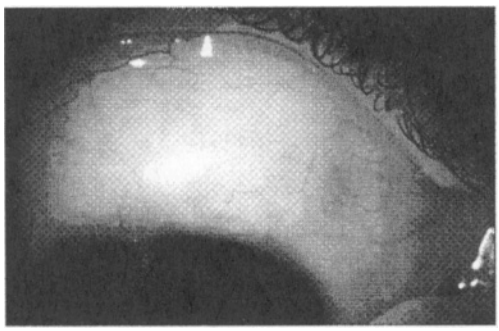

Figure 10. Functional filtering blab on the left eye

At present visual acuity is 20/200 on the right eye and 20/20 on the left eye. Since the begin of the interferon therapy, no relapse of the disease has occurred. The dose was lowered to 3 Mill. IU/3x week.

3.2 Side effects

Flu-like symptoms occurred in all of our patients, tachycardia in one patient, and dose-dependent reversible thrombocytopenia and reversible alopecia were the only additional side-effects. The flu-like symptoms were well tolerated under the addition of non-steroidal antiphlogistics. Antinuclear antibodies were detected in two patients. Considering the risk of

secondary ophthalmologic complications, these side effects have to be accepted.

4. CONCLUSION

Eye involvement is a serious manifestation of ABD, with a prevalence up to 70% and a bilateral inflammation up to 90%. It is characterised by anterior or posterior uveitis, retinal occlusive vasculitis and neuropathy of the optic nerve. Recurrent intraocular inflammation often leads to non-reversible avascular retinal changes, intraocular haemorrhage, secondary cataract, secondary glaucoma, and optic nerve atrophy, with subsequent reduced vision. Blindness occurs in 20% of the affected eyes[1].

Cyclosporine A in combination with steroids is the current treatment of choice for ABD[1]. In long-term therapy, severe side-effects can occur under both drugs. Cyclosporine A is responsible for dose-dependent nephrotoxic effects, steroids for Cushing syndrome, and osteoporosis.

Interferons have immunoregulatory, antiproliferative, antineoplastic and antiviral effects. The natural killer cell activity of patients with active ABD was found significantly lower than in patients with inactive disease and in normal controls. Interferon alfa was shown to increase the reduced activity of natural killer cells in ABD. Interferon alfa therapy is used in the treatment of mucocutaneous ABD since over 10 years, however, patients with ocular involvement were initially excluded[2]. In several case series and non-controlled studies high response rates were reported. Recent reports provide evidence that interferon alfa is also effective in ocular ABD[2,4-7]. Some advanced cases like retinal detachment and secondary cataract or glaucoma need surgical intervention. However, surgical intervention in Adamantiades-Behçet's-diseased eyes is often complicated by relapsing inflammation[8,9]. The case report describes a favourable outcome in such a serious situation in both eyes. Our patient had two different problems in his both eyes that required surgical intervention. Both surgical interventions did not initiate a relapse of the disease, and no postoperative complication occurred. We also did not see a closure of the trabeculectomy, that could be expected in chronically inflamed eyes.

The systemical treatment with interferon-alpha-2a in case of ocular involvement in Adamantiades-Behçet's disease seems to be a safe medical mesure, even when performed perioperatively. Our data confirm the remarkable effect of IFNα in the treatment of ocular ABD. International multicentre controlled studies are needed to compare the effectiveness of cyclosporine A and interferon alfa in the treatment of ocular ABD.

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Human Recombinant Interferon-α2a (rhIFNα2a) for the Treatment of Behçet's Disease with Sight-Threatening Retinal Vasculitis

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1. INTRODUCTION

Standard treatment of Behçet's Disease (BD) mainly comprises systemic immunosuppressive agents. Ocular involvement, mostly posterior uveitis with retinal vasculitis leads to blindness in 20-50% of the involved eyes within five years. We studied the efficacy of interferon-α2a in patients with sight threatening posterior uveitis or retinal vasculitis.

2. MATERIALS AND METHODS

Fifty patients with highly resistant ocular BD were included in four participating hospitals from March 1995 to March 2000. For entering the study, the patient had to have active posterior uveitis or panuveitis which had been refractory to at least one conventional immunosuppressive treatment or prednisolone in a dosage of at least 1 mg/kg bodyweight, and/or

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impossibility to taper the steroids to a maintenance dosage of less than 30 mg prednisolone equivalent daily. Forty-six patients fulfilled the International Study Group Criteria, four patients had incomplete BD with oral aphthous ulcers and panuveitis with typical occlusive retinal vasculitis and/or hypopyon. The study protocol had been approved by the institutional review board of each hospital and the patients had given informed consent. Previous therapy with immunosuppressants or other drugs had to be discontinued before the initiation of interferon treatment and systemic glucocorticosteroids had to be reduced to a maximum of 10 mg prednisolone equivalent per day. Topical nonsteroidal antirheumatic drugs and steroids were permitted for anterior uveitis. rhIFNα2a was applied at a dose of 6 million units subcutaneously per day. Dose reduction was performed according to a decision tree until discontinuation. Disease activity was evaluated every two weeks by the BD Activity Scoring System, and the Uveitis Scoring System. Statistical analysis was performed by ANCOVA.

3. RESULTS

Response rate of the ocular manifestations was 92% (3 non-responder, one incomplete response). Mean visual acuity rose significantly from 0.46 to 0.81 at week 24 ($p<0.0001$). Posterior uveitis score of the affected eyes fell by 46% every week ($p<0,001$). Remission of retinal inflammation was achieved by week 24. Mean BD Activity Score fell from 6.6 to 3.1 at week 24 and further to 1.8 at week 52. After a mean observation period of 36.4 months (range 12 to 72), 20 patients (40%) were off treatment and disease-free for 7-58 months (mean 29.5). In the other patients maintenance IFN dosage was 3 million units 3 times weekly.

4. DISCUSSION AND CONCLUSIONS

rhIFNα2a is effective in ocular BD, leading to significant improvement of vision and complete remission of ocular vasculitis in the majority of the patients. It may be superior to conventional immunosuppressants with respect to the possibility of discontinuation without relapse. Randomised studies comparing IFNα to cyclosporin or azathioprine are mandatory.

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Behçet's Disease: Visual Acuity after 5 Years in Patients with Alpha-Interferon Treatment

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1. BACKGROUND

Ocular involvement is present in 60-80% of patients with Behçet's disease (BD). Especially in the case of panuveitis or occlusive vasculitis visual prognosis is poorly irrespective of immunosuppressive treatment. This has been described by several studies during the last two decades: Despite treatment with immunosuppressants or steroids a loss or decrease of visual acuity occurred in approximately 50% of patients within 5 years of ocular BD[1-5].

Now recent open studies showed that interferon-α2a (IFN-α2a) is very effective for treating ocular BD and seems to be superior to conventional immunosuppressants like azathioprine or cyclosporine A[6-8].

The purpose of this study was to evaluate visual prognosis over a follow-up period of at least 5 years in patients with panuveitis and / or retinal vasculitis due to BD, who were treated with IFN-α2a at our hospital.

2. PATIENTS AND METHODS

We included 15 eyes of 9 patients (male:female = 6:3) with complete BD according to the diagnostic criteria of the International Study Group. HLA-B51 was positive in 5 patients. All patients had to have an active panuveitis and / or retinal vasculitis which had to be refractory to at least one

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conventional immunosuppressive treatment and / or prednisolone in a dosage of at least 1 mg/kg bodyweight.

Therapy was initiated as follows: Patients received IFN-α2a in a dosage of 6 million IU per day for at least two weeks. The dosage of interferon was tapered to 3 million IU twice a week over several months and then discontinued if possible. Previous treatment with immunosuppressants was stopped one day before starting interferon; systemic steroids were reduced to 10 mg prednisolone per day gradually.

We compared visual acuity at the start of interferon-therapy with visual acuity at the end of a follow-up time of at least 5 years.

3. RESULTS

Mean follow-up time from initiation of IFN-therapy to the last visit of patients at our clinic was 68.8 ± 10.6 months (60.0 – 93.5 months). Mean duration of IFN-treatment was 40.6 ± 17.0 months (9.0 – 61.0 months). In 7 of the 9 patients IFN-α2a could be discontinued as complete remission of ocular symptoms had occurred.

Mean visual acuity of all 15 eyes was 0.45 ± 0.40 at initiation of IFN-therapy, increasing to a maximum of 0.90 ± 0.41 during treatment, remaining at 0.85 ± 0.46 at the end of IFN-therapy and showing to be stable by the end of follow-up (0.82 ± 0.45).

A visual acuity of 0.1 or less was present in 6 eyes at initiation of IFN-therapy, but still in 2 eyes at the end of follow-up. In these 6 eyes also a remarkable increase of mean visual acuity occurred during follow-up (0.06 ± 0.03 at initiation of IFN-therapy, 0.46 ± 0.37 at the end of follow-up).

Summarizing the development of visual acuity in all eyes during follow-up of at least 5 years, it can be said that 10 eyes showed an increase of visual acuity of two lines or more. In 5 eyes visual acuity remained stable. This demonstrates that there was no decrease of visual acuity in any of these cases. Figure 1 shows the relationship between visual acuity at initiation of IFN-therapy and at the end of follow-up time.

No procedures for improvement of visual acuity (e.g. cataract surgery or vitrectomy) were performed during follow-up time. No eye developed a pale optic disc. If a macular oedema was present (11 eyes), a quick response to IFN-α2a alone was seen without any need of additional treatment (e.g. acetazolamide).

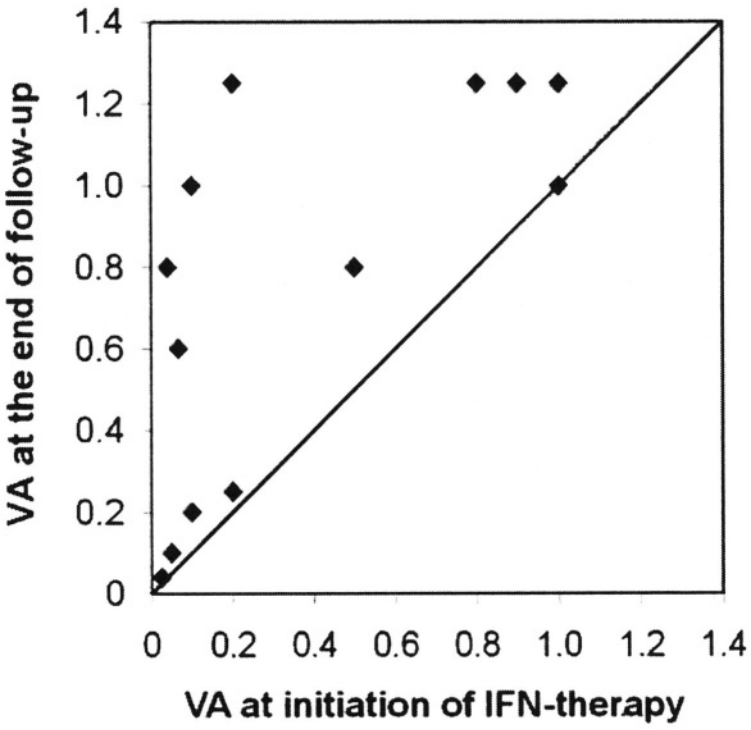

Figure 1. The relationship between visual acuity (VA) at initiation of IFN-therapy and at the end of follow-up time

4. CONCLUSION

Compared to conventional immunosuppressants, IFN-α2a seems to be much more effective to prevent a loss or decrease of visual acuity over a long period of time in patients with severe ocular BD.

As 15 eyes is only a small case series, further evaluation is needed. We expect that in approximately 12 months 30 eyes will have reached the 5-year follow-up mark, so more data will soon then be available.

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Multifocal ERG Changes in Patients with Ocular Behçet's Disease During Therapy with Interferon alpha 2a

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1. INTRODUCTION

One of the main problems in ocular Behçet's disease (BD) is severe posterior uveitis/panuveitis with retinal vasculitis leading to macular edema and to bad visual prognosis despite immunosuppression. Previously, we have shown the efficacy of interferon α2a (IFNα2a) in posterior uveitis demonstrated by marked improvement of vísual acuity due to reduction of inflammatory changes[1].

Despite the conventional full-field ERG in which patients with macular changes usually do not exhibit a pathologic examination, the multifocal electroretinography (mfERG) allows physiological mapping of the *central retina*[2-4].

The purpose of this study was to measure and to demonstrate the improvement of central retinal function under therapy with IFNa2a by means of the mfERG.

2. METHODS

Ten male BD patients (age range 21-42 years) with posterior uveitis or panuveitis (n=17 affected eyes) who had been diagnosed according to the

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criteria of the "International Study Group of Behçet's Disease" were treated with IFNα2a in a dosis range from 0.8 Mio lU/day to 6 Mio IU/day.

Fifteen of the 17 affected eyes disclosed macular edema. Eight of our patients were HLA-B51 positive.

These patients were examined before, 1 month, 3 months, 6 months, and 12 months after initiating interferon therapy with visual acuity, measurement of the viusal field, fluorescein-angiography, and the multifocal ERG system. Furthermore, correlation of changes in mfERG and visual acuity were investigated. The mean observation period was 24.7 ± 8.2 months.

The **mfERG** allows a quantification of the outer retinal layer function (photoreceptors, esp. cones)[3].

For the patients examination we used a resolution of *61 hexagonal stimulus elements* presented on a monitor within a visual field of 30°. Signals were filtered with a widened bandpass filter setting of 10-100 Hz.

For evaluation, responses were grouped by eccentricity (ring or area 1-5).

Amplitude and timing of the first positive peak (=**implicit time**) of each group response were determined.

3. RESULTS

In all affected 17 eyes the clinical macular dysfunction resulted in a reduced **amplitude** in the central area of the mfERG due to macular edema (present in 15 eyes, diagnosis was based on fluorescein-angiography), and to secondary changes (e.g. scars, epiretinal gliosis).

The implicit time was prolonged *only* in severe cases of recurrent uveitis (in cases of an acute uveitis onset the implicit time remained unchanged).

Successful treatment of the inflammatory macular changes resulted in:

- *improvement of the amplitudes* in acute retinal inflammation, not in chronic disease with secondary macular changes.
- *normalization of the implicit time* only in patients without central retinal changes due to chronic disease.
- *improvement of visual acuity*: before therapy start mean visual acuity was 0.54±0.33. After a mean observation period of 25 months the visual acuity improved during IFN-therapy to 0.95±0.30.
- in recurrent or chronic uveitis with secondary retinal changes the mfERG demonstrated *amplitude and implicit time impairment* despite good visual acuity, and no visible significant retinal changes in ophthalmoscopy.

3.1 Case Report

- Twenty-eight-year-old man of Turkish origin, diagnosis of Behçet's disease in January 1997
- recurrent oral and genital aphtosis, pustular skin lesions, erythema nodosum, arthritis, HLA B51 positive
- retinal vasculitis OD / OS
- treatment with methotrexate (15mg 1x/week) from January 1998 – January 1999, free of relapses
- relapse of ocular BD in OS in July 1999 (Figs, 1a and 2a).
- starting IFN-therapy in August 1999 with 6 Mio IU/day
- 8 months later visual acuity improved to 1.0 (IFN dosage meanwhile tapered down to 1.5 MioIU/day – Figs. 1b and 2b).
- in April 2001 relapse of posterior uveitis during reduction of IFN-treatment (IFN was augmented to 6 Mio IU/day)
- in May 2002 visual acuity was 1.0. IFN-therapy consists in 0.8 Mio IU/day and since April 2001 the patient has had no relapse.

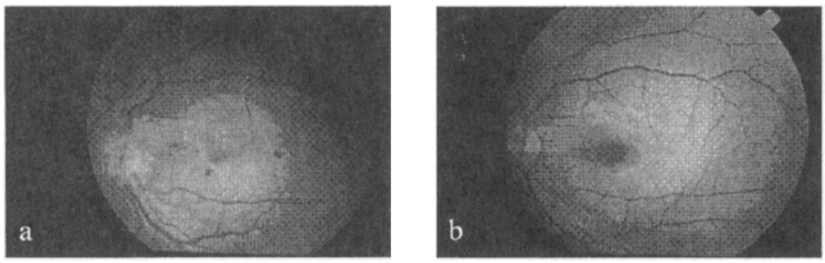

Figure 1. a) OS before IFNα2a therapy. The visual acuity was 0.3. The fundus disclosed optic disc edema, macular edema, central sanguinations, and retinal vasculitis. b) OS 8 months after starting IFN therapy. The visual acuity was 1.0 under IFN-treatment with 1.5 Mio IU/day. The fundus appearence was nearly normal and age-related.

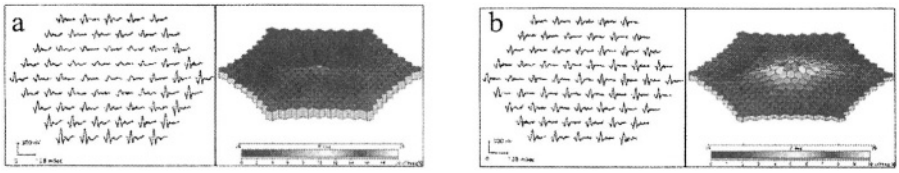

Figure 2. a) **MfERG** of OS before initiating IFNα2a therapy. *Left side*: in the central areas amplitude reduction and delayed peak times are present. *Right side*: Plot of response density with impaired macular function. b) **MfERG** of OS 8 months after initiating IFN therapy: improvement of the central areas. Despite visual acuity of 1.0 relative amplitude reduction is present. The implicit time was not longer delayed.

4. CONCLUSION

Previously, we have shown the efficacy of IFNα2a in BD patients with posterior uveitis or panuveitis achieved by marked improvement of visual acuity due to reduction of inflammatory retinal changes[1]. Now we could demonstrate this benefiting therapeutic effect by measurement of the multifocal ERG. Most of the affected eyes disclosed amplitude changes in the mfERG. These areas correspond to the macular- and the nearer perimacular region; therefore the amplitude alterations might be caused by inflammatory macular changes, esp. macular edema[3-5].

In patients with posterior uveitis or panuveitis due to BD treated with IFNα2a, the mfERG clearly reflects the clinical course and is an objective follow-up of macular dysfunction in recurrent uveitis. Reduced ERG amplitudes were observed in the macular region in all eyes with macular edema or secondary inflammatory changes with remarkable improvement in patients with acute uveitis. Implicit time changes were seen in progressed cases of chronic or recurrent macular edema. Thus, only in chronic eye disease with advanced stages no improvement of both amplitude and implicit time was detectable. The mfERG seems to have no direct correlation to visual acuity but indeed to duration and severity of ocular disease.

In conclusion, the mfERG reflects very well the extent of damage to the central retina due to ocular inflammation in BD patients with otherwise (visual acuity / ophthalmoscopy) absent pathological values.

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Influence of Interferon-alpha on Lymphocyte Subpopulations in Behçet's Disease

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1. INTRODUCTION

In Behçet's disease (BD), several abnormalities of lymphocyte subpopulations such as an elevation of NK and γδ T-cells[1] have been described. Standard treatment comprises immunosuppressive drugs. We successfully treated 50 patients with ocular BD with interferon α 2a (IFNα) (response rate 92%) which is counterintuitive because IFNα is immunostimulatory and can sometimes even induce autoimmune diseases such as SLE or RA. The aim of this study was to elucidate the immunomodulatory effects that IFNα might exert in BD.

2. MATERIALS AND METHODS

Fourteen patients (10 men, 4 women) with a median age of 31.5 years (range 23-44) with active ocular BD fulfilling the International Study Group Criteria for BD were treated with human recombinant IFNα2a. The patients were evaluated before treatment and at week 4 and 24 of IFNα treatment and compared with 10 healthy age- and sex-matched controls. PBMC were

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stained with monoclonal antibodies conjugated with four different fluorescent dyes and measured by flow cytometry. Statistical analysis was performed by ANOVA and Welch's test.

3. RESULTS

Compared with controls there was a significant elevation of monocytes ($CD14^+$), $CD3^+/\gamma\delta$ T-cells, $CD8^+/\gamma\delta$ T-cells, NK-cells ($CD56^+/CD16^+$), and activated/regulatory T-cells ($CD4^+/CD25^+$ and $CD8^+/CD25^+$) in the patients. Naive T-cells ($CD8^+/CD45^+RA^+/RO^-$, $CD4^+/CD45^+RA^+/RO^-$) were significantly lowered in patients. Under therapy, the number of NK-cells, $CD8^+/\gamma\delta$ T-cells, and $CD3^+/\gamma\delta$ T-cells decreased significantly, whereas B-cells increased. The reduced level of expression of HLA-class-I-molecules on monocytes in HLA-B-51-positive patients rose to levels comparable to HLA-B-51-negative patients (Table 1).

Table 1. Percentage of PBMC with different surface markers, baseline. P-values for patients versus healthy controls and p-values for changes in the course of IFN treatment. *significant

Surface marker	p vs. controls	Median controls	Median week 0	Median week 4	Median week 24	p course
CD3/αβ	0.344	71.15	68.35	75	67.15	0.184
CD4/αβ	0.512	43.7	43.9	46.5	43.7	0.927
CD8/αβ	0.099	25.25	29.75	30.55	25.75	0.032*
CD4/RA+/RO-	0.001*	49.35	26.6	18	26.1	0.706
CD4/RA-/RO+	0.59	20.3	28	31.95	26.75	0.075
CD8/RA+/RO-	0.009*	63.35	48.65	51.65	46.95	0.688
CD8/RA-/RO+	0.975	3.3	3.25	3.55	4.15	0.25
CD4+/CD25+	0.003*	7.7	17.25	18.75	17.45	0.27
CD8+/CD25+	0.0004*	0.6	2.1	1.45	2.1	0.059
CD3/γδ	0.0073*	2.7	6.7	7.35	5.6	0.011*
CD8/γδ	0.005*	0.95	2.5	2.7	2	0.005*
γδ/RA+/RO-	0.794	25.35	35.05	32.15	36.1	0.18
γδ/RA-/RO+	0.286	2.7	1.55	1.7	2.1	0.90
CD19+/CD20+	0.49	13	9.7	6.9	11.7	0.004*
CD56+/CD16+	0.023*	4.45	13	7.8	5.75	0.025*
CD14+	0.006*	14.5	25.05	31.75	20.2	0.069

4. DISCUSSION AND CONCLUSIONS

These results implicate a participation of NK-cells, $CD3^+/\gamma\delta$ T-cells, and $CD8^+/\gamma\delta$ T-cells in the pathogenesis of BD and may explain one mechanism by which IFNα exerts therapeutic effects. A direct participation of the activated/regulatory $CD4^+/CD25^+$ and $CD8^+/CD25^+$ T-cells is unlikely as they remained unchanged under IFNα. The reduced expression of HLA-class-I on monocytes in HLA-B-51 positive patients might reflect an impaired expression and antigen presentation by HLA-B51. The increase in B-cells and monocytes under IFNα might explain some of the side effects such as fever, arthralgia, and autoimmune phenomena.

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Interferon alpha 2a in IRPB-derived Peptide-induced EAU – Part I

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1. INTRODUCTION

In 1986 type I interferons (IFN) were introduced in the treatment for Behçet's Disease (BD). The efficacy of such interferons in ocular BD, even when it was refractory to other treatments, is very promising[1]. However, the mechanism of action is still unclear. Therefore we studied markers for immunocompetent cells and also, for the first time, the expression of the CD44 isoforms in an EAU (experimental autoimmune uveitis[2]) peptide-model during IFN-α treatment. CD44 isoforms constitute a family of cellular adhesion molecules exhibiting different biological functions including influence on immunopathology[3].

2. MATERIALS AND METHODS

B10.RIII mice were immunized subcutaneously with 100µg of the human IRBP-derived peptide aa 161-180. IFN-a dosage was adapted from our scheme in human patients correlating with weight. Twelve of 24 animals received IFN-α daily, injected subcutaneously ($3x10^3$-$6x10^3$ IU) until sacrifice. Histological and immunohistochemical analyses of the eyes were

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carried out on animals sacrificed on day 10 and 18 of post-immunization (n=6 for each treatment and time point). Histological analyses were performed on paraffin embedded sections using HE staining.

For immunohistochemical staining frozen sections were prepared using the alkaline phosphatase method with new fuchsin as chromogen. The applied monoclonal antibodies (MAbs) (Table 1) were directed against CD45, CD3, CD4, CD8, and CD44 isoforms, i.e. panCD44, splicing variants v3 (cytokine binding capacity), v6, v7 (exhibiting inhibitory function in different experimentally induced autoimmune diseases), and v10. Control staining was achieved on murine tongue and spleen sections.

Table 1. Monoclonal antibodies used in the study

MAbs	name	species	antigen
CD45*	30-F11	rat	leucocyte common antigen (LCA)
CD3ε*	145-2C11	hamster	CD3 associated differentiation antigen-pan-T-cell marker
CD4*	RM4-5	rat	T-cell differentiation antigen
CD8*	53-6.7	rat	T-cell differentiation antigen
panCD44	IM-7	rat	HA receptor
CD44v3	PTS-3	rat	CD44 variant isoform 3
CD44v6	11A6	rat	CD44 splicing variant
CD44v7	LN-7	mouse	CD44 splicing variant
CD44v10	K926	rat	CD44 splicing variant

* Commercially supplied by BD Pharmingen

3. RESULTS

1) At day 10 histological analyses revealed severe EAU (grading >3) in 4 of 6 control mice compared to only 2 of 6 IFN-α treated mice (Fig. 1 and Fig. 2). At day 18 there was histologically little difference between control and IFN-α treated mice.

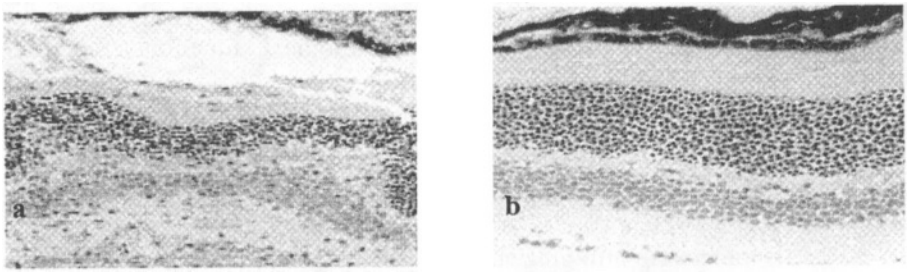

Figure 1. Ten days after immunization a) with peptide. Infiltrated retina, and b) with peptide and IFN-α treatment. Intact retina. HE-staining

2) Of all CD44 isoforms only panCD44 expression was detected in the retina exhibiting no difference between IFN-α treatment and control eyes on day 10 and on day 18. Staining for the CD44 isoforms v3, v6, v7, and v10 was not detectable (data not shown).

3) On day 10 in control animals CD45+ cell infiltration was detectable in the optic nerve, the ciliary body, in the retina and the retinal pigment epithelium (RPE) and appeared minimally in the retina in IFN-α treated animals. In contrast on day 18, CD45+ cell infiltration was detectable in the optic nerve and in the destroyed retina without difference between IFN-α treatment and control.

4) Ten days after immunization CD3+ T-cell infiltration was detectable in the destroyed retina. CD4+ reactions (stained cells) were in minority. In contrast, 18 days after immunization CD3+ and CD4+ T-cell infiltrations appeared in the destroyed retina but CD4+ cells were in the minority. In both cases the staining was performed on sections derived from the same eyes.

4. CONCLUSION

Clinically, the IFN-α treated animals exhibited minimal disease, compared to the control group. These findings have been histologically confirmed.

Using a variety of monoclonal antibodies, we were not able to demonstrate any differences for panCD44 expression. The similar expression pattern of panCD44 in the retina between control and IFN-α treated mice indicates that this expression may be not influenced by the applied IFN-α dosage. The panCD44 homing receptor may be important for the regulation of EAU induction by CD45+ and CD3+ T-cells.

Differences have been observed for CD45+ infiltration between control and IFN-α treated animals. This may be an important hint to characterize their function by markers others than CD8 and CD4 specific antibodies, especially for their T-cell receptor (TCR) expression (α/ß versus γ/δ).

To identify further differences between IFN-α treated and control mice our analyses will be extended to antigen presenting cells (APC), especially to dendritic cells (DC) capable to regulate the Th1/Th2 balance. Additionally, these analyses will be extended to cell adhesion molecules and/or homing receptor expression kinetics regulated by chemo-and cytokines.

EAU induction experiments are presently performed with an increased IFN-α dosage correlating with the surface (m^2) of human beings to clarify our results.

ACKNOWLEDGEMENTS

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A Case of Behçet's Disease with Pathergy Reaction at Interferon Injection Site

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1. INTRODUCTION

It has previously been reported that interferon alfa (IFN-α) injection sites may develop pyoderma gangrenosum, interface dermatitis, vasculitis or ulcers characterised by intravascular thrombi and mixed inflammatory cell infiltrate. Injection site reactions are noninfectious inflammatory reactions at the site of an injected substance.

We had used IFN-α treatment for Behçet patients with the dosage of 3 million i.u. 3 times a week[1]. Interestingly, in one of our patients treated with IFN the eruption developed sparing of vaccination sites. It was accepted as skin pathergy reaction.

2. CASE REPORT

A 32-year-old man with a history of Behçet's disease was evaluated for the treatment. He had aphthous ulcers, genital ulcers and uveitis. He had been treated with colchicine and azothioprine for several years. However, the treatment has been ineffective in controlling the symptoms. Because of disease progression and its resistant to treatment we planned to use IFN-α treatment at a dosage of 9 million i.u. a week. After 4 weeks, painless pustular skin lesions developed at IFN-α injection sites. He had fatigue and mild flue-like symptoms as well. Examination of the arm revealed 0.5 mm pustules and surrounding erythema. We did not take a biopsy. Bacterial

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culture was negative. The clinical impression was of a pathergy reaction or a local reaction to the IFN-α. His pathergy was positive at 48 hours. During the IFN treatment pathergy reaction at forearm was positive.

3. DISCUSSION

We report on a case with pustules at IFN-α injection sites. The clinical presentation suggested a pathergy reaction or injection reaction to IFN-α.

Interferons are a family of glycosylated proteins which have antiproliferative, antitumoral, and immunomodulatory effects. IFN-α inhibits angiogenesis, limits endothelial cell migration, and promotes endothelial cell apoptosis[2]. Most IFN treatment protocols call for subcutaneous administration. A variety of reactions have been reported at IFN-α injection sites (Table 1)[3-7].

Table 1. IFN-α injection site reactions (from ref. 6)

Pyoderma gangrenosum
Leucocytoclastic vasculitis
Interface dermatitis
Dermal hypersensitivity
Necrotising ulcerations
Suppurative and granulomatous dermatitis

In our case we were assuming inhibition of mucocutaneous symptoms as well as pathergy reaction during IFN-α treatment. Interestingly, eruption developed sparing of vaccination sites. It is accepted as skin pathergy reaction. Pathergy occurs in approximately 40% of the cases but apparently in non-treated population, especially during the exacerbation period. Pathergy positivity has been decreasing in recent years in Turkey[10,11]. Any treatment for Behçet's disease and age of the patients may affect the rate of prevalence of pathergy positivity. We noticed pathergy-like lesions during IFN-α treatment at the injection sites. Budak et al.[12] made similar observations in their Behçet cases with chronic myelogenous leukemia being treated with IFN-α. Sanders et al.[6] pointed out that IFN-α injection site reactions have clinical and histological similarities with pyoderma gangrenosum and Crohn's disease. We add, therefore, an adverse cutaneous reaction related to IFN-α treatment. Skin pathergy reaction may also develop following IFN-α treatment in Behçet's disease.

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Current and Future Use of Anti-TNF Agents in the Treatment of Autoimmune, Inflammatory Disorders

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1. INTRODUCTION

Not since the use of cortisone in the treatment of rheumatoid arthritis (RA) over 50 years ago, has there been as much excitement over anti-inflammatory treatment. Agents that inhibit the effects of tumor necrosis factor α (TNFα) have demonstrated striking efficacy in studies of RA, juvenile RA, psoriatic arthritis, and Crohn's disease, mirrored or exceeded by their benefits in clinical practice. Data is rapidly accumulating that anti-TNF agents may be effective in the treatment of several other immune mediated inflammatory diseases.

2. APPROVED INDICATIONS FOR ANTI-TNFα AGENTS IN THE UNITED STATES

There are two anti-TNF α agents that have been approved for use and are currently available in the United States. In 1998, etanercept (Enbrel) received an indication for the treatment of RA. Shortly thereafter infliximab (Remicade) was approved for the treatment of Crohn's disease, and six months later the drug received the indication for the treatment of RA. More recent indications for etanercept are the treatment of juvenile RA and psoriatic arthritis. These two agents differ in their structure as well as their mechanism of TNFα inhibition. Etanercept is a genetically engineered

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molecule which contains the soluble p75 TNF receptor which binds circulating TNFα, making the ligand unavailable to bind to it's cell bound receptor. Infliximab is a chimeric monoclonal antibody to TNFα which inactivates circulating or cell bound ligand. Infliximab is administered in an intermittent intravenous infusion while etanercept is given by subcutaneous injection. In both cases, anti-TNF activity is recognized clinically in the reduction of inflammatory disease activity.

Anti-TNFα drugs are effective in the treatment of the signs and symptoms of RA, in inhibition of radiographic progression, and for improvement of function in patients with the disease. Infliximab is used to treat Crohn's disease which is unresponsive to conventional therapy, to induce and maintain remission of the disease, and to treat disease related fistulas. Trials are ongoing in the treatment of ulcerative colitis with infliximab. Both agents are being studied in psoriasis, ankylosing spondylitis, and vasculitis.

Potential side effects of TNFα inhibitors include the occurrence of serious infections and these agents have been associated with the reactivation of latent tuberculosis in some susceptible individuals.

3. WHY IS TNFα SO IMPORTANT IN INFLAMMATORY DISEASES?

Research continues to unravel the details of the pathogenesis of inflammatory disorders, TNFα participates in many of these conditions as a proinflammatory cytokine, very "upstream" in the inflammatory process, responsible for the recruitment of other proinflammatory cytokines such as IL-1, as well as the stimulation of factors responsible for attraction of inflammatory cells, synthesis of acute phase proteins, angiogenesis, and metalloproteinase enzyme synthesis. In some cases TNFα works together with other cytokines to drive the inflammatory process. Inhibition of this key mediator has been demonstrated to have a potent anti-inflammatory effect in several models. The role of this cytokine in the pathogenesis of osteoporosis and loss of bone in inflammatory conditions is currently under intense investigation.

While TNFα is undoubtedly a major player in inflammation, it would be naive to believe that this complex process can be controlled completely in all of our patients through the inhibition of this single molecule. There are patients whose inflammatory disease seems resistant to TNFα blockade. The possibility of disease subsets, each driven by specific dominant cytokines or combinations of cytokines in an attractive hypothesis. The important role of other cytokines such as IL-1, IL-15, IL-17, and others can be demonstrated

in conditions of inflammation. Combination cytokine inhibition is a strategy just now being tested in the clinical setting.

4. ANTI-TNFα INHIBITION IN BEHÇET'S DISEASE

In BD, several case reports and small case series support the possibility that anti-TNF agents may be of benefit:

Hassard[1]: infliximab therapy for gastrointestinal BD (N=1)

Robertson[2]: infliximab for oro-genital ulcers (N=1)

Goossens[3]: remission of BD with infliximab (N=1)

Rozenbaum[4]: mucocutaneous lesions treated (N=1)

Sfikakis[5]: infliximab for panuveitis in BD (N=5)

Banares: infliximab in the treatment of refractory post uveitis

Travis[6]: anti-TNFα treatment of gastrointestinal BD (N=2)

Munoz-Fernandez[7]: ocular disease treated with infliximab (N=1)

While anecdotal reports suffer from publication bias, this preliminary experience supports a need for additional study of these agents in the treatment of BD.

Abstracts were presented at the annual EULAR meeting in Stockholm, including the long-awaited first report of a double-blind study by Melikoglu and colleagues from Istanbul[8]. This study with etanercept therapy for mucocutaneous manifestations of BD provides strong evidence for the efficacy of this agent. Of interest, the pathergy reaction in these patients was not suppressed during the course of treatment. In addition, there were other reports presented at this congress:

Bagnato[9]: infliximab treatment of BD, a case report (N=1)

Erkan[10]: infliximab treatment of aphthosis in BD (N=2)

Reports from this International Conference on Behcet's Disease include an open experience in 9 patients with severe eye disease, failing combination conventional therapy of azathioprine, cyclosporine and steroids, by

Melikoglu: These were patients with very severe ocular disease and it remains to be seen whether patients might be more responsive if treated sooner. Additional reports included:

Elezoglou: Serum soluble TNF-RII levels increased in active BD

Wallace: TNF-103C polymorphisms is associated with BD in a UK cohort

Sable-Fourtassou: infliximab in panuveitis (N=3)

Joseph: infliximab in refractory post uveitis (N=3)

Triolo: unresponsive ocular and central nervous system disease (N=3)

Morris: treatment of an 18-year-old with panuveitis (N=l)

Sfikakis: long term follow up after treatment in two patients reported (N=2)

Behrens: a case report with multiple complications (N=1)

The magnitude of the response to anti-TNF therapy in many of these individual cases is quite remarkable, including the induction of remission in some of these patients.

Multicenter studies of anti-TNFα therapies are essential to confirm the beneficial role of these agents in BD. Most appropriate studies would seem to be directed against those complications associated with the greatest morbidity: ocular disease, central nervous system disease, and large vessel disease.

5. IMMUNE MEDIATED INFLAMMATORY DISEASES

Research into the pathogenesis of autoimmune inflammatory diseases, as well as clinical experience with disorders affecting a variety of different organs, support a strong bond between these disorders. This commonality mandates that we continue to modify our approach to this group of diseases that would be in the best interest of our patients. More and more are specialists needed whose therapeutic expertise crosses traditional boundaries to encompass a much wider group diseases which share the features of immune mediated inflammatory disorders.

6. CONCLUSIONS

In addition to the recognized efficacy in RA, Crohn's disease, and psoriatic arthritis, data on the therapeutic value of anti-TNF agents continues to accumulate in the treatment of a number of other autoimmune, inflammatory diseases. Could these agents become the corticosteroid of the 21st century?

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Successful Long-term Treatment of Refractory Adamantiades-Behçet's Disease (ABD) with Infliximab: Report of Two Patients

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1. INTRODUCTION

Adamantiades-Behçet's disease (ABD) is a multisystemic, chronic, inflammatory disorder characterised by spontaneous exacerbations and remissions. Mucocutaneous, ocular, articular, vascular, gastrointestinal and central nervous system involvement may be present, while the severity of the disease is varying to a great extent among different patients[1]. In this commonly disabling disease, ocular involvement is the most frequent cause of morbidity, leading to blindness in 20-25% of those affected[1,2]. Since the etiology of ABD is unknown, therapeutic intervention remains empiric. Although various non-specific immunosupressive agents have been used, a significant number of patients fails to respond[1-3]. Recently, the blockade of tumour necrosis factor (TNF) actions has emerged as a possible new therapeutic target in ABD. In a previous study, we examined the effects of a single infusion of the anti-TNF mAb infliximab in our 5 patients who suffered from refractory sight-threatening panuveitis[4]. Moreover, a total of 11 cases being refractory to conventional disease treatment has been reported to receive short-term infliximab treatment, including 2 patients with severe mucocutaneous manifestations[5,6], 3 patients with severe gastrointestinal involvement[7,8] and 1 more patient with severe ocular

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disease[9] during 2001. In all cases short-term infliximab treatment had impressive results. We report here our preliminary experience in two Greek patients who completed 20 and 16 months, respectively, of continuous i.v. treatment with infliximab.

2. CASE REPORTS

2.1 Case 1

The first patient was a 42-year-old female, with a 20-year disease duration, who presented with severe orogenital ulcerations, oligoarthritis, and low-grade fever. During the past years her orogenital ulcerations responded only to moderate-to-high doses of steroids, which had resulted in severe, steroid-induced osteoporosis. Any attempt in the past to reduce the daily dose of methylprednisolone below 8 mg using cyclosporine A and/or azathioprine was unsuccessful. At the initiation of infliximab treatment, which was decided mainly to treat severe genital ulcers, she was on methylprednisolone 0.2 mg/kgr/day and colchicine.

Infliximab (Remicade®) was administrated intravenously at a dose of 5 mg/kg at day 1, day 30, and every 8 weeks therafter. To date she has completed 20 months of continuous treatment. The acute response to infliximab treatment was the resolution of all symptoms within 10 days. Infliximab proved to be efficacious in the long term; ulcerations have not appeared and no episodes of arthritis and fever have occurred ever since the second infusion. Moreover, methylprednisolone was rapidly tapered and for the last 18 months the patient was receiving, in addition to infliximab, 3 mg/day of methylprednisolone only. Up to the present day no adverse events have been noted. Interestingly, during the following 9 months after the initiation of infliximab, the patient developed moderate titres of serum antinuclear antibodies, which were negative at baseline, as well as low titres of anti-dsDNA antibodies. During the subsequent 11 months of follow-up no clinical signs suggestive of systemic lupus erythematosus were ever noted, despite continuous treatment.

2.2 Case 2

The second patient was a 21-year-old male, with a 4-year history of ABD, who presented with a bilateral panuveitis relapse. Despite combination treatment with cyclosporine A (3 mg/kgr/day), azathioprine (2mg/kgr/day), and prednisolone (0.2-0.3 mg/kgr/day), he suffered from

recurrent episodes of ocular inflammation. During the last year prior to infliximab administration, 11 such episodes had occurred. At his last ocular relapse (day 0) we decided to treat him with infliximab. Remicade® was administrated intravenously at a dose of 5 mg/kg at day 1, day 30, and every 8 weeks therafter. In addition to visual acuity evaluation, a standard scoring system was used to assess the presence of inflammatory cells in anterior chamber (on a scale from 0 to 4), the presence of vitreous haze (on a scale from 0-3), and retinal involvement (presence of vasculitis and number of retinal lesions). As shown in Table 1, the acute response to infliximab treatment was a dramatic improvement of ocular inflammation within 48 hours, and the resolution of symptoms within 7 days. The resolution of vitreous haze and of a retinal lesion following infliximab administration is depicted in Fig. 1.

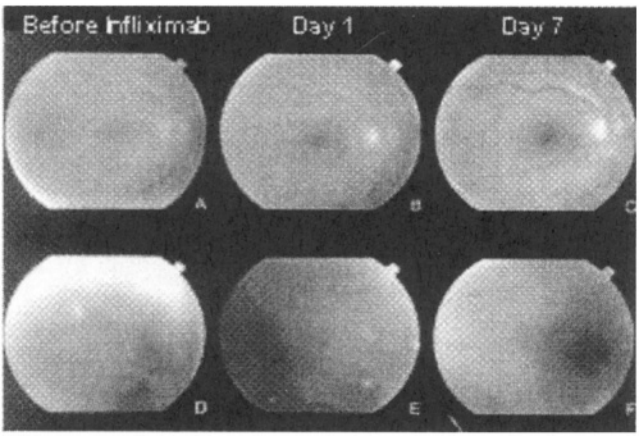

Figure 1. Resolution of vitreous haze and a retinal lesion after infliximab therapy. Significant (2+) vitreous haze (A) and retinal lesion in the mid-periphery (D) of the right eye before infliximab administration. Vitreous haze has decreased (1+) at day 1 (B) and cleared at day 7 (C). The retinal lesion has diminished by 50% at day 1 (E) and is barely detectable at day 7 (F).

Infliximab proved efficacious in the long term; the patient is in remission ever since the first infusion. The evaluation of visual acuity, and ocular inflammation score during treatment is shown in Table 1. Moreover, prednisolone was tapered and withdrawn in the 9th month of infliximab treatment, whereas azathioprine and cyclosporine A were both withdrawn within 7 and 14 months, respectively. For the last 2 months the patient was only receiving infliximab. During monotherapy with infliximab, the patient's overall clinical status remained stable. Infliximab administration was well tolerated and no adverse events were noted.

Table 1. Visual acuity and ocular score of inflammation at panuveitis relapse (day 0), and 1,4, 14, 28 days up to 16 months after the initiation of infliximab therapy

	Visual Acuity		Anterior Chamber Cells (0 – 4)		Vitreous Haze (0-3)		Vasculitis (+/-)		Retinal Lesions (number)	
Eye	L	R	L	R	L	R	L	R	L	R
Day 0	0.4	0.2	1	2	1	2	+	+	1	3
Day 1	0.5	0.5	Trace	Trace	0	1	+	+	1	1
Day 4	0.8	0.6	0	0	0	Trace	-	-	0	0
Day 14	1	0.7	0	0	0	0	-	-	0	0
Day 28	1	0.8	0	0	0	0	-	-	0	0
Months 2 –16	1	0.8	0	0	0	0	-	-	0	0

3. DISCUSSION

Inflammation in ABD is thought to be mediated by various cytokines, including TNF-α[10]. Increased serum levels of circulating TNF-α and soluble TNF-receptors in the peripheral blood of patients with active disease[11,12], as well as high levels of TNF-α in the aqueous humor of patients with ABD-associated uveitis have been recently reported[13,14]. The beneficial results in ABD patients who have received the anti-TNF antibody infliximab[5-9,15], including our 5 patients with sight-threatening panuveitits[4], are further supporting a possibly central role of TNF-α in ABD pathogenesis. However, only the short-term effects of infliximab have been reported in these small studies; whether the blockade of TNF-α has a long-term efficacy in preventing relapses and progression of the disease is currently unknown.

The two patients described here represent our first experience in continuous infliximab treatment in ABD. To the best of our knowledge, there are no reports on ABD patients who have been treated with infliximab for more than 6 months. Several additional patients being refractory to conventional disease treatment are currently enrolled in our therapeutic protocol. Although various infliximab doses have been used[4-9,15,16], according to our previous experience[4] we chose the dose of 5 mg/kg, administered every 8 weeks. Under this treatment schedule, both patients displayed a rapid and sustained response of all symptoms. Infliximab therapy maintained remission and allowed the reduction or the withdrawal of concomitant medications. No adverse effects were noted, but, as has been frequently reported in patients with rheumatoid arthritis receiving infliximab[17], the female patient developed antinuclear and anti-dsDNA autoantibodies soon after the initiation of the treatment. Although no lupus-related signs or symptoms have appeared we are currently following-up

these values every 6 months. Both patients continue to receive infliximab every 8 weeks and we plan to gradually broaden the intervals between the doses under observation for sustained remission. These preliminary results suggest that efficacy is not reduced during long-term treatment; even though clearly more patients are needed to confirm that TNF-blockade is a safe, effective in the long-term, steroid-sparing approach for ABD.

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The Effect of Anti-Tumour Necrosis Factor alpha (Infliximab) on Sight-Threatening Uveitis in a Patient with Behçet's Disease

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1. INTRODUCTION AND CASE HISTORY

A 14-year-old boy presented in 1998 with oral and genital liberation, bilateral blurred vision and floaters, polyarthralgia and a facial rash. He was diagnosed with Behçet's disease. He was immediately started on high dose steroids but when the dose of prednisolone was reduced he was plagued with bouts of retinal infiltrates and vasculitis and systemic symptoms of Behçet's disease.

He developed right cystoid macular oedema which responded to pulsed intravenous methylprednisolone and his visual acuity varied considerably over 3 years at best 6/9 and at worst 6/60. However, there was concern about the iatrogenic effects of such large doses of steroids in a teenager so other immunosuppressants were tried in an attempt to bring his panuveitis under control on a dose of prednisolone below 20 mg.

There was little success with tacrolimus, azathioprine, mycophenolate mofetil, thalidomide, cyclophosphamide, and alpha interferon. He developed many problems including weight gain, renal impairment, hypertension, osteoporosis and avascular necrosis of his hip and knee joints requiring hip decompression surgery and bilateral knee replacements. He has also required bilateral cataract surgery.

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2. ANTI-TUMOUR NECROSIS FACTOR-ALPHA

At the end of 2001, the patient's fundi were typical of advanced Behcet's disease with mild vitritis, pale atrophic optic discs and corresponding visual field defects. A recent paper by Sfikakis et al.[1] had advocated the use of anti-tumour necrosis factor-alpha (infliximab) in panuveitis associated with Behçet's disease, so we gave this patient his first dose in December 2001.

An immediate decrease of inflammation was observed in the eyes and the systemic disease, in a similar fashion described by Sfikakis et al.[1]. He was free of inflammation for four months and his visual acuity improved to 6/6 N4.5 in both eyes. For the first time since diagnosis his prednisolone dosage was reduced below 20 mg, eventually to 11 mg in conjunction with azathioprine and mycophenolate mofetil. He has also lost 11 kg in weight.

3. A NEW SIDE EFFECT OF INFLIXIMAB

Sfikakis et al.[1] found transient side effects of flu-like symptoms, headache, nausea, and abdominal pain after infliximab infusion. This patient only complained of mild nausea during administration of the drug which passed quickly. Unfortunately, after four infusions he developed a right upper arm pyomyositis which required intravenous antibiotics.

This side effect has not been reported previously with infliximab and may be due to the increased immunosuppression. He then missed two doses of infliximab and unfortunately suffered a relapse in his uveitis with his visual acuity dropping to 6/36 in both eyes. He has now recovered from his pyomyosistis with no persistent muscular inflammation and it has been decided to re-institute infliximab treatment.

4. CONCLUSION

This case highlights the management problems encountered when dealing with Behçet's disease and emphasises the significant co-morbidity which can be encountered as a result of treatment. Anti-tumour necrosis factor shows promise as an effective new therapy for sight-threatening uveitis and perhaps other vasculitic manifestations of this disease in which tumour necrosis factor appears to play a central pathogenic role.

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Antitumor Necrosis Factor Monoclonal Antibody Therapy in a Woman with Severe Adamantiades-Behçet's Disease

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1. INTRODUCTION

Individual patients with Adamantiades-Behçet's disease (ABD) experience a chronic deterioriating course despite intensive, chronic immunosuppressive therapy. Since tumor necrosis factor-α (TNFα) might play an important pathogenetic role in this disorder, we used anti-TNFα monoclonal antibody (TNFαAB) therapy in a woman recalcitrant to conventional therapy.

2. CASE REPORT

A 44-year old woman of Greek origin presented with systemic ABD known for more than 5 years. Diagnostic criteria were bipolar ulcers, papulopustular lesions, positive pathergy phenomenon and polyarthritis. Despite different treatments in adequate dosage over sufficient time (corticosteroids, colchicine, azathiophrin, dapsone, interferon alpha, mycophenolate mofetil, methotrexate, chlorambucil, cyclosporin A) reduction of daily doses of prednisolone below 12-15 mg were followed by glottis edema, pharyngeal ulcerations, and dyspnea. The patient presented with a Cushing appearance, insulin-dependent diabetes mellitus, oral and

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genital ulcers, polyarthritis in 3 major joints, papulopustular lesions and a non-healing ulceration of her lower left leg, probably due to a cutaneous vasculitis, despite 32 mg methylprednisolone (MP) and cyclosporin A (CSA) 4 mg/kg/d. As the reduction of the MP dose was followed by difficulties to breathe and swallow due to large pharyngeal ulcerations, CSA was stopped and TNF α AB therapy (Infliximab, 3 mg/kg) was instituted.

Although the oral ulcers healed within 1 week, new papulopustular lesions evolved at the abdomen. The planned application of Infliximab had to be postponed for 4 months (persisting muculopurulent secretions, personal reasons). Before the 2nd application the findings were similar to the initial presentation, but the MP dose was lower (14 mg/d). After the 2nd application (5 mg/kg; ciprofloxacin prophylaxis) the oral ulcers healed but the ulceration on the leg persisted. 14 days after the 3rd dose new bipolar ulcers developed. The patient returned to our clinic 4 months later with fever and severe inflammation of the throat despite 40 mg MP and 4 mg/kg CSA. Infliximab, given 3x at intervals of 14 days, again induced a rapid response (fever 1d, ulcer improvement 2d) and allowed to taper corticosteroids (15 mg). TNF α AB therapy was tolerated well at all times.

3. CONCLUSION

ABD is a T helper cell type 1-mediated disease and TNFα plays a pivotal role in its evolution. This case illustrates the therapeutic difficulties in ABD. Monoclonal TNFα antibody therapy improved the bipolar ulcers and arthritis initially and allowed to reduce the daily MP dose. No sepsis occurred under antibiotic prophylaxis and daily wound care. However, a complete remission could not be achieved. Despite some promising case reports on TNFα antibody therapy[1-3], it could not induce a remission in our patient. So this treatment may be an alternative adjunctive experimental approach in recalcitrant cases.

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Efficacy of Corticosteroids and Cyclosporin in the Treatment of Retinal Vasculitis in Patients with Behçet's Disease

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1. INTRODUCTION

Posterior uveitis and retinal vasculitis (RV) are the main cause of severe loss of vision or blindness in patients with Behçet's disease (BD). Treatment of RV in BD is difficult since the agents that are effective in posterior uveitis fail in RV.

This study was designed to evaluate the efficacy of corticosteroids and cyclosporine in the treatment of BD patients with RV.

2. MATERIAL AND METHODS

BD patients (20 men, 6 women, mean age 30.8±6.5 years with a range of 18-46 years) were included in this study. All patients fulfilled the International Study Group criteria for BD. Corticosteroids were used in active period of RV by pulse therapy (metipred 3 g), per os (prednisolone 40 mg/d), and locally (periocular injections of 3 mg dexamethasone). Cyclosporine was given as monotherapy (5 mg/kg/d) and in combination with prednisolone per os (10-15 mg/d).

Chi-square tests and Student's t-test were used for statistical analysis.

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3. RESULTS

Pulse therapy and per os corticosteroids were more effective than periocular injections in active phase of retinal vasculitis (respectively 92.8%, 90.9%, and 45.5%, p<0.009).

The rate of inflammatory reduction and the degree of visual acuity improvement was more prominent in patients with steroid pulse-therapy (respectively 12.7±2.4 days and 0.19±0.21) compared to patients with per os steroids (respectively 17.7±1.8 days, p<0.0000 and 0.08±0.07, p=0.0121) (Table 1). For a long control of RV in BD patients prednisolone and cyclosporine were used (Table 2).

Table 1. The rate of visual improvement and inflammatory reduction in RV BD patients receiving corticosteroid pulse-therapy and corticosteroids per os

Methods of therapy	Visual acuity (M±SD)			The rate of inflammatory reduction (days, M±SD).
	At the beginning	After 10 days	Difference	
Corticosteroid pulse therapy	0.18±0.19	0.38±0.30	0.19±0.21 p=0.0121	12.7±2.4 p<0.0001
Corticosteroids per os	0.29±0.36	0.37±0.31	0.08±0.07	17.7±1.8

Comparison: p- corticosteroid pulse therapy vs corticosteroids per os

Table 2. Efficacy of prednisolone and cyclosporine for a long control of RV in BD patients

	Remission		Decrease of recurrences		Improvement of visual acuity		Stabilization of visual acuity	
	n	%	n	%	n	%	n	%
Prednisolone 10-15 mg/d, n=14	-	-	1	7.1	-	-	1	7.1
Cyclosporine 5 mg/kg/d, n=7	2	28.6	2	28.6	3	42.9	1	14.3
Cyclosporine 3.5 mg/kg/d + prednisolone 10-15 mg/d, n=11	5	45.5	4	36.4	3	27.3	6	54.5

In 3 cases cyclosporine and in 3 cases cyclosporine with prednisolone was administered after prednisolone.

Prednisolone (10-15 mg/d) was ineffective for prevention of recurrences and for visual stabilisation. The more prominent effect has been received by

using combination of cyclosporine (3.5 mg/kg/d) with prednisolone (10-15 mg/d). Cyclosporine 5 mg/kg/d was less effective (ns).

4. CONCLUSION

Corticosteroid pulse therapy is effective in active period of RV. For a long control of RV in BD patients, cyclosporine 3.5 mg/kg/d with prednisolone (10-15 mg/d) is an effective combination, less effective is cyclosporine 5 mg/kg/d, and prednisolone alone (10-15 mg/d) is ineffective.

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Enhancement of Transmucosal Permeation of Cyclosporine by Benzalkonium Chloride

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1. INTRODUCTION

Topical cyclosporine A (CsA) has been used in the treatment of oral ulcerations associated with Behçet's disease[1], but local tissue penetration of this highly hydrophobic and high molecular mass (1203 Da) drug may not be optimal[2,3]. Tissue levels measured in biopsied buccal mucosa specimens, in the presence of low blood levels, suggested a localised mode of action of this immunosuppressant, indicating that topical drug absorption is an important factor in eliciting CsA's beneficial effects on oral mucosal lesions. For this reason we studied the permeation of this drug through intact and mechanically de-epithelialised human vaginal mucosa, which may be used as a model of buccal mucosa[4-8], in the presence and absence of the penetration enhancer 0.01% benzalkonium chloride (BZCl)[9].

2. MATERIALS AND METHODS

Human vaginal mucosa was obtained from excess tissue removed from eight postmenopausal patients following vaginal hysterectomies. No specimens were obtained where there was clinical evidence of any disease that might have influenced the permeability characteristics of the vaginal

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mucosa. On arrival in our laboratory, excess connective tissue was trimmed away and all specimens were snap-frozen in liquid nitrogen and stored at -85°C for a period of up to 6 months[10]. The study was approved by the Ethics Committee of the University of Stellenbosch and Tygerberg Hospital.

Prior to each permeability experiment, tissue specimens were thawed at room temperature in phosphate-buffered saline (PBS; pH 7.9). Each thawed specimen was divided into two halves. The one half, with intact epithelial layer, was used unaltered as control specimen, while the other half was carefully scraped with a scalpel to remove the epithelial layer without damaging the underlying connective tissue layer. CsA permeation through thawed frozen intact and mechanically de-epithelialised vaginal mucosa was determined using a flow-through diffusion apparatus (20°C, 24 h), as previously described[4-8].

Flux rates for CsA across these two mucosa were determined in both the presence and absence of 0.01% BZCl. ANOVA and Duncan's multiple range tests were used to test for steady state over at least two consecutive 2-h time intervals. An unpaired *t*-test with Welch's correction was used to investigate possible differences between CsA flux means across vaginal tissues at 2-h intervals. A significance level of $P<0.05$ was used for all tests and comparisons.

A section of thawed tissue obtained from each patient, before and after de-epithelialisation, was placed in formalin and histologically examined using paraffin-embedded 5-μm sections stained with haematoxylin and eosin.

3. RESULTS

Overall mean flux values for CsA across intact and de-epithelialised vaginal mucosa, both in the presence and absence of 0.01% BZCl are shown in Fig. 1. Steady-state diffusion kinetics was reached after approximately 4 h in the absence of 0.01% BZCl for both intact and de-epithelialised vaginal mucosa. Flux rates of CsA across intact vaginal mucosa tended to increase by 28 to 46% in the presence of 0.01% BZCl, the differences becoming statistically significantly different after 12 h. There was also a clear tendency for flux rates of CsA across de-epithelialised vaginal mucosa to be approximately 28% higher than across intact mucosa over the entire course of the experiment, but these differences were statistically significantly higher ($P<0.05$) only after 10 h. Flux rates across de-epithelialised mucosa were 52% to 140% higher in the presence of 0.01% BZCl than for de-epithelialised mucosa in the absence of BZCl, but statistically significant differences ($P<0.05$) were only observed after 12 hours of the experiment.

Histological examination of formalin-fixed specimens showed that structural integrity of the tissue was preserved after freezing as previously demonstrated[10], although individual epithelial cells demonstrated early signs of autolysis. After mechanical de-epithelialisation, microscopic examination revealed that separation between the epithelium and the lamina propria occurred at the basement membrane.

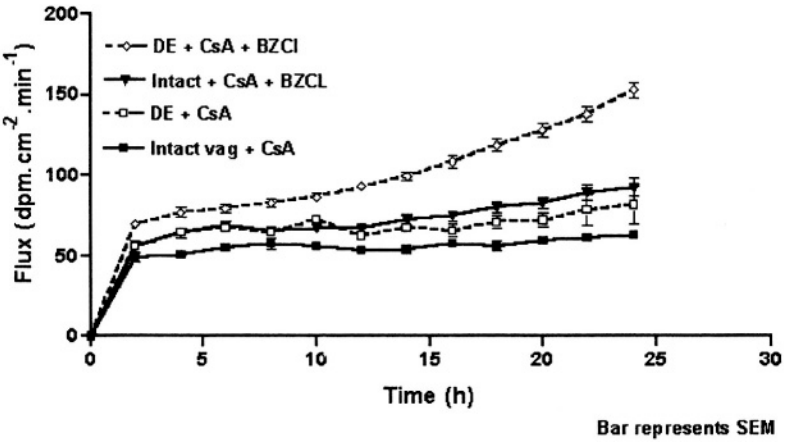

Figure 1. Overall mean flux values for CsA across intact and de-epithelialised (DE) human vaginal mucosa in the absence and presence of 0.01% BZCl

4. CONCLUSION

In conclusion, we demonstrated that the diffusion characteristics of CsA across human vagina were significantly enhanced in the presence of 0.01% BZCl. This was found to be the case particularly where the epithelial layers had been removed from the mucosal surface. It is suggested that this may have clinical applications in those situations where epithelial layers have been damaged or sloughed off due to disease processes causing oral ulcerations, e.g. Behçet's disease. Better penetration of CsA and relief of symptoms may therefore be obtained when this surfactant is added to mouth-rinses.

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Azathioprine and Low Dose Pulse Cyclophosphamide in Severe Ocular Lesions of Behçet's Disease

A preliminary report

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1. INTRODUCTION

Combination of cytotoxic drugs was suggested to decrease the rate of non-responders in severe ocular lesions of Behçet's disease (BD)[1]. Uncontrolled experience with combinations such as Cyclosporin and Azathioprine (AZA)[2], or low dose pulse cyclophosphamide (LDP) and methotrexate[3] showed their efficacy in these lesions.

In this study we are going to find out if the combination of AZA and LDP is effective in severe ocular lesions of BD without increasing side effects.

2. MATERIALS AND METHODS

Twelve BD patients with posterior uveitis and retinal vasculitis were selected. They all received LDP as 0.5 g/m^2 body surface/month by intravenous infusion, AZA 2-3 mg/kg/day and prednisolone 0.5 mg/kg/day orally. Visual acuity, Disease Activity Index (DAI) based on the inflammatory state of each section of each eye, and a total adjusted DAI for each patient was calculated[3]. Improvement and stabilization of the lesions

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were classified as *good result.* The threshold level was set to 20% change from the baseline. Comparisons were made by *student t test.*

3. RESULTS

This combination therapy was effective in the treatment of all parts of the eyes. The mean total adjusted DAI decreased from 40.6±10.5 to 30.6±10.4 ($p>0.08$). Side effect was nausea seen only in one case. Comparison of these results with patients who received LDP or AZA alone, showed no statistically significant difference after the same mean follow-up time (Table 1).

Table 1 Comparison the good results of 3 methods of treatment

	LDP*(~)	AZA†(~)	AZALDP‡(~)
No. of patients	98	66	12
Mean follow-up	9.8(0.7)	9.7(1.1)	9.3(2.6)
Anterior uveitis	89(4.4)	85(6.1)	88(13.2)
Posterior uveitis	87(4.8)	87(6.0)	85(15.6)
Retinitis	76(6.5)	82(7.3)	79(21.5)
Visual acuity	79(5.7)	76(7.3)	79(16.3)
TAI DAI ζ	74(8.7)	79(9.8)	75(24.5)
TA DAI φ	84(7.3)	85(8.6)	92(15.3)

* Low dose pulse cyclophosphamide, ~ Confidence interval, † azathioprine,
‡ AZA + LDP, ζ Total Adjusted Inflammatory Disease Activity Index,
φ Total Adjusted Disease Activity Index

4. DISCUSSION

Combination therapy with LDP and AZA was effective in ocular lesions of BD, with no superiority to single therapy with the same drugs in short term. Considering the disease-modifying nature of these two immunosuppressive drugs, it seems that a longer follow-up time with more patients is needed to confirm the efficacy of this regimen.

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Low Dose MTX for Progressive Neuro-Behçet's Disease

A follow-up study for 4 years

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1. INTRODUCTION

Progressive neuro-Behçet's disease (progressive NB) can ultimately lead to the deterioration of the patients health[1]. Since conventional treatment with prednisolone, colchicine, and cytotoxic drugs has not been effective in progressive NB[1-3] an open trial was designed to investigate the efficacy and the safety of low-dose weekly methotrexate (MTX) for progressive NB for as long as 4 years.

2. PATIENTS AND METHODS

Ten patients who had satisfied the 1982 Japanese diagnostic criteria as well as the International criteria for Behçet's disease, whose neuropsychiatric manifestations were judged to be progressive in spite of conventional treatment were enrolled in this study (Table 1). All patients gave informed consent. The patients were given oral MTX until the end of the 4-year-trial. The initial dose of MTX was 5.0-7.5 mg/week, and the dose was increased by 2.5 mg every 2 weeks up to 5.0-15 mg/week according to cerebrospinal fluid (CSF) IL-6 concentrations. The patients were allowed to continue to take small doses of prednisolone (less than 15 mg/day) and colchicine (1.0 mg/day) without changes in doses. The clinical responses of

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the patients were judged every 12 months by neuropsychiatric findings, revised Wechsler adult intelligence scale (WAIS-R), brain MRI scans and CSF IL-6 levels.

Table 1. Profile and clinical data of patients with progressive NB.

No.	Age	Sex	Manifestations	CNS manifestations	Findings on MRI scans
1	59	male	OA	Dementia, Meningitis, Ataxia	Bs and Cbr atrophy
2	64	male	OA	Psychosis, Dementia, Ataxia	T2 high, Bs atrophy
3	55	male	OA	Dementia, Ataxia	Cerebral atrophy
4	47	female	(-)	Dementia, Psychosis, Myoclonus	Bs and Cbr atrophy
5	61	female	OA	Dementia, Psychosis, Ataxia	T2 high, Severe Bs atrophy
6	72	male	(-)	Dementia, Left hemiparesis	T2 high, Brain atrophy
7	31	male	Uv, Skin	Dementia, Ataxia	Bs and Cbr atrophy
8	38	male	Uv Skin	Psychosis, Impotence	Unremarkable
9	51	male	Skin	Psychosis, Ataxia	T2 high, Bs and Cbr atrophy
10	52	male	OA, Skin	Psychosis, Ataxia	Bs atrophy

OA: oral aphthae; Uv: uveitis; Skin: skin lesions; GU: genital ulcers
T2 high: High intensities on T2 weighted images; Bs: Brain stem; Cbr: Cerebrum.

3. RESULTS

Two of the 10 patients dropped out of the trial due to complications other than MTX toxicity. After 4 years of the trial, CSF IL-6 levels were significantly decreased (Fig. 1). The neuropsychiatric manifestations as well as the findings on MRI scans and intelligence quotients were not significantly worsened (Fig. 2). Three patients presented with mild liver dysfunction, which returned to normal by decreasing the doses of MTX or supplementing folate (5.0 mg-10 mg/week).

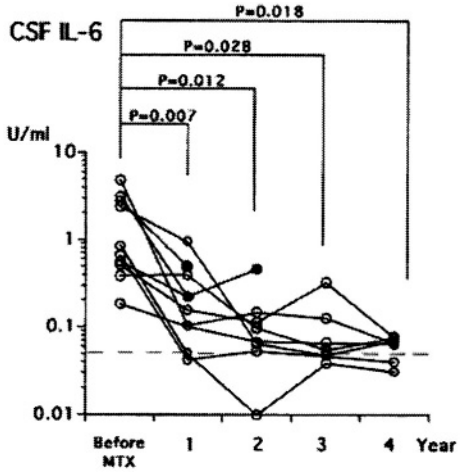

Figure 1. Cerebrospinal fluid (CSF) IL-6 in patients with progressive NB: Normal range for CSF IL-6 is below 0.05 U/ml (10 pg/ml). Solid circle indicates the point of drop out. Statistical analysis was done by Wilcoxon signed test.

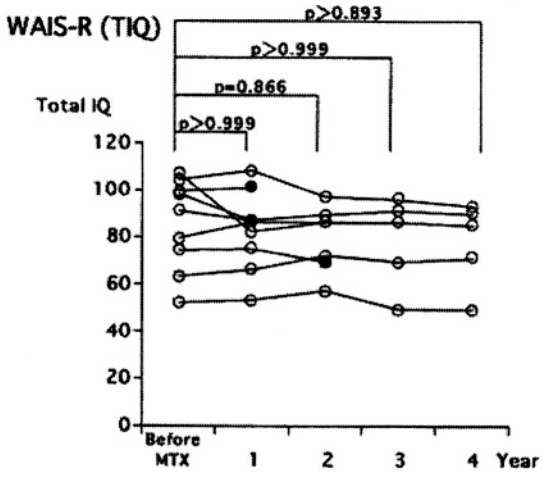

Figure 2. Results of revised Wechsler adult intelligence scale in patients with progressive NB: TIQ: total intelligence quotient. Solid circle indicates the point of drop out. Statistical analysis was done by Wilcoxon signed test.

4. CONCLUSION

The results suggest that low dose weekly MTX therapy might be tolerable and have a beneficial effect on the treatment of patients with progressive NB since it prevented the progression of the neuropsychiatric manifestations by markedly decreasing CSF IL-6 levels.

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High Dose Methotrexate for Ocular Lesions of Behçet's Disease

Preliminary short-term results

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1. INTRODUCTION

Ocular lesions are the major cause of morbidity in Behçet's disease (BD). They are frequently seen (58% of cases[1]). They progress by successive attacks. In posterior uveitis (PU) and in retinal vasculitis (RV) the healing process is very slow because a new attack occurs before the healing of a former one is completed. As a result, lesions will accumulate from one attack to another. Moreover, the healing is usually accompanied by sequelae, and frequently complicated by hemorrhage. All these lesions will progress gradually toward severe loss of vision or blindness. Cytotoxic drugs are the main therapeutic agents that can be used to prevent the critical outcome[2]. Methotrexate (MTX) was demonstrated in previous studies to be effective in ocular lesions of BD[3-4]. However, as for rheumatoid arthritis higher doses of MTX showed better efficacy than the original low dose of 7.5 mg weekly, it was rational to find out if the same would be true for ocular lesions of BD. The aim of this study was therefore to evaluate the efficacy of high dose MTX (HMTX) in ocular lesions of BD, and to compare its efficacy with the low dose MTX (LMTX). We present here a preliminary study, assessing the efficacy of HMTX in a short treatment course.

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2. MATERIALS AND METHODS

New patients with ocular lesions of BD were randomly selected for this study. They had to have an active posterior uveitis or an active retinal vasculitis. Up to now, 23 patients have entered the study. The following data are their short-term preliminary results. For the comparison, the data of the LMTX group from the Ocular Behçet's Disease Treatment Registry were used (297 patients in January 2002).

2.1 Visual acuity measurement

The visual acuity (VA) before the treatment was calculated for each eye by the Snellen chart on a scale of 10/10.

2.2 Ocular inflammatory indices

An activity index was calculated for anterior uveitis (AU), posterior uveitis (PU), and retinal vasculitis (RV) upon the inflammatory state of each eye. Each of the inflammatory parameters was graded from zero (none) to 4 (highest degree of inflammation). These indices were determined as follows. For AU: cells, flares, keratic precipitates, and hypopyon. For PU: cells, snow ball and snow banking. For RV: Periarteritis, periphlebitis, edema of disk, edema of macula, edema of retina, papillitis, and active peripheral lesions were selected for index calculation.

2.3 Patients Activity Indices

A Total Inflammatory Activity Index (TIAI) was calculated for both eyes of each patient on the inflammatory state of each section of each eye. A coefficient was used for each section of each eye to adjust the gravity of the lesion. The coefficient was 1 for AU, 2 for PU, and 3 for RV. The inflammatory index of each section was multiplied by its coefficient and then all the indices were added to obtain the TIAI.

Total Adjusted Disease Activity Index (TADAI) was calculated for both eyes of each patient by adding the TIAI to the result of (10-VA) multiplied by 2 (the coefficient of gravity for VA impairment).

2.4 Statistical analysis

Mean value, standard deviation (SD), and confidence interval (CI) at 95% were calculated for VA and all activity indices before and after the

treatment. Student t test was used to compare the mean VA and different mean activity indices before and after the treatment.

3. RESULTS

3.1 General data

The minimum follow-up time was 3 months and the maximum was 15.4 months. The mean follow-up time was 9.1 months (SD: 3.7 months, CI: 1.6). The mean duration of ocular lesions at the entry of the study was 47.5 months (SD: 63.1, CI: 27.2).

3.2 Visual acuity

Before the treatment:

In HMTX group: From 46 eyes, 18 were normal before and during the treatment. The remaining ones (28 eyes) had a mean VA of 5/10 (SD: 3.6, CI: 1.4).

In LMTX group: From 593 eyes, 124 were normal before and during the treatment. The remaining ones (469 eyes) had a mean VA of 4.9/10 (SD: 3.6, CI: 0.4).

Both groups had similar entry data.

After the treatment:

In HMTX group: The mean VA improved to 6.7/10 (SD: 3.8, CI: 1.5). The mean improvement was 1.7/10 (t: 3.299, $p<0.003$). Referring to the individual eye, 68% improved, 18% were stabilised, and 5% aggravated.

In LMTX group: The mean VA improved to 5.6/10 (SD: 4.8, CI: 0.4). The mean improvement was 0.7/10 (t: 3.590, $p<0.0004$). Referring to the individual eye, 49% improved, 16% stabilised, and 35% aggravated.

3.3 Anterior uveitis

Before the treatment:

In HMTX group, 27 eyes were free of AU before and during the treatment. The remaining ones (19 eyes) had a mean AU of 2.9 (SD: 2.4, CI: 1.2).

In LMTX group, 320 eyes were free of AU before and during the treatment. The remaining ones (272 eyes) had a mean AU of 2.4 (SD: 2.3, CI: 0.3).

AU in HTMX group was more severe than in LMTX group.

After the treatment:

In HMTX group: The mean AU improved to 0.4 (SD: 1.2, CI: 0.6). The mean improvement was 2.5 (t: 4.388, $p<0.0004$). Referring to the individual eye, 95% improved, and 5% aggravated.

In LMTX group: The mean AU improved to 1 (SD: 1.8, CI: 0.2). The mean improvement was 1.4 (t: 7.445, $p<0.000001$). Referring to the individual eye, 72% improved, 5% stabilised, and 23% aggravated.

3.4 Posterior uveitis

Before the treatment:

In HMTX group, 13 eyes were free of PU before and during the treatment. The remaining ones (32 eyes) had a mean PU of 1.8 (SD: 1.0, CI: 0.4).

In LMTX group, 157 eyes were free of PU before and during the treatment. The remaining ones (406 eyes) had a mean AU of 1.8 (SD: 1.2, CI: 0.1).

Both groups had similar entry data.

After the treatment:

In HMTX group: The mean PU improved to 0.4 (SD: 0.7, CI: 0.3). The mean improvement was 1.4 (t: 9.292, $p<0.000001$). Referring to the individual eye, 94% improved, and 4% were stabilised.

In LMTX group: The mean PU improved to 0.7 (SD: 1.1, CI: 0.1). The mean improvement was 1.1 (t: 14.223, $p<0.000001$). Referring to the individual eye, 75% improved, 10% stabilised, and 15% aggravated.

3.5 Retinal vasculitis

Before the treatment:

In HMTX group, 16 eyes were free of RV before and during the treatment. The remaining ones (25 eyes) had a mean RV of 2 (SD: 1.7, CI: 0.7).

In LMTX group, 211 eyes were free of RV before and during the treatment. The remaining ones (315 eyes) had a mean RV of 1.9 (SD: 1.9, CI: 0.2).

Both groups had approximately similar entry data.

After the treatment:

In HMTX group: The mean RV improved to 1.1 (SD: 1.1, CI: 0.5). The mean improvement was 0.9 (t: 2.579, $p<0.02$). Referring to the individual eye, 60% improved, 16% stabilised, and 24% aggravated.

In LMTX group: The mean RV improved to 1.5 (SD: 1.9, CI: 0.2). The mean improvement was 0.4 (t: 2.661, p<0.009). Referring to the individual eye, 52% improved, 18% stabilised, and 30% aggravated.

3.6 Total inflammatory activity index

Before the treatment:

In HMTX group: The mean TIAI was 12.2 (SD: 7.7, CI: 3.3). In LMTX group: The mean TIAI was 12.3 (SD: 8.7, CI: 1.0). Both groups had similar entry data.

After the treatment:

In HMTX group: The mean TIAI improved to 4.1 (SD: 6.0, CI: 2.6). The mean improvement was 8.1 (t: 6.168, p: 0.000003). Regarding the individual patient, 96% improved, and 4% stabilised.

In LMTX group: The mean TIAI improved to 6.5 (SD: 9.1, CI: 1.0). The mean improvement was 5.8 (t: 8.832, p<0.000001). Regarding the individual patient, 73% improved, 5% stabilised, and 22% aggravated.

3.7 Total adjusted disease activity index

Before the treatment:

In HMTX group, the mean TADAI was 24.4 (SD: 16.2, CI: 7.0). In LMTX group, the mean TADAI was 28.3 (SD: 16.8, CI: 1.9). HMTX group had lower TADAI as entry data.than LMTX group.

After the treatment:

In HMTX group: The mean TADAI improved to 12.1 (SD: 15.3, CI: 6.6). The mean improvement was 12.3 (t: 5.962, p: 0.000005). Regarding the individual patient, 87% improved, 9% stabilised, and 4% aggravated.

In LMTX group: The mean TADAI improved to 20.3 (SD: 20.1, CI: 2.3). The mean improvement was 8 (t: 7.499, p<0.000001). Regarding the individual patient, 71% improved, 1% stabilised, and 28% aggravated.

4. DISCUSSION

As shown by the detailed results, both LMTX and HMTX methods were efficient, and the difference in mean improvement was statistically significant for all inflammatory parameters whether calculated for each eye separately or for both eyes in a patient. However, HMTX was more powerful and provided better improvement than LMTX (Table 1).

Table 1. Results of high dose and low dose methotrexate

	Mean Before	*Mean After*	*Mean Improve*	*Mean Before*	*Mean After*	*Mean Improve*
VA	5	6.7	1.7	4.9	5.6	0.7
AU	2.9	0.4	2.5	2.4	1	1.4
PU	1.8	0.4	1.4	1.8	0.7	1.1
RV	2.0	1.1	0.9	1.9	1.5	0.4
TIAI	12.2	4.1	8.1	12.3	6.5	5.8
TADAI	24.4	12.1	12.3	28.3	20.3	8.0

5. CONCLUSION

In BD like in rheumatoid arthritis, higher doses of methotrexate are more efficient. Regarding the low cost of methotrexate, the low incidence of sides effects even with higher doses, and the ease of use and control, we strongly recommend high dose methotrexate for the treatment of ocular manifestations.

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Therapeutic Effect of Thalidomide through Cytokine and Chemokine Regulation in Herpes Simplex Virus-Induced Behçet's Disease-Like Animal Model

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1. INTRODUCTION

Thalidomide has proven over the years to be of clinical use in a small number of immunological diseases[1-8], including Behçet's disease (BD)[9]. Nevertheless, the mechanism of thalidomide action in patients with BD remains poorly understood. A BD-like mouse model was developed by herpes simplex virus (HSV) inoculation[10]. However, HSV alone is not sufficient to explain the pathogenesis of BD, and there are evidences to suggest that immunological abnormalities play an important role in its pathogenesis[11]. In fact, the BD-like mouse model was also thought up to investigate immunological abnormalities. Therefore, in order to study the mechanism of action of thalidomide on immune regulation in BD patients, and the possibility of improving HSV-induced BD-like symptoms in mice, thalidomide was administered to this mouse model.

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2. MATERIALS AND METHODS

2.1 Thalidomide treatment

100 μg of thalidomide was orally administered to BD-like mice for 5 consecutive days.

2.2 Animals and induction of BD symptoms

Four to 5-week-old male ICR mice were used for this study. The earlobes of the mice were scratched with a needle, then inoculated with 1.0 x 10^6 plaque forming units/ml of HSV type 1 (F strain). Virus inoculation was performed twice with a 10-day interval, followed by 16 weeks of observation. Mice were bred in temperature- and light-controlled conventional rooms (20-22°C, 12 h light cycle starting at 8:00 a.m.). The mice had free access to food and water. In order to classify symptomatic mice as having BD, we followed a revised Japanese classification with minor modifications[10]. Oral, genital and other skin ulcers (including bulla and crust), and eye symptoms were classified as major symptoms. Arthritis, gastrointestinal ulcers and neurological disorders were identified as minor symptoms. Mice with at least one major and one minor symptom were classified as having BD.

2.3 RNA Isolation and RT-PCR

Total RNA was isolated using TRIzol™ (Gibco-BRL, MA, USA), and cDNA was prepared using Superscript™ II first-strand synthesis system (Gibco-BRL, MA, USA) according to the manufacturer's instructions. PCR reaction was performed using PCR SuperMix (Gibco-BRL, MA, USA). The amplification was processed in a Perkin Elmer Thermo Cycler 900 with an initial 5 minutes denaturation at 94°C, followed by 35 cycles of the profile: 94°C for 30 seconds; 56°C for 30 seconds; and 72°C for 1 minute. The products were subjected to electrophoresis on a 1.8% agarose gel and visualized under UV light.

2.4 Flow cytometry analysis of Fas- and annexin V-stained cells

Before intracellular cytokine staining, splenocytes were freshly isolated. Brefeldin A (5 μg/ml) (Sigma, St. Louis, MO) was added for the last 4 h of incubation to accumulate cytokines in the Golgi complex. Cells were

harvested, washed in culture medium containing brefeldin A and fixed with 4% formaldehyde in 1% fetal bovine serum containing PBS for 20 min at room temperature. Then, cells were permeabilised with 0.1% saponin in PBS containing 1% fetal bovine serum and 0.1% sodium azide (saponin buffer) for 10 min at room temperature. Cell suspensions were then treated with FITC- or PE-conjugated antibody suspended in permeabilised buffer. Samples were analyzed on a flow cytometer FACS Vantage (Becton Dickinson) collecting at least 20,000 gated lymphocytes.

3. RESULTS

3.1 Improvement of BD-like symptoms in thalidomide-administered mice

Eight out of 10 BD-like mice showed definite signs of improvement in skin ulcer, bulla and crust, intestinal and genital symptoms. One mouse, which also had arthritis, did not show improved symptoms, while the last mouse showed improvements in skin symptoms but developed keratitis after thalidomide administration. The latter mouse also had hepatitis. The control group, mice treated with PBS instead of thalidomide, did not show any change in BD-like symptoms.

3.2 Evidence of down-regulation of TNFα and up-regulation of perforin, Fas and MIP-1α mRNA expression following thalidomide treatment

In order to investigate the possible role played by cytokines affected by thalidomide in the improvement of BD-like symptoms, RT-PCR was used in the spleens of BD-like mice. In the thalidomide administered group, TNFα was found to have decreased whereas perform, Fas and MIP-1α expression had increased. Other cytokines and chemokines, such as IL-2, IL-4, IL-10, IL-1β, IL-6, LPT, MCP-1, RANTES, IP-10, perforin, Fas L, and MIP-1α did not show any significant differences in expression levels.

3.3 Thalidomide treatment can up-regulate Fas and MIP-1α protein expression

Fas expression of splenocytes in thalidomide-administered BD-like mice was significantly up-regulated (54.4%) when compared with those not administered with thalidomide (22.8%) by FACS analysis. When analysed

using immunohistochemistry, MIP-1α was found to stain strongly in the spleen tissues of thalidomide-administered BD-like mice when compared with non-administered BD-like mice.

3.4 RT-PCR analysis of MIP-1α mRNA in peripheral blood of the thalidomide-treated group

Thalidomide-administered BD-like mice were also found to express up-regulated levels of MIP-1α mRNA in peripheral blood, when compared to those not administered with thalidomide

3.5 Western blot analysis of MIP-1α protein levels in the thalidomide treated group

Thalidomide-administered BD-like mice were also shown to express up-regulated protein levels of MIP-1α in spleen tissues, when compared to those not administered with thalidomide.

3.6 Effect of anti-MIP-1α antibody on the expression levels of perforin mRNA in thalidomide-treated splenocytes using RT-PCR analysis

Splenocytes cultured with anti-MIP-1α antibody and thalidomide for 1 day were found to express decreased level of perforin mRNA when compared to those cultured in thalidomide alone. From this, we can draw the conclusion that MIP-1α up-regulates perforin expression. Therefore, thalidomide is connected to the induction of cell death mechanism in splenocytes of BD-like mice.

3.7 Annexin V staining in splenocytes of thalidomide-administered BD-like mice

Splenocytes isolated from thalidomide administered BD-like mice were analyzed by flow cytometry to investigate the expression of annexin V. Thalidomide administered for 5 consecutive days improved BD-like symptoms. These mice were found to express more annexin V stained cells (22.03%) than control BD-like mice (12.30%). Thalidomide was also found to induce cell death through the expression of Fas in the splenocytes of spleen tissue from BD-like mice.

4. CONCLUSION

These results suggest that thalidomide can modulate both cytokine and chemokine expression and also induce death in cells involved in producing excessive inflammatory reactions in order to produce measurable improvements in the symptoms of BD-like mice.

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Pentoxifylline Use for Behçet's Disease

The result of a survey among rheumatologists in North America

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1. INTRODUCTION

Pentoxifylline has been reported to be efficacious in the treatment of aphthous stomatitis[1] and Behçet's disease[2]. We have previously reported that pentoxifylline with or without colchicine was effective in ameliorating oral or genital ulcers of most Behçet's patients and its side effects were usually minimal and reversible[3]. In this paper, we will report beneficial results with pentoxifylline in the treatment of mucocutaneous lesions of Behçet's disease among rheumatologists in the United States and Canada.

2. METHOD

A two-page questionnaire was devised and sent to all fellow members listed in the membership directory of American College of Rheumatology who indicated in clinical practice and resided in the USA, Puerto Rico or Canada (N=3709 from November 2000-January 2001). In this survey, the rheumatologists provided the number of Behçet's patients in their practice and whether they used pentoxifylline with or without colchicine for their patients. The sex of these patients, the doses of pentoxifylline and colchicine they used and the encountered toxicities were ascertained. The effectiveness of these agents was reported for the following categories: oral ulcers, genital ulcers, ocular manifestations or other manifestations; and response to therapy was rated as follows: complete resolution, improved (partial resolution), or no response. The improved outcomes were characterised as a

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decrease in frequency and/or decrease in severity of symptoms. The duration of improvement or remission with either therapy was reported in intervals of less than 6 months, 6-12 months, 12-24 months, or more. If pentoxifylline was discontinued, the question was asked if there was a relapse of disease activity. Additionally, the survey ascertained whether any unexpected side effects occurred with concurrent use of colchicine. Surveys were returned via postage paid envelopes. Results were logged manually and entered into an excel spreadsheet database. Frequency statistics were computed with excel and verified by SAS. Further statistical analysis of chi-square was computed by SAS.

3. SURVEY OUTCOME

Eight hundred and sixty-four surveys were returned (response rate = 23.5%) with the total 1504 patients with Behçet's disease. Thirty-one pieces of mail were undeliverable. One hundred thirty-five of the rheumatologists (16%) had used pentoxifylline in 274 (52%) of their 524 Behçet's patients. Of these 274 patients used pentoxifylline, 73 were male, 181 female, and sex was not reported for 20 patients. Seven hundred and twenty-nine rheumatologists had seen 980 patients with Behçet's disease without using pentoxifylline at all.

3.1 Pentoxifylline use alone

The average dose of pentoxifylline prescribed was 400 mg tid. The use of pentoxifylline alone resulted in complete resolution of oral ulcers in 6% (16/264) of patients and partial resolution in 44% (115/264) of patients with a total response of 50% (131/264). For genital ulcers, complete resolution was reported in 9% (23/252) of patients, partial response in 30% (76/252) of patients, yielding a total response of 39% (99/252). Less impressive results were reported for ocular lesions (complete resolution 5%, partial resolution 8%, and total response 13%) and other symptoms such as arthritis, skin lesions, vasculitis, esophageal or rectal ulcers (complete resolution 8%, partial resolution 26%, and total response 34%) (Table 1).

The duration of pentoxifylline therapy was available for 131 patients and the results were as follows: twenty-four (17.8%) were maintained for less than 6 months, 47 (34.8%) for 6-12 months, 30 (22.2%) for 12-24 months, and 30 (22.2%) for more than 24 months. If pentoxifylline was discontinued (137 patients), twenty-six (19%) relapsed; 88 (64%) did not relapse and 23 (17%) were unknown.

Table 1. Efficacy of pentoxifylline and/or colchicine for different manifestations of Behçet's disease

	Pentoxifylline alone	Pentoxifylline and colchicine	chi-square
Oral ulcers			
Complete resolution	6% (16/264)	4% (5/114)	
Partial resolution	44% (115/264)	50% (57/114)	
Total response	**50% (131/264)**	**54% (62/114)**	**=71.6 p<0.001**
Genital ulcers			
Complete resolution	9% (23/252)	6% (6/105)	
Partial resolution	30% (76/252)	42% (44/105)	
Total response	**39% (99/252)**	**48% (50/105)**	**=193.1 p<0.001**
Ocular lesions			
Complete resolution	5% (8/176)	Combined with	
Partial resolution	8% (14/176)	other symptoms	
Total response	**13% (22/176)**	(see below)	
Other symptoms			
Complete resolution	8% (5/ 65)	3% (2/ 71)	
Partial resolution	26% (17/ 65)	13% (9/ 71)	
Total response	**34% (22/ 65)**	**16% (11/ 71)**	

3.2 Pentoxifylline and colchicine

Colchicine was used concurrently in 122 patients with the average dose of 1.8 mg a day. When pentoxifylline was combined with colchicine, oral ulcers completely resolved in 4% of patients and partially resolved in 50% of patients (N=115), yielding 54% of total response and statistically improved compared with the use of pentoxifylline alone (P< 0.001). Genital ulcers completely resolved in 6% of patients, and partially resolved in 42% of patients (N=105 with 8 non-applicable). The total response was 48% and was significantly improved compared with the use of pentoxifylline alone (p<0.001). Combined use of pentoxifylline and colchicine had no additional benefits for other manifestations of Behçet's disease with only a 16% of total response rate (N=71). The duration of combination therapy with pentoxifylline and colchicine was reported for 75 patients: 4 (5.3%) were maintained for less than 6 months, 28 (37.3%) for 6-12 months, 18 (24%) for 12-24 months, 23 (30.7%) for more than 24 months and 2 (2.7%) unknown. The relapse rate was not ascertained for those patients who discontinued combination therapy.

3.3 Toxicities

The following side effects were observed with the use of pentoxifylline: gastrointestinal (GI) toxicities; GI upset, nausea or diarrhea 45% (62/138), nervousness or agitation 3.6% (5/138), rash 1.5% (2/138), and swollen hands and feet 0.7% (1/138). Discontinuation of pentoxifylline only occurred in one patient secondary to GI toxicity. In the combination therapy group, there were only 3 out of 82 patients (3.6%) with reported side effects (one each with diarrhea, diarrhea/nausea and GI upset).

4. CONCLUSIONS

Behçet's disease was infrequently encountered in North America, 1.7 cases per rheumatologist, with female to male ratio of 2.5/1. As previously reported[2,3], pentoxifylline was useful in alleviating 50% of oral ulcers and 39% of genital ulcers of Behçet's patients in North America, at least partially. In conjunction with colchicine, the efficacy significantly improved to 54% for oral ulcers and 48% for genital ulcers ($P < 0.001$). Pentoxifylline and/or colchicine appeared to have limited efficacy for the other manifestations of Behçet's disease. Pentoxifylline and/or colchicine was under-utilised by rheumatologists who had patients with Behçet's disease.

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Cataract Surgery in Behçet's Disease Patients

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1. INTRODUCTION

Cataract formation is a common complication of uveitis in Behçet's disease (BD) patients and a cause of decreased visual acuity. Treatment of this cataract is not easy to deal with since surgical procedure can provoke inflammation[1]. Methods of cataract extraction and the possibility of intraocular lens implantation in BD patients still need to be discussed[1-5].

The aim of this study was to evaluate visual outcome and inflammation recurrences after different methods of cataract extraction in BD patients.

2. MATERIAL AND METHODS

The retrospective clinical trial was performed to review the outcome of cataract extraction in 36 eyes of 25 patients (21 men, 4 women; mean age 36.3±6.5 years with a range of 27-47 years) with complicated cataract due to ocular BD and compared with results of cataract surgery in 47 eyes of 41 patients (34 men, 7 women) with age-related cataract. All BD patients fulfilled the International Study Group criteria for BD. The surgical methods were intracapsular cataract extraction (ICCE), extracapsular cataract extraction (ECCE), ECCE+intraocular lens (ECCE+IOL), and lensvitrectomy. Chi-square tests and Student's t-test were used for statistical analysis.

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3. RESULTS

Visual acuity improved post-operatively in all eyes, but more prominently in control group (0.54±0.18 vs BD patients 0.26±0.14, p<0.0000) and depended on retinal and optic nerve function before operation. Inflammatory response just after cataract surgery was significantly higher in BD patients than that of controls with age-related cataract (Table 1).

Table 1. Frequency of inflammatory reaction just after operation

Surgical methods of cataract extraction	Number of eyes with inflammatory reaction/ number of operated eyes (%)		
	BD patients	p value	Control group
ICCE	3/7 (42.8)	0.0428	0/11
ECCE	7/12 (58.3)	0.0012	1/21 (4.8)
ECCE + IOL	4/6 (66.6)	0.0113	1/15 (6.7)
Lensvitrectomy	4/11 (36.4)	-	-

Comparison of different methods of cataract extraction revealed that inflammatory reaction just after operation was less often in lensvitrectomy group and more prominent in ECCE+IOL group. The frequency of ocular attacks was significantly increased in BD patients undergoing extracapsular cataract extraction with intraocular lens (vs ICCE p=0.0045, vs ECCE p=0.0396, vs lensvitrectomy p=0.0033) (Table 2).

Table 2. Frequency of recurrences in BD patients before and after cataract surgery

Surgical methods of cataract extraction	Frequency of recurrences (M±σ)		
	Within 6 months before operation	p value	Within 6 months after operation
ICCE	0.28 ± 0.48	ns	0.43±0.53; p*=0,0344, p**=0,0045
ECCE	0.17 ± 0.39	0.0054	1.17 + 1.11; p***=0,0396; p****=0,0037
ECCE+IOL	0.17 ± 0.41	0.0029	2.33 ± 1.21; p*****=0,0033
Lensvitrectomy	0.18 ± 0.40	ns	0.09±0.3

Indication p*– ICCA vs ECCA, p**– ICCA vs ECCA+IOL, p***– ECCA vs ECCA+IOL, p****– ECCA vs lensvitrectomy, p*****– ECCA+IOL vs lensvitrectomy

4. CONCLUSION

Improvement of visual acuity after cataract surgery in BD patients mainly depends on retinal and optic nerve function before operation. Inflammatory reaction just after operation and frequency of recurrences after cataract surgery are more prominent in BD patients undergoing extracapsular cataract extraction with intraocular lens. Lensvitrectomy is preferable for complicated cataract due to ocular BD.

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Do Metal Surgical Staples Induce Post-Surgical Intestinal Ulcer in Behçet's Disease?

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1. INTRODUCTION

Behçet's disease is classified as a hyper-reactive disease, and one typical phenomenon associated with it is the needle reaction of the skin. Hyperchemotaxis of polymorphonuclear leukocytes plays an important role in this reaction. We present a case with intestinal Behçet's disease showing recurrent post-surgical ulcer formation of the colon due to surgical stapling.

2. CASE REPORT

A 24-year-old female who had oral aphthae, erythema nodosum, and genital ulcers had been diagnosed of Behçet's disease when she was 21. Two years later, she developed severe right lower abdominal pain. Endoscopic examination revealed an active ulcer in her cecum and she underwent ileocecal resection. Six months after surgery she developed right lower abdominal pain and her colon and ileum were observed using a fiberscope. Multiple ulcer formation around the staple site was observed at the anastomotic portion of the intestine (Fig. 1). These findings suggest that these intestinal ulcers were induced by surgical staples because this phenomenon was a typical manifestaion of hyper-reactivity to surgical stapling which is known as the needle reaction.

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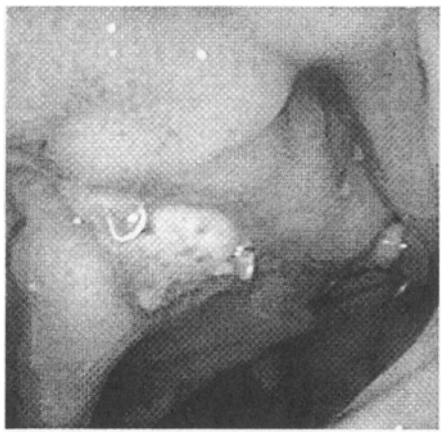

Figure 1. Multiple ulcer formation around the staple site

3. DISCUSSION

Post-surgically recurrence of the ulcers was frequency observed at the anastomotic portion of intestine in patients with Behçet's disease[1]. Staples cause inflammation with formation of intestine ulcers in some Behçet's patients. Recently, surgical staples have been used almost always for anastomosis in colonostomy. In Behçet's patients, however, stapling may induce hyper-reactive ulcer formation due to a specific pathogenic pathway[2]. When Behçet's disease with intestinal lesions undergoes intestinal resection we suggest that melt-away thread should be used in order to prevent post-surgical hyper-reactive ulcer formation.

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PATIENT - PHYSICIAN RELATIONSHIP

Clinicans, Scientists and Patients

A discussion paper

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1. SELF-HELP GROUPS

In common with many other diseases, patients with Behçet's Disease (BD) have formed self-help groups, largely on national lines with developing international cooperation. Their functions will be described more accurately by their representatives.

2. LIAISON BETWEEN CLINICIANS AND RESEARCH SCIENTISTS AND THE PATIENT GROUPS

It is appropriate to discuss what liaison is appropriate between clinicians and research scientists, as represented by the International Society for Behçet's Disease, and the patient groups. This can be considered under several headings:

1. How much should patient groups assist regarding diagnosis and treatment?
2. Should there be a register of patients, and of the spectrum of manifestations of their disease, in individual countries or internationally?
3. Should patient groups assist in the recruitment of patients to research programmes?
4. Should clinicians / scientists, known to be actively engaged in the management and research of BD, be eligible for membership of a patient

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liaison group? Similarly should a patient group be eligible for membership of a medical / research society?

5. Should patients and doctors/scientists attend and contribute to each other's conferences, should conferences be entirely separate, or should there be parallel conferences with overlap of contributions and attendance?

3. CONCLUSION

It may be that there are not entirely agreed answers to these questions. However, this is a necessary discussion, to be held in a professional, friendly and constructive atmosphere, for the ultimate benefit of patients through research based improved understanding and treatment of BD.

Hopes of Patients with Behçet's Disease in Japan Towards Researchers and the Relationship between Patients and Medical Staff Concerning Social Work

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1. INTRODUCTION

Behçet's disease occurs with multi-organic recurrent inflammation. We held the 1st International Convention for Patients with the Silk Road Disease (Behçet's Disease) in May, 2000[1]. Now, with the patients' requests, we need to appeal to researchers world-wide. Also, we would like to discuss the relationship between patients and medical staffs concerning social work on Behçet's disease.

2. REQUESTS FROM PATIENTS WITH BEHÇET'S DISEASE

At the 1st International Convention for Patients with the Silk Road Disease in Japan in May, 2000, there were 316 participants. Most of them considered it very valuable as a result of the questionnaire. To commemorate the 1st international convention, we released "The declaration of Yokohama 2000" which says that May 20th is regarded as the "International Behçet's disease day". The declaration includes 5 statements and all are our sincere

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hopes (Table 1). We think that its contents represents universal hopes for patients in the whole world.

According to the questionnaire of the 1st convention, the patient participants hoped for more co-operation and closer communication with the researchers. We understood that the patients sincerely hoped for investigation of the etiology of Behçet's disease, and for effective new medicine.

Table 1. The declaration has 5 statements and all are universal for all patients with Behcet's disease

	The Declaration of Yokohama 2000 -Our 5 Hopes-
1	To investigate the etiology of Behçet's disease and establish the treatment.
2	Equal opportunity of treatment for all patients all over the world.
3	To gain the understandings from the society that how hard it is to live with a disability caused by Behçet's disease.
4	To establish an international Behçet's disease association.
5	To hold the international convention for patients with Behçet's disease regularly.

3. NON-PROFIT ORGANIZATION (NPO)

Basing on the comments and opinions of patients, their families and medical staff, we established the new non-profit organization (NPO) in Japan, in October, 2001[2]. It's called "Ocular Inflammation Study Group". Mr. Minoru Nishida is both patient and president. Ms Michiko Wakayama (patient) and Prof. Shigeaki Ohno are vice-presidents. Now, 23 members are enrolled among them patients, their families, medical staff, and volunteers. We just opened the website and some people applied through it. For the next campaign, we are planning to deliver the NPO leaflet. It's very important for us to broaden our NPO and other activities to reach as many people as possible.

Our NPO aims a couple of things. First of all, to inform non-affected people as well as patients about the ocular inflammation diseases, especially Behçet's disease. It's very important to avoid a lot of misunderstandings about patients and their families. Second, to send therapeutic medicine for Behçet's disease to foreign countries in need. We sincerely learned through the 1st convention that it's very hard for a lot of countries to gain therapeutic medicine for Behçet's disease, even those kind we can easily purchase in Japan. We have just sent colchicine to Mongolia, and the medical doctors

inform us regularly how it has been used for Mongolian patients. We hope that this project can be continued for some years. Mongolian doctors told us that they are negotiating with the Ministry of Health and Welfare to obtain colchicine domestically. We wish that this could come true.

Our next objection is to support for the research proceedings. In this year, we selected a researcher who has been working on Behçet's disease. We hope that this small contribution will be continued as long as we can.

Fund-raising is a very severe problem for such activities. However, we never lost our objectives. We list up our URL and email address. If you are interested in it, we welcome your access.

URL: http://www007.upp.so-net.ne.jp/ganen/
E-mail: ganen@lily.freemail.ne.jp

4. RELATIONSHIP BETWEEN PATIENTS AND MEDICAL STAFF

Patients with Behçet's disease feel mostly very uneasy about their future and face a lot of problems about their occupation and daily life because the etiology is still unknown and the symptoms are recurrent. Especially the ocular symptoms always irritate the patients a great deal. As previously described, we think that a combined association with patients and medical staff should be very helpful. Especially these days, when most patients have a lot of medical knowledge, they can select their own treatment and examination. Until a few decades ago, it was very hard for most patients to select.

From now on, patients and medical staff will tie up more strongly through instruments such as our NPO or conventions. Medical staff have to be careful about the conditions of patients and always try by their work for improvement. A stronger relationship between patients and medical staffs will also support research progress about the disease. The most important thing is always to care about patients in means of social work as well. There is already some combined social work with patients and medical staff in several countries, however, we have first experienced it regarding Behçet's disease in Japan in 2000. Through both works, we realised that we have to communicate smoothly with patients and mostly just escort them through all the procedures, as we suggest here.

First of all, the physical condition of the patients is not always stable. Medical staffs have to examine the condition of patients and provide assistance if patients cannot work temporarily. Next, to take an initiative of importance, to support patients intensively is rather very appreciated.

Finally, to listen to the patient's opinions may lead to the improvement of our research. Patients hope that the medical staff will keep them currently up-dated with news about the disease. More activities associated with the bond between patients and medical staff will be increasing in a near future. At the moment, we have to reflect on how to communicate with the patients in the most satisfying way. It is a key to make the joint events with patients and medical staff a success.

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Jordan Friends of Behçet's Disease Patients Society

A fruit of joint efforts between doctors and patients

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1. INTRODUCTION

The idea of establishing a support group for Behçet's disease patients in Jordan was a fruit of an international medical cooperation. After a workshop with a group of British and Japanese researchers, patients had the opportunity to meet and share experiences. Jordan Friends of Behçet's Disease Society is registered as a charity organization, it was established in 1998 as a result of joint efforts of doctors, patients and their families to fulfill the need for better understanding and care of Behçet's disease patients.

2. GOALS

1. Raising awareness among Jordanians about Behçet's disease.
2. Provide contact and support for sufferers and their families.
3. Helping disabled Behçet's patients to become active members of the society.
4. Promote research.

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3. ACTIVITIES

Since its establishment, the society worked on different aspects and cooperated with various institutions, societies and organizations to achieve its goals.

Regarding patients:

- The society hosted medical lectures presented by doctors of different specialties related to Behçet's disease, in an attempt to give patients a better idea about the disease and how to deal with it.
- The society was able to support some patients with vital expensive medication that was mostly donated by other patients.
- The society was also a place for members to find some diversion.

Regarding the community:

The society organized lectures in schools, clubs and other charity societies, distributing pamphlets. Members (both doctors and patients) participated in different health oriented television and radio programs to raise awareness about Behçet's disease.

After participation in the 1st International Convention for Patients with the Silk Route Disease (Behçet's Disease) held 2000 in Yokohama, Japan, the society was convinced that patients worldwide had the same difficulties. Especially the patient-doctors relationship was considered a major aspect as it was found an irksome experience by many patients. Thus, the society was encouraged to continue its activities targeting not only the public and patients but doctors as well, we had the privilege to bring both doctors and patients together in local medical conferences and to throw light on different aspects of the disease. Also, we participated in the conferences of general practitioners and orthopedic doctors. It was the first time in Jordan that a patient presented a paper on behalf of Behçet's patients to the 5th International Ophthalmology Conference.

The society is the first to be established in our region, it became a source of information for many Arab patients who contacted us for information and support, it became an example which might encourage patients from other Arab countries to form such a society.

The society has excellent relations with the Behçet's Syndrome Society in the UK, we were able to establish some sort of a network for helping patients together.

4. OUR AIM... OUR CALL

The Jordan Friends of Behçet's Disease Patients Society calls for creating an International database for Behçet's disease. The database will be

a forum for both patients and doctors. We look forward to establishing an institutionalised cooperation between patients and doctors to reduce the anonymous character of the disease for all patients, especially the newly diagnosed.

The database we call for will provide information that covers routine medications and psychological aspects as well as suggesting diversions that will suit the specific patients disabilities. We strongly believe that the Behçet's database will have the following benefits:

- Giving the right information about the disease.
- Creating a space for Behçet's patients to express themselves and help newly affected patients to overcome the anxiety of being lonely.
- Giving advice about whom and where to ask about the different aspects of the disease.
- Increasing level of cooperation between doctors and patients.
- Raising more awareness of Behçet's disease, and the fears and hopes of patients among doctors. Reducing the information gap among different regions in the world.
- Contributing to International cooperation and promoting research.

5. CONCLUSION

The society is deeply convinced that international co-operation with similar societies (and hopefully a future International Society for Behçet's Support Groups) will help considerably to achieve a better future for patients with Behçet's disease.

ACKNOWLEDGMENTS

The society wishes to thank the following pharmaceutical companies: Glaxo Smith and Kline, Novartis Pharma and Hikma company for their continuous support of the society. We hope they will support our call for an International database for Behçet's patients.

The Relationship Between Patient Groups and Physicians from a UK Support Group Perspective

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Behçet's Syndrome Society, Lambourn, Hungerford, UK

1. LAY-LED SUPPORT GROUPS

Associations of this type have often been started and inspired by benefactors who were not experts but who saw a need for them to meet a shortfall in their communities.

They are mostly non-profit organisations

Examples of these have been in existence for centuries:

- Monasterial hospitals and places of education
- Charity hospitals and schools
- Craft and trades guilds
- Workforce unions
- Mutual benefit societies for insurance or mortgages
- Thrift groups
- Community projects
- Research organisations
- Patient support groups

1.1 Patient support groups

Provide information about specific diseases in a situation outside the medical environment.

Provide socio-economic support where state agencies fail.

Provide services or engage in projects to better the lives of their members.

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Encourage research into their particular disease by funding, lobbying and enlisting members to projects.

Run political or publicity campaigns to enhance awareness and obtain better provision for their members.

Provide an educational service to health professionals.

Facilitate better liaison between health professionals and their patients.

1.2 The UK Behçet's Syndrome Society

Founded in 1983 by a sufferer, Mrs. Judith Buckle.
Membership initially at 60+, currently at 1450+.
Estimated people with Behçet's in the UK ~ 2000 – 2500.
Registered as a UK charity.
Income around £7,500 p.a., capital around £40,000.
Provides

- leaflets
- twice-yearly newsletter
- web site
- telephone help-lines
- charitable grants
- Run by volunteers
- from home after work
- in their spare time
- no paid staff
- no offices

Probably very typical of hundreds of patient support groups especially for rare disorders.

Little or no socio-economic or state support for small groups.

UK Department of Health encouraging the patient support group as a service.

2. HOW DOCTORS CAN HELP PATIENTS

2.1 Good practice

Members have found the following things to be very helpful:

- Multi-disciplinary clinics or access to other specialists on the same day.
- Direct access by phone for crises and problems.
- Rapid "emergency" appointments.
- Seeing the same specialist each visit.

- Psychiatrists, accident and emergency who will send patients to the relevant Behçet's specialist.
- Proper pain-relief.
- Getting a diagnosis.
- Imaginative and inventive treatment.
- Doctors who do not feel bound by text-book concepts.
- A firm letter to a general practitioner (GP) who has been obstructive or disbelieving.
- Doctors who tell their patients about support groups.
- Doctors who involve the support groups in their research.

2.2 Issues that need addressing

Unfortunately members sometimes have less helpful experiences and a few are listed below:

- Hospital doctors who refuse to refer them on to an acknowledged expert.
- GPs who refuse to refer them to anyone at all.
- Being passed on to junior doctors or other colleagues who do not have the same interest or enthusiasm for this rare disease.
- "Shared care" that is not within the same institution does not appear to be very satisfactory - there can be communication or professional resentment problems.
- Being returned to a less expert local hospital for care.
- Incompetent or negligent doctors who are protected by professional ethics.
- Training of doctors does not seem to encourage imaginative approaches towards diagnosis of diseases that do not have a conventional picture.

2.3 Suggestions for the future

- When a Behçet's specialist moves from a hospital or clinic, it is of great help if they tell patients and the patient support group where they have moved on to. This saves hours of hard work for hard-pressed volunteers trying to trace them (Doctors are allowed to retire or die!).
- Patients to keep full copies of their notes to bring at each appointment.
- Doctors to write down for patients the names of colleagues they may visit and the names of treatments or procedures under consideration.
- Tests and examinations done during flare-ups, not later when they have remitted.

- The types of expertise and skills amongst the active representatives of the support groups should be considered. Non-medical does not mean non-expert.
- Collaboration should be on an equal basis with support groups – they probably have intellectual abilities to match those of doctors. Respect their knowledge and experience.

2.4 Finally

Doctors have been trained to cure, but there is another group of people they can help, those who are incurable but look to them for help and relief.

They have not failed because these patients are not cured.

They have succeeded if they have made their life a little easier.

3. HOW PATIENTS CAN HELP DOCTORS

3.1 Feedback

The doctor's burden should be made a little easier. There is little or no feed-back to patients or support groups to help with this. Please send suggestions to patient support groups. We can then inform our members.

3.2 'Problem' patients

Please forgive the problem patients. They may have upset local doctors and caused angry correspondence from colleagues, but they may not have been believed, and can become aggressive, mute, tearful, 'emotional' in the clinic. They behave like this from relief at finding a safe haven.

3.3 What makes a good patient?

- Those who do not expect to be cured but hope for less frequent, less aggressive flare-ups and perhaps occasional spells of feeling really well?
- Those who keep comments and replies to questions short and neat?
- Those who describe and record symptoms or side-effects of drugs clearly?
- Those who write a list of problems and questions before arrival at the appointment so best use is made of the time available?

- Those who know proper names for the parts of the body and a little bit about how it works?
- Those who take their medication as asked?
- Those who notify the clinic if they cannot turn up so their 'slot' is available for another patient's emergency visit?

4. CONCLUSION

Behçet's disease can be very distressing and almost always interferes seriously with a patient's quality of life. The patient's comfort and morale can be greatly enhanced by sympathetic and imaginative Behçet's doctors and by the services of a well-informed lay support group. Co-operation and understanding between these two main props of the patient's day to day experience is desirable and achievable. In the UK this doctor-support group relationship is developing in a positive manner and thriving well.

"Living with Behçet" - A Young Patients' Group

ADELTRAUD MÜLLER
Self Aid Group „Leben mit Behcet", Kassel, Germany

1. INTRODUCTION

Adamantiades-Behçet's disease is a complex disorder and we are aware of its symptoms. For patients it is usually a very long period of suffering

the large number of different manifestations in the intestine (DD Crohn's disease), heart, genital aphthous ulcers, joints (DD arthritis), skin lesions (papules/pustules, erythema nodosum), eye involvement (uveitis), nervous system involvement ("neuro-Behçet"), and legs (vasculitis), and the lack of information on Adamantiades-Behçet's disease. Adamantiades-Behçet's disease is a systemic disease and a long odyssey.

Certainly the main obstacle is that Adamantiades-Behçet's disease is still considered a very rare illness. However, it is not, which is justified by the large number of members of our group here in Germany and, of course, the groups in Great Britain, the US, Japan, and many others.

2. MEDICAL TREATMENT IN GERMANY

It is encouraging for us that the two German research centres in Berlin and Tübingen focus on Adamantiades-Behçet's disease. But still it is a long and troublesome way to the first medical advice until a specialist is found. In some cases much of the suffered pain is not at all necessary.

There are good physicians here in Germany. And there exist methods to treat the disease. However, treatment is not always easy to administer because of all the side effects of available medicine. It is, therefore, our great

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hope that in the near future there will be possible treatments which may relieve Adamantiades-Behçet's disease with less unwanted side-effects.

At the moment there exists a limited therapeutic spectrum with colchicin, corticosteroids, thalidomide, interferon-alpha, and no drugs that have been registered for the disease (with the exception of cyclosporin A for uveitis). It is our wish addressing to the pharmaceutical industry to provide further resources.

3. THE PATIENT AND HIS DISEASE

There is an endless list of questions that patients ask, such as the influence of hormones to the disease, the risks for pregnant women, children involvement, whether there are restrictions for vaccination, whether a specific diet should be given, and so on. The information of and given by physicians also has to be improved.

Most of the patients have to stand a variety of social, psychological, and financial problems and undergo even more trouble to get approved as a disabled person by the authorities.

An effort is being made towards the public recognition of Adamantiades-Behçet's disease and the improvement of co-operation with our physicians and other organised patient groups world wide. The aim is quicker recognition of the disease and the support of patients within the community. An intensification of research activities is also requested.

Private Practice Offices Serve as Centers for Adamantiades-Behçet's Disease

The German way

KLAUS FRITZ
Dermatologist, Private Practice, Landau, Germany

1. INTRODUCTION

Adamantiades-Behçet's disease (ABD) is a rare, chronic, life-long disorder that involves inflammation throughout the body with multiple symptoms[1-4].

To the dermatologist, the disorder is known mainly because of mouth and genital ulcers. Since a skin problem is the first symptom in 80% of the patients, dermatologists should take a leading position in diagnosis and treatment of patients with ABD, especially because internal symptoms like inflammation of the arteries, the eyes (retina), brain, joints, skin, and bowel often are misinterpreted as a different disease.

ABD-patients have multiple symptoms which are often misdiagnosed because it is a rare disease, little known by physicians, and there is no leading specialty (Table 1). Patients need an early diagnosis which can be difficult, since many cases remain monosymptomatic throughout a long period, they need a multidiscipline approach, a guiding specialist and the cooperation with patient groups and the public. Patient associations speculate that the number of people suffering from symptoms not yet diagnosed as ABD is far higher than the number of patients who are diagnosed.

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Table 1. Organ system-associated classification of clinical signs

Skin	**Joints**
Erythema nodosum-like eruptions	Arthritis
Superficial thrombophlebitis	
Pustular skin lesions	**Eyes**
Hyperirritability	Hypopyon
Raynaud phenomenon	Uveitis
	Iritis
Nervous System	Iridocyclitis
Brainstem syndrome	Chorioretinitis
Meningoencephalomyelitic syndrome	
Organic confusional state	**Inheritance**
Schizoaffective disorder	Familial cases reported

2. THE GERMAN WAY

In order to meet this need, the charity German Registry of Adamantiades-Behçet's Disease[5] and additionally a German net of dermatological practice offices were established; the latter are familiar with the various faces of ABD.

With their help, patients are led in their struggle through the right diagnostic procedures in cooperation with other specialists who are also instructed by the dermatologists.

Dermatologists take a leading position in ABD. In contrast to other countries, dermatological symptoms like aphthous ulcers, genital ulcers and other skin disorders are the first symptoms in Germany and by far more frequent than other disorders. Eye diseases are less common than in Japan for instance, the same is true for arthritis. In addition dermatologists are available throughout Germany, in short distances and without waiting time.

More than 3500 dermatologists in private practices and more than 500 in hospitals provide dermatological health care. Direct access to dermatologists for all patients is granted, no matter how much a patient earns or which health insurance covers the costs. This is different from health care systems like, for instance, in the UK.

A study on the reputation of dermatology in spring 2002, when 1000 people of all ages and social classes were interviewed, showed that dermatologists enjoy a high reputation for all skin related disorders.77% would immediately see a dermatologist for these symptoms. In 19% they first consult the general practitioner, who usually refers ABD patients to dermatologists, at least when the first symptoms reoccur or when patients do not find relief by symptomatic treatment that was offered.There are 5 steps providing appropriate service for ABD-patients. Step 1 is a basic diagnosis at first consultation which can be offered by any dermatologist and by those

of other specialties who are familiar with ABD. This means it must be checked whether there is a main symptom like oral aphthous ulcers (min. 3x/year; Fig. 1) plus 2 of the 4 following criteria:

– Genital ulcers
– Uveitis (iritis, retinitis)
– Skin disorders (erythema nodosum, folliculitis, sterile pustules)
– Pathergy test positive

based on the diagnostic criteria of the „International Study Group for Behçet's disease".

If ABD is considered at step 2 physicians may refer patients to one of the approx. 25 dermatological centers for ABD, now built up on a private initiative throughout the whole country. There, blood test and extensive clinical examination can be performed (Table 2). The patient is referred to a number of specialists who cooperate in a 3rd step when results are compiled and finally offer an appropriate treatment as step 4.

Table 2. Laboratory examinations to be advised in Adamantiades-Behçet's disease

Laboratory parameters for differential diagnostic purposes	**Disease-associated laboratory parameters**
Blood count	Anticardiolipin antibodies
Differential blood count	HLA-Typing
Iron	c-reactive protein
Vit.B12 / Ferritin	Erythrocyte sedimentation rate (ESR)
Transferrin	
Rheumatic factor	
Complement C3,C4	
Antinuclear antibodies (ANA)	
Liver tests	
Creatinine	
Antistreptolysin titer	
Yersinia serological tests	
Herpes simplex virus – Ak	
Coxsackie – Ak	
Echoverus – Ak	
Hepatitits – Serological tests	

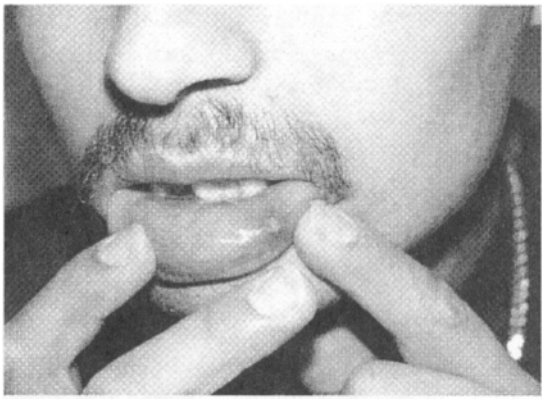

Figure 1. Characteristic aphthous ulcers at the mucosa of the lower lip

3. TREATMENT

Treatment for ABD is symptomatic and supportive. Medication may be prescribed to reduce inflammation and/or regulate the immune system.

ABD is initially treated with corticosteroids to suppress acute inflammation. Other therapeutic regimens have to substitute or be added to corticosteroids on a long term basis [6]. Many of these drugs can have serious side effects, including liver or kidney damage.

Also, patients want to be informed about alternative treatments which sometimes are useless, sometimes maybe supportive and sometimes might be even very powerful but do have uncontrolled side effects. As step 5, these centers of special competence are supposed to cooperate with patient groups in order to inform other patients and the public, and decrease isolation and stress in the lives of people with ABD and their families.

4. CONTACT ADDRESSES

For more information, especially on research programs and therapy, there is a number of organisations, most of them to be found on the web:

International Society for Behçet's Disease
c/o Dr. Colin G. Barnes, President
Little Hoopern
Chagford TQ13 8BZ, UK
http://www.behcet.ws

Deutsches Register Morbus Adamantiades-Behçet e.V.
Department of Dermatology
University Medical Center Benjamin Franklin
The Free University of Berlin
Fabeckstrasse 60-62
14195 Berlin, Germany
postmaster@behcet.de
http://www.behcet.de
Fax: 49-30-84456908

American Behcet's Disease Association
P.O. 15247
Chattanooga, TN 37415, USA
mharting@.ix.netcom.com
http://www.behcets.com
Tel: 800-7-BEHCETS (723-4238) National Eye Institute (NEI)

National Institutes of Health
Bldg. 31,Rm. 6A32
Bethesda, MD 20892-2510, USA
2020@b31.nei.nih.gov
http://www.nei.nih.gov
Tel: 301-496-5248 Professionals 800-869-2020

National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
National Institutes of Health
Bldg. 31, Rm. 4C05
Bethesda, MD 20892-2350, USA
NIAMSInfo@mail.nih.gov
http://www.nih.gov/niams
Tel: 301-496-8188 877-22-NIAMS (226-4267)

National Institute of Dental and Craniofacial Research (NIDCR)
National Institutes of Health
Bldg. 45, Rm.4AS19
Bethesda, MD 20892-6400, USA
nidrinfo@od31.nidr.nih.gov
http://www.nidr.nih.gov
Tel: 301-496-4261

National Organization for Rare Disorders (NORD)
P.O. Box 8923
(100 Route 37)
New Fairfield, CT 06812-8923, USA
orphan@rarediseases.org
http://www.rarediseases.org
Tel: 203-746-6518 800-999-NORD (6673)
Fax: 203-746-6481

Or:

http://www.behcet.de
www.behcet.ws
www.behcet.de
www.behcets.org.uk
www.niams.nih.gov
www.behcet.asso.fr
www.behcetsdisease. com
www.behcet.it
www.ser.es/pacientes/behcet.html
lvasculitis.med.jhu.edu/behcet's.htm
www.behcetscanada.com

5. CONCLUSION

Efficient information from the German Registry of Adamantiades-Behçet's disease e.V. (Chair: Prof. Ch.C. Zouboulis, Berlin), own efforts of dermatologists and the cooperation with the German Association of Dermatologists will make possible to help affected people find professional help.

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The Editor

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Born 1960 in Athens, Greece. Medical studies at the National and Capodistrian University of Athens. Residency in Dermatology, Venereology and Allergology at the Freie Universität Berlin. Dissertation 1988 in Berlin. Research Fellowship 1993-94 at the University of Michigan (Ann Arbor, Michigan), Cleveland Clinic Foundation (Cleveland, Ohio), and Massachusetts General Hospital, Harvard Medical School (Boston, Massachusetts). Habilitation 1995 in Berlin. Since 2000 Professor of Dermatology and Venereology and Vice-Chair of the Department of Dermatology, University Medical Center Benjamin Franklin, Freie Universität Berlin. Major clinical and research subjects: Dermato-Pharmacology, Dermato-Endocrinology, Physiology of the Sebaceous Gland and Acne, Adamantiades-Behçet's Disease, Cryosurgery. Major scientific awards: Oskar Gans Prize of the German Dermatological Society (1993), European Academy of Dermatology and Venereology Dermatology Research Fellowship (1997), Felix Wankel Animal Protection Research Award (2000), Beauty Care Prize for excellent Dermatological Research (2000 and 2002). Member of many scientific committees, organizer of several international conferences and author of many scientific publications. Associate Editor of *The Journal of Investigative Dermatology,* Assistant Editor of *Dermatology,* and member of the Editorial Academy of the *International Journal of Molecuilar Medicine.* Member of the Executive Committee and the Council of the *International Society for Behçet's Disease* and President of the *10th International Conference of Behçet's Disease* and the registered charity *Deutsches Register Morbus Adamantiades-Behçet e. V.*

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