When it looks like Behçet's syndrome but is something else: Differential diagnosis of Behcet's syndrome: a two-centre retrospective analysis

Authors: Fabian Lötscher[1*/], Floor Kerstens[2,3**], Martin Krusche[4], Nikolas Ruffer[4], Ina Kötter[4,5], Franktien Turkstra[2,3], EULAR study group on MHC-I-opathy.**

[1] Department of Rheumatology and Immunology, Inselspital Bern, Bern University Hospital, University of Bern, Switzerland

[2] Amsterdam Rheumatology & Immunology Center, Reade, Amsterdam, The Netherlands

[3] Reade location Jan van Breemen, department of Rheumatology, Amsterdam the Netherlands

[4] III. Department of Medicine, University Medical Center Hamburg-Eppendorf, Germany

[5] Department of Rheumatology and Immunology, Klinikum Bad Bramstedt, Germany

*Corresponding author:

Dr. Fabian Lötscher

fabu99@gmx.ch

ORCID iD 0000000216269931

Mobile: +41763226196

**Fabian Lötscher and Floor Kerstens equally contributed to the manuscript and share first authorship

ABSTRACT

Objective: To investigate the differential diagnostic spectrum in patients with suspected Behçet's syndrome (BS) in low prevalence regions. In addition, the number of patients fulfilling the ICBD criteria despite not having BS was evaluated.

Methods: This retrospective analysis was performed in two referral centers for BS. Patients with confirmed BS (clinical diagnosis with fulfilment of ISG criteria or a score of ≥ 5 points in the ICBD criteria) were excluded. The remaining patients were divided into eleven differential diagnosis categories. If no definitive alternative diagnosis could be established, patients were termed 'probable BS' in case of (1) relapsing orogenital aphthosis in the absence of other causes and either *HLA-B51* positivity, origin from an endemic area or presence of an additional typical BS symptom that is not part of the classification criteria or (2) with 3-4 points scored in the ICBD criteria.

Results: In total 202 patients were included and categorized as follows: 58 patients (28.7%) as 'probable BS', 57 (28.2%) skin disease, 26 (12.9%) chronic pain syndrome, 14 (6.9%) eye disease, 11 (5.4%) spondyloarthropathy, 9 (4.5%) gastrointestinal disease, 7 (3.5%) neurological disease, 4 (2%) arthritis, 3 (1.5%) auto-inflammation, 3 (1.5%) connective tissue disease, 10 (5.0%) miscellaneous disease. *HLA-B51* was positive in 55/132 (41.6%); 75/202 (37.1%) of the patients fulfilled the ICBD criteria.

Conclusion: In a low disease prevalence setting the straightforward application of the ICBD criteria may lead to overdiagnosis of BS. The differential diagnosis of BS is enormously broad. Clinicians should be aware that *HLA-B51* positivity is still not considered as a diagnostic feature in BS.

Keywords:

Behçet's syndrome, differential diagnosis, classification criteria, HLA-B*51, cohort

Key Messages

- The spectrum of differential diagnoses for Behçet's syndrome (BS) is broad, interdisciplinary care plays a key role.
- ICBD criteria may lead to overdiagnosis of BS in areas with low disease prevalence.
- *HLA-B51* positivity should still not be considered as a diagnostic feature in BS.

1. Introduction

Behçet's syndrome (BS) is an inflammatory systemic disorder of unknown aetiology, with mucocutaneous, ocular, articular, vascular, neurological, and gastrointestinal involvement. At present, multiple pathophysiologic mechanisms are being discussed (1,3) and different age- and sex-related clinical phenotypes of disease presentation have been proposed (3,4,5).

Genetic factors play an important role in pathogenesis. For example, the Class I MHC antigen *HLA-B51* has been recognized as the strongest and worldwide replicated susceptibility factor associated with BS and recent findings have provided insights into its pathogenic role (6). BS is more frequent around the ancient 'Silk Road', which overlaps with a high prevalence of *HLA-B51* antigen in the healthy population in this region (7). The estimated prevalence differs according to geographical region and publication method: pooled prevalence ranges from 120 in 100 000 inhabitants in Turkey, to 4.5 in the Middle East and 3.3 in Europe (8).

Due to the absence of pathognomonic histologic or laboratory features, the diagnosis of BS solely relies on clinical presentation. Various sets of diagnostic/classification criteria were developed for research settings (9). However, these criteria are commonly used by physicians in routine practice to assist the diagnostic process (9,10). While providing acceptable sensitivity and specificity as classification criteria, the International Study Group Criteria for Behçet's Disease (ISG criteria (11)) and the more recently established International Criteria for Behçet's Disease (ICBD criteria (12)) do not reflect regional differences in prevalence and clinical manifestations (amongst others severity) of BS (13,14,15). ISG and ICBD criteria are used both as classification criteria for study purposes and by many clinicians worldwide as diagnostic criteria in everyday clinical practice. In fact, studies showed that the application of the ICBD-criteria in a clinical setting entails the risk of overdiagnosis and could therefore lead to overtreatment (16).

Furthermore, given the low prevalence of BS, most physicians in low prevalence countries are unfamiliar with the disease and its differentials: Studies in Germany, the Netherlands or Switzerland have demonstrated a diagnostic delay of up to 8 years from disease onset to time of diagnosis (17). In addition, clinicians falsely invoke fulfilment of the above-mentioned

diagnostic/classification criteria as well as *HLA-B51* positivity when establishing the diagnosis of BS (10).

The aim of this study was to analyse the true differential diagnostic spectrum of established special consultations for BS in regions with a low disease prevalence of BS.

2. Methods

This retrospective study was conducted at two referral centers (Hamburg, Germany; Amsterdam, Netherlands) specialized in the care of BS.

Patients referred for the evaluation of BS were analysed retrospectively and anonymously. Demographic characteristics as well as clinical manifestations and serological data were collected from blinded patient charts and added to Microsoft Excel ® (Hamburg) or were added to a RedCAP database (Amsterdam).

In Hamburg, data were collected from January 2015 to December 2018. In Amsterdam data of the study entry were collected between September 2009 and June 2017, follow up data (in case present) until April 2019.

The range of data collected is presented in **Table 1** and **Table 2**. Organ manifestations of BS were according to the ISG and ICBD criteria. Manifestations that are not clearly defined in the ISG and ICBD criteria (such as ocular, gastrointestinal, and neurologic disease) were classified according to the literature. The following eye manifestations were classified as typical for BS: chronic relapsing and nongranulomatous bilateral anterior uveitis with posterior involvement (including 'vitritis and retinitis'), uni- or bilateral posterior uveitis (vitritis, retinal vasculitis, retinitis), panuveitis (18,19). Gastrointestinal manifestations were considered as being typical for BS if they appeared accordingly on endoscopic examination (typical localization: ileocecal region; typical ulcers: single or few (<5), large, deep penetrating, discrete and round or oval-shaped) (20). Neurologic manifestations of BS were classified according to international consensus recommendations by Kalra et al. (21).

The origin of the patients (based on origin nationality but taking parental origin into account) was divided into endemic and non-endemic. In this work, the following regions were considered as endemic (7,8): Turkey, Asia and Middle East, Arabian Peninsula, North Africa (Morocco).

BS was diagnosed clinically in combination with either fulfilment of ISG criteria (11) or scoring ≥ 5 points in the calculation of the more recent ICBD criteria (12). This particular cut-off point was determined to minimize false-positive BS classifications. Using ≥4 as cut-off would increase the number of patients incorrectly considered as definitive BS (particularly all patients with OGA, since OGA in western society is more often not BS (22)). Patients with confirmed BS were only assessed for diagnostic performance of the ICBD criteria (see below) and excluded from further analysis. Subjects who had an alternative diagnosis, but met the classification criteria for BS, were labelled with the corresponding alternative diagnosis.

Patients that did not fulfil the ISG-criteria and scored 3 to 4 points of the ICBD-criteria were labelled 'probable BS' if no definitive alternative diagnosis could be established. Importantly, patients with exclusively relapsing bipolar aphthosis were not automatically classified as 'probable BS' despite scoring 4 points in the ICBD criteria. Only patients with otherwise

unknown cause of the bipolar aphthae and presence of one of the following criteria were assessed as 'probable BS': (1) *HLA-B51* positivity, (2) origin from the endemic area, (3) presence of an additional BS symptom that is not considered a diagnostic criterion according to the ISG and ICBD criteria (e.g. epididymitis), as illustrated in **Figure 1**. Patients with ambiguous presentation were classified according to a case-based discussion (physician's judgement) by the authors (I. Kötter, F. Turkstra and F. Lötscher). Patients with clinically confirmed arthritis were consequently also labelled with arthralgias.

All subjects who did not meet the classification criteria mentioned above, were divided into eleven different differential diagnosis categories reflecting the main differential diagnostic considerations in clinical practice: probable BS, skin disease, chronic pain syndrome, eye disease, spondyloarthropathy (SpA), gastrointestinal disease, neurological disease, arthritis, autoinflammatory disease, connective tissue disease and miscellaneous disorders. Patients were assigned to these categories according to their most prominent clinical features. In some patients, features from other categories were also present (e.g. chronic pain with oral aphthosis).

The diagnostic performance of the ICBD criteria was calculated using expert opinion as the diagnostic gold standard. The patients in the differential diagnosis subcategory 'probable BS' were handled as definite BS in the analysis (it can be assumed that the performance is therefore overestimated). Receiver operation characteristic curves were calculated for different scenarios with the 'probable BS' category.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki. The data collection in Amsterdam was approved by the local ethics committee of the Amsterdam University Medical Center, location VUmc. According to the local ethics committee in Hamburg, the retrospective and anonymised data collection in this study was not considered a "research project on humans" according to §9 of the Hamburg Chamber Act for the Health Professions. Therefore, no ethical approval was required.

3. Results

A total of 384 patients were examined for the presence of BS, and 182 were diagnosed with BS. The remaining 202 were included in the study (**Figure 1**). The mean age was 39.3 years and 134 (66.3%) were female (**Table 1**). 32.2% of patients were from Germany, 7.4% from the Netherlands, 5.0% from other European countries, 35.1% from Turkey, 5.9% from Morocco, 10.4% from Asia or Middle Eastern countries, 0.5% from Africa. In total 101 (50.0%) patients originated from endemic countries.

The ICBD criteria performed as follows in our cohort: With an ICBD score cut-off of 4 points, sensitivity and specificity were 93.5% and 75.7%, respectively. Applying an ICBD score cut-off of 5 points, sensitivity and specificity were 75.8% and 97.2%, respectively (see **Supplementary Data S1**, available at *Rheumatology* online). The discriminatory power of the ICBD criteria is illustrated in **Figure 2** and presented in more detail in the supplementary data (**Figure 2, Supplementary Data S1**). In 75 patients (37.1%) the ICBD criteria were fulfilled with a score of ≥ 4 points. Furthermore, 3 female patients from Hamburg fulfilling the ISG criteria were included in this study, because the clinical presentation was more consistent with an alternative diagnosis than with BS according to the physician's judgement (see notes at **Table 1**).

Table 2 displays the distribution of clinical and laboratory features of BS among the analysed patients. Fifty-eight patients (28.7%) were categorised as 'probable BS'. The remaining patients were classified into ten differential diagnostic categories as follows: 57 (28.2%) patients with skin disease, 26 (12.9%) with chronic pain syndrome, 14 (6.9%) with eye disease, 11 (5.4%) with SpA, 9 (4.5%) with gastrointestinal disease, 7 (3.5%) with neurological disease, 4 (2.0%) with arthritis, 3 (1.5%) each with auto-inflammatory diseases and connective tissue diseases, 10 (5.0%) with miscellaneous disorders. Overall, *HLA-B51* was positive in 27.2% (55/202); if only the tested patients are considered, the proportion was 41.6% (55/132). Pathergy testing was positive in 2.5% (5/202) of all patients, in 44 patients the pathergy test was not performed.

The majority (34/58, 58.6%) of patients classified as 'probable BS' were female. Twelve percent had a family history of BS. Clinical manifestations consisted mainly of oral aphthosis (57/58 patients, 98.3%), genital aphthosis (26/58 patients, 44.8%), skin disease (22/58, 37.9%; 8 patients with erythema nodosum and 14 with papulopustular lesions), arthralgias (38/58 patients, 65.5%) and arthritis (14/58 patients, 24.1%) as well as eye disease (10/58 patients, 17.2%).

More than one fourth of all patients (57/202, 28.2%) suffered from recurrent orogenital aphthosis (OGA). Most of them were categorised as 'probable BS' but were represented in every differential diagnosis group except for eye disease and miscellaneous disorders.
Skin lesions were mainly papulopustular and occurred most frequently in patients classified as 'probable BS'. Genital aphthosis, vascular disease, CNS disease, gastrointestinal disease and epididymitis were present in a minority of patients, across all categories (**Table 2**). No arterial involvement including aneurysms was observed in this cohort. Joint involvement was frequent and consisted mainly of arthralgia.

Table 3 provides an overview of the individual differential diagnosis groups and the corresponding diagnoses.

4. Discussion

In this two-centre retrospective study of patients who were referred for the evaluation of suspected BS, more than one third (75/202, 37.1%) with a diagnosis other than BS formally fulfil the ICBD criteria for BS (**Tables 1, 2 and 3**). The specificity of the ICBD criteria with an applied cut-off score of four is low in our cohort (sensitivity 93.5%, specificity 75.7%). This is despite the fact that the patients with diagnostic uncertainty (those in the 'probable BS' category) were treated as 'true BS' patients in the calculation (**Supplementary Data S1**). Even after exclusion of the 'probable BS' category, almost a quarter of the patients still fulfils the ICBD criteria (35 out of 144 subjects, 24.2%), mainly due to the manifestation of recurrent OGA. Three patients in our cohort fulfilled the ISG criteria, but expert clinical evaluation concluded that an alternative diagnosis was explanatory for the presenting symptoms (see notes at **Table 1**). For scientific research purposes, most experts still rely on the 1990 ISG criteria (11), which were originally intended to define patients participating in research programs but proofed to perform well in a clinical context (23). In the following decades, the evaluation of the ISG-criteria revealed lower sensitivity in comparison to other diagnostic/classification criteria, leading to the establishment of the new ICBD criteria in 2014 (12). The ICBD criteria include a broader spectrum of clinical manifestations, since vascular and neurologic features are included. In addition, the score prioritizes OGA and ocular manifestations. Consequently, solely OGA can lead to a diagnosis of BS. To complicate matters, there are various other sets of classification and diagnostic criteria that are preferred in different geographic regions (9). Diagnostic criteria only perform well in regions with high disease prevalence and other groups have already recognized the possible over-diagnosis of BS in non-endemic regions when applying the ICBD criteria (16). The significant positivity rate of the ICBD criteria in our cohort with differential diagnoses reflects this problem and should caution clinicians to be careful when applying the ICBD as diagnostic criteria in a clinical setting. In a uveitis cohort, Zhong et al. (24) recently detected optimal discriminatory property by using a cut-off of 5 points when applying the ICBD criteria. In our cohort, a cut-off of 5 points raises the specificity to 97.2%, as expected at the expense of sensitivity (75.7%). In our view, this could be a valid option to counteract overdiagnosis in the future and deserves further evaluation in different BS cohorts, especially in clinical settings in low-prevalence regions.

OGA is the hallmark feature of BS but can occur in various other conditions. It can be a challange to distinguish mimics from true OGA for physicians who do not diagnose and treat ulcerative lesions on a regular basis. A small proportion of patients referred with suspected BS based on presumed OGA, do not actually suffer from aphthosis, but from non-aphthous ulcerative lesions. Our cohort reflects the broad range of differential diagnoses of OGA (**Tables 2 and 3**), which should always be considered by the specialist in the low-prevalence region.

Differentials include simple recurring OGA, blistering diseases (bullous pemphigoid, pemphigus vulgaris), lichen sclerosus/lichen planus, herpes virus infections, connective tissue disease and inflammatory bowel disease (IBD; Crohn's disease and ulcerative colitis, as well as SpA with enteropathy) to rarely seen manifestations such as the pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) syndrome. Interestingly, an older study in a Caucasian population showed that recurrent OGA can rarely be attributed to BS (22). However, genetic susceptibility loci, linking periodic fever, aphthous stomatitis, pharyngitis, adenitis (PFAPA) syndrome, BS and recurrent aphthous stomatitis, were recently identified, implying a disease spectrum ranging from recurrent aphthous stomatitis to BS (25). In patients with close fulfilment of the ICBD criteria (cut-off of 4 points, especially with recurrent OGA) located in low prevalence regions, we suggest establishing the diagnosis of BS only after broad differential diagnostic considerations and longitudinal follow-up. This seems to be of particular importance for a subgroup of patients in our cohort: around 30% of patients were labelled as probable BS, meaning that they share some features of BS but do not exhibit sufficient clinical criteria to reliably diagnose the disease. 57/58 patients in this group had relapsing OA and approximately 50% of them suffered from recurrent GA, in the remaining OA was associated with eye, skin, CNS, gastrointestinal or rarely vascular disease (**Table 2**). It is known that a significant proportion of patients in this category eventually presents with 'definite BS', and probability scores can predict future disease manifestation in these subjects (26).

HLA-B51 testing was performed in 132 (65.3%) of our patients, with 42% positivity (55/132). The high proportion of patients in our cohort without confirmed BS could imply, that many clinicians rely on *HLA-B51* positivity as a diagnostic parameter for BS and refer patients to specialized centers for further evaluation. It has long been known that BS is associated with the major histocompatibility complex *HLA-B51* (27). A metanalysis, performed in 2009 calculated a pooled odds ratio of 5.78 to develop BS for *HLA-B51* allele carriers compared with non-carriers (28), which suggests a significant contribution of *HLA-B51* to the genetic susceptibility but does not make it a diagnostic marker. *HLA-B51* positivity is found to be 3-8% in healthy subjects living in non-endemic countries (USA, Europe, UK), and in up to 24% of the healthy population in endemic regions (29). Thus, routine *HLA-B51* testing is not recommended in the diagnostic process in patients with suspected BS.

Our results display a broad spectrum of differential diagnoses (**Table 3**). These include above all the commonly suspected inflammatory systemic diseases (such as IBD, SpA and inflammatory arthritis, connective tissue diseases, sarcoidosis and autoinflammatory syndromes) but also infections (incl. herpes virus and urinary tract infections), a broad variety of skin diseases, neurological disorders (such as moyamoya disease, multiple sclerosis, and

CNS vasculitis) and chronic pain syndrome. Five percent (11/202) of the patients were finally classified as suffering from SpA, psoriatic arthritis being the most common. BS and the SpA can be considered to share a mutual immunopathogenic basis. As these disorders are associated with specific MHC class I (MHC-I) molecules (BS: *HLA-B51*, SpA: *HLA-B27*) and share many clinical features they have been recently termed 'MHC I-opathies' (30).

Arthritis is a common feature of BS, with a tendency to affect the large joints of the lower extremities (31). In our cohort, peripheral arthritis as well as SpA was accompanied by oral or genital aphthae in almost all patients (**Tables 2 and 3**). According to the findings on colonoscopy, 7 patients in our cohort could be classified as suffering from either Crohn's disease or ulcerative colitis. Apart from shared clinical features, endoscopic findings in IBD (especially Crohn's disease) can be difficult to distinguish from intestinal lesions found in BS (32,33,34). Coexistence of BS and IBD has been reported to be rare (35). As shown in **Table 1**, in case of multiple extraintestinal symptoms, patients with IBD may even meet the ISG criteria in addition to the ICBD criteria, which again underlines the clinical difficulty of differentiating these disorders (**Table 1**). In addition to manifestations that are easier to delineate (such as episcleritis/scleritis or vitreal bleeding), the largest proportion of our patients with ocular differentials suffered from uveitis, which also resembles the most frequent ocular manifestation in BS (19). In these cases, longitudinal observation is indispensable for further differential diagnostic classification.

To the best of our knowledge, this study represents the first analysis of the differential diagnostic spectrum of BS in a real-world setting in low prevalence regions. Taken together, our results underline the difficulty of applying diagnostic/classification criteria in clinical practice and our work should remind clinicians in low prevalence settings not to rely solely on the diagnostic and classification criteria (ICBD and ISG criteria) when diagnosing BS, especially in the presence of isolated OGA. Furthermore *HLA-B51* should not be used as a diagnostic criterion. Consequently, the evaluation of these patients still requires an intensive collaboration of experts from various clinical disciplines who are familiar with the disease and the differential diagnostic spectrum.

We acknowledge that our work has some limitations: Due to the retrospective study design and the limited recruitment in two centers, our results may not be applicable in different settings. The lack of a longitudinal analysis prevents from drawing conclusions regarding obvious BS diagnoses during the further course of the disease, which is particularly problematic in view of the patients in the subgroup with possible BS. We therefore suggest the following future considerations: To improve diagnostic confidence in BS, additional diagnostic tools such as vascular sonographic features (36) or laboratory parameters are desirable,

especially in low-prevalence countries. The rising migration of people from high prevalence countries to other regions of the world requires increased expertise in diagnostics and therapy in the respective countries. Thus, it is useful to establish specialized reference centers for BS in low-prevalence countries to improve patient care through clinical expertise and prospective data collection.

5. Statements

Funding

FL was supported by a grant of the Swiss Society of Rheumatology.

Conflicts of interest

The authors have declared no conflicts of interest.

Data availability

As no informed consent for data sharing was given by the patients included, supporting data is not available.

6. References

1. Leccese P, Alpsoy E. Behçet's Disease: An Overview of Etiopathogenesis. Front Immunol. 2019 May 10;10:1067. doi: 10.3389/fimmu.2019.01067. PMID: 31134098; PMCID: PMC6523006.

2. Mattioli I, Bettiol A, Saruhan-Direskeneli G, Direskeneli H, Emmi G. Pathogenesis of Behçet's Syndrome: Genetic, Environmental and Immunological Factors. Front Med (Lausanne). 2021 Oct 8;8:713052. doi: 10.3389/fmed.2021.713052. PMID: 34692721; PMCID: PMC8531401.

3. Bettiol A, Prisco D, Emmi G. Behçet: the syndrome. Rheumatology (Oxford). 2020 May 1;59(Suppl 3):iii101-iii107. doi: 10.1093/rheumatology/kez626. PMID: 32348523.

4. Zou J, Luo D, Shen Y, Guan JL. Characteristics and phenotype heterogeneity in late-onset Behçet's syndrome: a cohort from a referral center in China. Clin Rheumatol. 2021 Jun;40(6):2319-2326. doi: 10.1007/s10067-020-05536-z. Epub 2021 Jan 7. PMID: 33411142.

5. Zou J, Luo JF, Shen Y, Cai JF, Guan JL. Cluster analysis of phenotypes of patients with Behçet's syndrome: a large cohort study from a referral center in China. Arthritis Res Ther. 2021 Jan 30;23(1):45. doi: 10.1186/s13075-021-02429-7. PMID: 33514418; PMCID: PMC7847001.

6. Gur M, Golcuk M, Gul A, Erman B. Molecular dynamics simulations provide molecular insights into the role of HLA-B51 in Behçet's disease pathogenesis. Chem Biol Drug Des. 2020 Jul;96(1):644-658. doi: 10.1111/cbdd.13658. PMID: 32691964.

7. Verity DH, Marr JE, Ohno S, Wallace GR, Stanford MR. Behçet's disease, the Silk Road and HLA-B51: historical and geographical perspectives. Tissue Antigens. 1999 Sep;54(3):213-20. doi: 10.1034/j.1399-0039.1999.540301.x. PMID: 10519357.

8. Maldini C, Druce K, Basu N, LaValley MP, Mahr A. Exploring the variability in Behçet's disease prevalence: a meta-analytical approach. Rheumatology (Oxford). 2018 Jan 1;57(1):185-195. doi: 10.1093/rheumatology/kew486. PMID: 28339670.

9. Davatchi F, Sadeghi Abdollahi B, Chams-Davatchi C, Shahram F, Shams H, Nadji A, et al. The saga of diagnostic/classification criteria in Behcet's disease. Int J Rheum Dis. 2015 Jul;18(6):594-605. doi: 10.1111/1756-185X.12520. Epub 2015 Apr 16. PMID: 25879654.

10. Kilian NC, Sawalha AH. Behçet's disease in the United States: A single center descriptive and comparative study. Eur J Rheumatol. 2017 Dec;4(4):239-244. doi: 10.5152/eurjrheum.2017.17112. Epub 2017 Nov 3. PMID: 29308276; PMCID: PMC5741334.

11. Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's Disease. Lancet. 1990 May 5;335(8697):1078-80. PMID: 1970380.

12. International Team for the Revision of the International Criteria for Behçet's Disease (ITR-ICBD). The International Criteria for Behçet's Disease (ICBD): a collaborative study of 27 countries on the sensitivity and specificity of the new criteria. J Eur Acad Dermatol Venereol. 2014 Mar;28(3):338-47. doi: 10.1111/jdv.12107. Epub 2013 Feb 26. PMID: 23441863.

13. Leccese P, Yazici Y, Olivieri I. Behcet's syndrome in nonendemic regions. Curr Opin Rheumatol. 2017 Jan;29(1):12-16. doi: 10.1097/BOR.0000000000000349. PMID: 27684358.

14. Celik S, Yazici Y, Sut N, Yazici H. Pulmonary artery aneurysms in Behçet's syndrome: a review of the literature with emphasis on geographical differences. Clin Exp Rheumatol. 2015 Nov-Dec;33(6 Suppl 94):S54-9. Epub 2015 Jul 23. PMID: 26211653.

15. Kappen JH, van Dijk EH, Baak-Dijkstra M, van Daele PL, Lam-Tse WK, van Hagen PM, et al. Behçet's disease, hospital-based prevalence and manifestations in the Rotterdam area. Neth J Med. 2015 Dec;73(10):471-7. PMID: 26687263.

16. Blake T, Pickup L, Carruthers D, Damato EM, Denniston A, Hamburger J, et al. Birmingham Behçet's service: classification of disease and application of the 2014 International Criteria for Behçet's Disease (ICBD) to a UK cohort. BMC Musculoskelet Disord. 2017 Mar 11;18(1):101. doi: 10.1186/s12891-017-1463-y. PMID: 28283043; PMCID: PMC5346254.

17. Villiger RA, Stefanski AL, Grobéty V, Adler S, Villiger PM. Behçet's syndrome: clinical presentation and prevalence in Switzerland. Swiss Med Wkly. 2019 Jul 22;149:w20072. doi: 10.4414/smw.2019.20072. PMID: 31329265.

18. Ksiaa I, Abroug N, Kechida M, Zina S, Jelliti B, Khochtali S, et al. Eye and Behçet's disease. J Fr Ophtalmol. 2019 Apr;42(4):e133-e146. doi: 10.1016/j.jfo.2019.02.002. Epub 2019 Mar 5. PMID: 30850197.

19. Tugal-Tutkun I, Onal S, Altan-Yaycioglu R, Huseyin Altunbas H, Urgancioglu M. Uveitis in Behçet disease: an analysis of 880 patients. Am J Ophthalmol. 2004 Sep;138(3):373-80. doi: 10.1016/j.ajo.2004.03.022. PMID: 15364218.

20. Lee CR, Kim WH, Cho YS, Kim MH, Kim JH, Park IS, et al. Colonoscopic findings in intestinal Behçet's disease. Inflamm Bowel Dis. 2001 Aug;7(3):243-9. doi: 10.1097/00054725-200108000-00010. PMID: 11515851.

21. Kalra S, Silman A, Akman-Demir G, Bohlega S, Borhani-Haghighi A, Constantinescu CS, et al. Diagnosis and management of Neuro-Behçet's disease: international consensus recommendations. J Neurol. 2014 Sep;261(9):1662-76. doi: 10.1007/s00415-013-7209-3. Epub 2013 Dec 24. PMID: 24366648; PMCID: PMC4155170.

22. Letsinger JA, McCarty MA, Jorizzo JL. Complex aphthosis: a large case series with evaluation algorithm and therapeutic ladder from topicals to thalidomide. J Am Acad Dermatol. 2005 Mar;52(3 Pt 1):500-8. doi: 10.1016/j.jaad.2004.10.863. PMID: 15761429.

23. O'Neill TW, Rigby AS, Silman AJ, Barnes C. Validation of the International Study Group criteria for Behçet's disease. Br J Rheumatol. 1994 Feb;33(2):115-7. doi: 10.1093/rheumatology/33.2.115. PMID: 8162473.

24. Zhong Z, Liao W, Gao Y, Su G, Feng X, Yang P. Evaluation of sensitivity and specificity of diagnostic criteria for Behçet's disease in the absence of a gold standard. Rheumatology (Oxford). 2022 Aug 30;61(9):3667-3676. doi: 10.1093/rheumatology/keac018. PMID: 35021208.

25. Manthiram K, Preite S, Dedeoglu F, Demir S, Ozen S, Edwards KM, et al. Common genetic susceptibility loci link PFAPA syndrome, Behçet's disease, and recurrent aphthous stomatitis. Proc Natl Acad Sci U S A. 2020 Jun 23;117(25):14405-14411. doi: 10.1073/pnas.2002051117. Epub 2020 Jun 9. PMID: 32518111; PMCID: PMC7322016.

26. Kerstens FG, Turkstra F, Swearingen CJ, Yazici Y. Initial visit symptoms in probable Behçet's syndrome is predictive of ISG criteria fulfillment in Behçet's syndrome: data from

New York and Amsterdam cohorts. Clin Exp Rheumatol. 2021 Sep-Oct;39 Suppl 132(5):43-46. doi: 10.55563/clinexprheumatol/0hia54. Epub 2021 Sep 13. PMID: 34524080.

27. Ohno S, Aoki K, Sugiura S, Nakayama E, Itakura K, Aizawa M. Letter: HL-A5 and Behçet's disease. Lancet. 1973 Dec 15;2(7842):1383-4. doi: 10.1016/s0140-6736(73)93343-6. PMID: 4128069.

28. de Menthon M, Lavalley MP, Maldini C, Guillevin L, Mahr A. HLA-B51/B5 and the risk of Behçet's disease: a systematic review and meta-analysis of case-control genetic association studies. Arthritis Rheum. 2009 Oct 15;61(10):1287-96. doi: 10.1002/art.24642. PMID: 19790126; PMCID: PMC3867978.

29. Giza M, Koftori D, Chen L, Bowness P. Is Behçet's disease a 'class 1-opathy'? The role of HLA-B*51 in the pathogenesis of Behçet's disease. Clin Exp Immunol. 2018 Jan;191(1):11-18. doi: 10.1111/cei.13049. Epub 2017 Oct 6. Erratum in: Clin Exp Immunol. 2018 Mar;191(3):373. PMID: 28898393; PMCID: PMC5721257.

30. McGonagle D, Aydin SZ, Gül A, Mahr A, Direskeneli H. 'MHC-I-opathy'-unified concept for spondyloarthritis and Behçet disease. Nat Rev Rheumatol. 2015 Dec;11(12):731-40. doi: 10.1038/nrrheum.2015.147. Epub 2015 Nov 3. PMID: 26526644.

31. Kötter I, Lötscher F. Behçet's Syndrome Apart From the Triple Symptom Complex: Vascular, Neurologic, Gastrointestinal, and Musculoskeletal Manifestations. A Mini Review. Front Med (Lausanne). 2021 Apr 9;8:639758. doi: 10.3389/fmed.2021.639758. PMID: 33898481; PMCID: PMC8063110.

32. Cheon JH, Kim ES, Shin SJ, Kim TI, Lee KM, Kim SW, et al. Development and validation of novel diagnostic criteria for intestinal Behçet's disease in Korean patients with ileocolonic ulcers. Am J Gastroenterol. 2009 Oct;104(10):2492-9. doi: 10.1038/ajg.2009.331. Epub 2009 Jun 16. PMID: 19532129.

33. Lee SK, Kim BK, Kim TI, Kim WH. Differential diagnosis of intestinal Behçet's disease and Crohn's disease by colonoscopic findings. Endoscopy. 2009 Jan;41(1):9-16. doi: 10.1055/s-0028-1103481. Epub 2009 Jan 21. PMID: 19160153.

34. Valenti S, Gallizzi R, De Vivo D, Romano C. Intestinal Behçet and Crohn's disease: two sides of the same coin. Pediatr Rheumatol Online J. 2017 Apr 20;15(1):33. doi: 10.1186/s12969-017-0162-4. PMID: 28427473; PMCID: PMC5397832.

35. Hatemi I, Hatemi G, Celik AF, Melikoglu M, Arzuhal N, Mat C, et al. Frequency of pathergy phenomenon and other features of Behçet's syndrome among patients with inflammatory bowel disease. Clin Exp Rheumatol. 2008 Jul-Aug;26(4 Suppl 50):S91-5. PMID: 19026122.

36. Alibaz-Oner F, Ergelen R, Yıldız Y, Aldag M, Yazici A, Cefle A, et al. Femoral vein wall thickness measurement: A new diagnostic tool for Behçet's disease. Rheumatology (Oxford). 2021 Jan 5;60(1):288-296. doi: 10.1093/rheumatology/keaa264. PMID: 32756998.

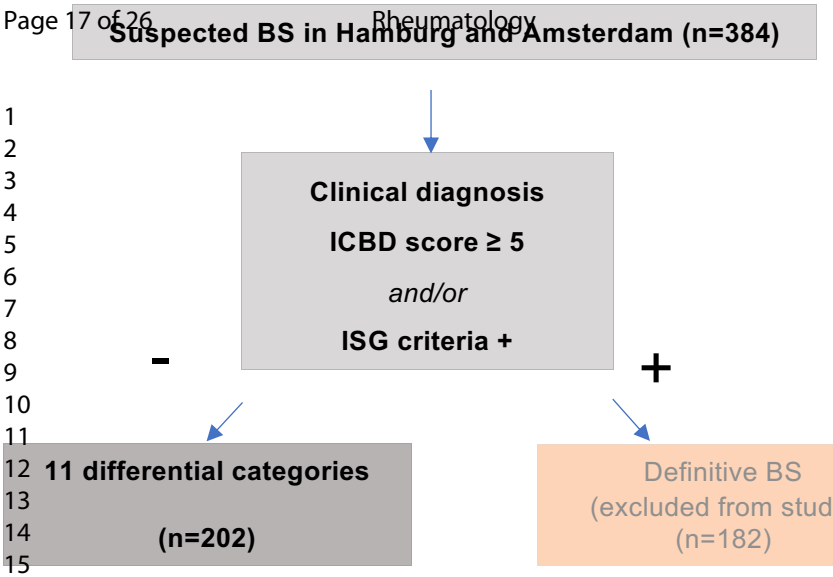
7. Figure captions

Figure 1: Illustration of the methods of the study, including the definition of the 'Probable BS' category.

Abbreviations: BS, Behçet Syndrome; ICBD, International Criteria for Behcet's Disease; ISG, International Study Group; OGA, oral and genital aphthae; w/o, without

Figure 2: Receiver operating characteristic (ROC) curve of the discrimination power of the ICBD Criteria in this cohort.

Solid line with filled-in dots: Patients from the 'probable BS' category were counted as BS patients. Dotted line with circles: Patients from the 'probable BS' category were counted as differential diagnoses without BS. X: indicating ICBD score of 5 points (in both graphs)
The individual graphs and the corresponding ICBD figures can be viewed in the supplementary data (see Supplementary Data S1, available at *Rheumatology* online).



Note:

Definition of 'Probable BS' category (n=58)

with **relapsing OGA**

and

HLA-B51 + *or*

endemic origin *or*

other BS symptoms (not included in ICBD and ISG criteria)

or

w/o **relapsing OGA**

and ICBD score of 3 - 4

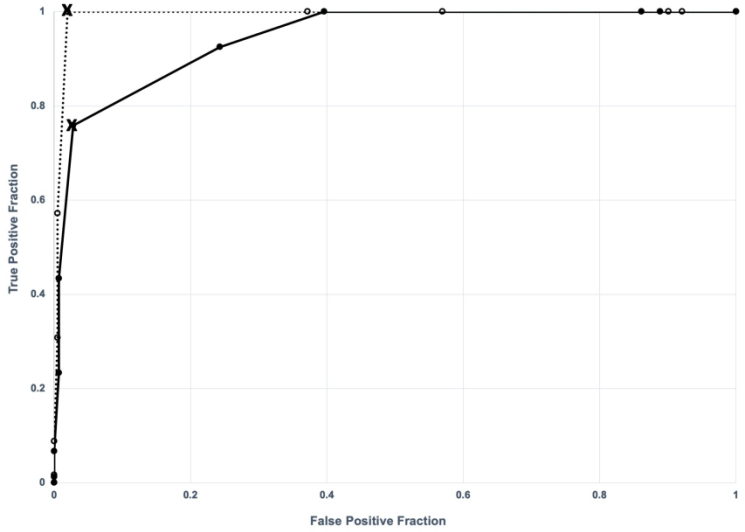


Figure 2: Receiver operating characteristic (ROC) curve of the discrimination power of the ICBD Criteria in this cohort.

Solid line with filled-in dots: Patients from the 'probable BS' category were counted as BS patients. Dotted line with circles: Patients from the 'probable BS' category were counted as differential diagnoses without BS.
X: indicating ICBD score of 5 points (in both graphs)
The individual graphs and the corresponding ICBD figures can be viewed in the supplementary data (see Supplementary Data S1).

338x338mm (225 x 225 DPI)

Characteristics	Description	Number	%
Patients	Hamburg	145	71.8
	Amsterdam	57	28.2
Gender	Male	68	33.7
	Female	134	66.3
Age (years)	Hamburg	39.7	
	Amsterdam	38.2	
	Mean Age whole Group	39.3	
Family History (of BS)	pos.	11	5.4
	neg.	174	86.1
	unknown	17	8.4
Origin	Endemic†	101	50.0
	Non-endemic	96	47.5
	Unclear	5	2.5
ICBD Criteria	<4 points	127	62.9
	≥ 4 points	75	37.1
ISG Criteria	positive	3‡	1.5

Table 1: **Patient characteristics.**

Notes:
† The following regions are considered endemic in this study: Turkey, Asia and the Middle East, Arabian Peninsula, North Africa (Morocco).
‡3 females in this study fulfil the ISG criteria:
#1) Turkish, Crohn's disease with OGA (oral and genital aphthae) and ocular manifestation.
#2) German, Ulcerative colitis, OGA, arthritis and erythema nodosum.
#3) Iranian, anti-desmoglein-antibody positive bullous pemphigoid with OGA and papulopustulosis of the skin.

Differential Category		Probable BS	Skin	Chronic pain	Eye	SpA	GI Disease	Neuro-logical	Arthritis	AID	CTD	Misc	All	%
Count		**58**	**57**	**26**	**14**	**11**	**9**	**7**	**4**	**3**	**3**	**10**	**202**	
% of all		*28.7*	*28.2*	*12.9*	*6.9*	*5.4*	*4.5*	*3.5*	*2.0*	*1.5*	*1.5*	*5.0*		
Origin	Endemic	36	21	17	6	6	3	3	3	1	2	3	**101**	*50.0*
	non-endemic	22	34	9	6	5	5	4	1	2	1	7	**96**	*47.5*
	unclear	0	2	0	2	0	1	0	0	0	0	0	**5**	*2.5*
Oral Aphthosis	pos.	57	46	24	4	10	7	6	3	3	2	6	**168**	*83.2*
Genital Aphthosis	pos.	26	21	6	1	2	2	1	1	1	1	0	**62**	*30.7*
Skin Manifestation	Erythema nodosum	8	3	1	0	0	2	1	0	0	0	2	**17**	*8.4*
	Papulopustulosis	14	4	1	2	2	3	1	0	0	0	0	**27**	*13.4*
	Acneiform	0	0	1	0	0	1	0	0	0	0	0	**2**	*1.0*
Pathergy	pos.	2	1	1	1	0	0	0	0	0	0	0	**5**	*2.5*
	not performed	7	11	10	4	2	2	1	1	1	1	4	**44**	*21.8*
Eye	pos.	10	0	0	7	2	2	0	0	0	0	3	**24**	*11.9*
Joints	Arthralgia	38	23	22	3	11	6	2	4	1	3	4	**117**	*57.9*
	Arthritis	14	1	3†	0	10	3	0	4	0	1	1	**37**	*18.3*
Vascular*	Thrombophlebitis, thrombosis or both*	5	1	4	1	2	0	1	0	0	2	0	**16**	7.9
CNS	pos.	4	0	0	1	0	0	1	0	0	0	0	**6**	*3.0*
GIT	pos.	4	1	1	1	3	8	2	0	0	0	0	**20**	*9.9*
	unclear.	1	2	3	0	1	0	0	0	0	0	1	**8**	*4.0*
Epididymitis	pos.	1	0	0	0	0	0	0	0	0	0	0	**1**	*0.5*
HLA-B51	pos.	20	16	5	7	1	3	1	0	0	0	2	**55**	*27.2*
	not performed	11	19	16	4	5	3	2	4	1	3	2	**70**	*34.7*
ICBD Criteria	0-3 Points	18	41	18	13	8	7	6	3	2	2	9	**127**	*62.9*
	4 Points	40	15	8	1	2	0	1	1	1	1	1	**71**	*35.1*
	> 4 Points	0	1	0	0	1	2	0	0	0	0	0	**4**	*2.0*

Table 2: Overview of clinical manifestations according to differential categories.
Abbreviations: AID, autoinflammatory disease; BS, Behçet Syndrome; CNS, central nervous system; CTD, connective tissue disease; GI disease, gastrointestinal disease; GIT, gastrointestinal disease; Misc, miscellaneous disorders; SpA, spondyloarthropathy.

Notes:
* no arterial manifestations in this cohort
† A small proportion of patients categorized as suffering from chronic pain syndrome also had arthritis: Patients with arthritis and chronic pain syndrome were considered suffering from primarily chronic pain syndrome, if arthritis was not evident anymore, and chronic pain syndrome was the clinically leading manifestation. Patients with chronic pain syndrome but no arthralgias suffered from myalgias and other non-articular pain (such as abdominal pain).

Differential Categories	*Total (%)*	*ICBD + (%)*
Probable BS	**58 (28.7)**	**40 (69.0)**
Skin Disease	**57 (28.2)**	**16 (28.1)**
Habitual oral aphthosis †	17	0
Recurrent orogenital aphthosis ‡	11	11
Erythema exsudative multiforme	5	1
Herpes virus infection	4	0
Blistering disease (bullous pemphigoid, pemphigus)	4	2
Lichen sclerosus / lichen planus	4	1
Erythema nodosum	3	0
Ulcerative vulvitis	1	0
Acne inversa	1	0
Ulcer of the tongue	1	0
Psoriasis (of the nails)	1	0
Lymphomatoid papulosis	1	1
Unclassifiable skin disease #	4	0
Chronic Pain (in combination with)	**26 (12.9)**	**8 (30.8)**
Habitual oral aphthosis	11	1
Oral aphthosis with or w/o other symptoms §	6	1
Herpes virus infection	2	1
Recurrent orogenital aphthosis	5	5
Recurrent folliculitis	1	0
Fibroma w. paraceratosis	1	0
Eye Disease	**14 (6.9)**	**1 (7.1)**
Idiopathic anterior uveitis	3	1
Idiopathic posterior uveitis (including retinal vasculitis and retinitis)	3	0
Ocular sarcoidosis	2	0
Episcleritis	2	0
Optic disc vasculitis	1	0
Idiopathic intermediate uveitis	1	0
Scleritis	1	0
Vitreal Bleeding	1	0
Spondyloarthropathy	**11 (5.4)**	**3 (27.3)**
Psoriatic arthritis	5	0
Axial SpA	3	1
SpA w. enteropathy	2	2
Reactive arthritis	1	0
Gastrointestinal Disease	**9 (4.5)**	**2 (22.2)**
Ulcerative colitis	4	0
Crohn's disease	3	1
Unclassified	2	1
Neurological Disease	**7 (3.5)**	**1 (14.2)**
Myopathy/myositis	2	0
Multiple sclerosis	2	0
Moyamoya disease (with recurrent orogenital aphthosis)	1	1
CNS vasculitis	1	0
CNS disease of unknown aetiology	1	0
Arthritis	**4 (2.0)**	**1 (25.0)**
Undifferentiated arthritis	3	0
Rheumatoid arthritis (with orogenital aphthosis)	1	1
Autoinflammatory Disease	**3 (1.5)**	**1 (33.3)**
Familial mediterranean fever	1	0
Unclassified *(PFAPA syndrome possible)*	1	0
PAPA syndrome	1	1
Connective Tissue Disease ±	**3 (1.5)**	**1 (33.3)**
Miscellaneous Disorders	**10 (5.0)**	**1 (10.0)**
Sarcoidosis	2	0
Infection		
Urinary tract infection	1	0
Rectal ulceration in Hepatitis B	1	0
Further		
Tolosa-Hunt-Syndrome (possible)	1	0
C1 esterase inhibitor deficiency (possible)	1	0
Osteoarthritis ∞	3	0
MAGIC	1	1
All	**202**	**75 (37.1)**

Table 3: Overview of differential categories and corresponding diagnoses with indication of ICBD-criteria positivity.

Abbreviations: BS, Behçet Syndrome; CNS, Central Nervous System; MAGIC, mouth and genital ulcers with inflamed cartilage syndrome; PAPA, Pyogenic Arthritis, Pyoderma gangrenosum, Acne; PFAPA, Periodic Fever, Aphthous Stomatitis, Pharyngitis, Adenitis; SpA, spondyloarthropathy.

Notes *(number of patients):*

† Including concomitant genital furunculosis *(1)*

‡ Including recurrent orogenital aphthosis in Steven Johnson Syndrome due to mycoplasma infection *(1)*

Including: gingivitis of unknown etiology *(1)*, atypical 'cut like' abnormality in the cheek mucosa *(1)*, recurrent furunculosis / panniculitis on arms, with severe scarring *(1)*, oral ulceration of unknown etiology *(1)*

§ w/o other symptoms *(1)*, pathergy test and DVT *(1)*, polyarthritis *(1)*, proctitis *(1)*, acneiform skin lesions *(1)*, thrombophlebitis *(1)*

± Including undifferentiated connective tissue disease *(2)* and limited cutaneous systemic sclerosis *(1)*

∞ Including osteoarthritis with open angle glaucoma, glomerulonephritis with nephrotic syndrome and non-healing wounds *(1)*