Correlation of Serum Amyloid-A Levels, Clinical Manifestations, Treatment, and Disease Activity in Patients with Behçet's Disease

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ABSTRACT: **Background:** Behçet's disease (BD) is an inflammatory disorder potentially leading to life- and sight-threatening complications. No laboratory marker associated with disease activity or predicting the occurrence of disease manifestations is currently available.

Objectives: To determine an association between serum amyloid-A (SAA) levels and disease activity via the BD Current Activity Form (BDCAF), to evaluate disease activity in relation to different SAA thresholds, to examine the association between single organ involvement and the overall major organ involvement with different SAA thresholds, and to assess the influence of biologic therapy on SAA levels.

Methods: We collected 95 serum samples from 64 BD patients. Related demographic, clinical, and therapeutic data were retrospectively gathered.

Results: No association was identified between SAA levels and BD disease activity (Spearman's rho = 0.085, $P = 0.411$). A significant difference was found in the mean BDCAF score between patients presenting with SAA levels ‹ 200 mg/L and those with SAA levels › 200 mg/L ($P = 0.027$). SAA levels › 200 mg/L were associated with major organ involvement ($P = 0.008$). A significant association was found between SAA levels › 150 mg/dl and ocular ($P = 0.008$), skin ($P = 0.002$), and mucosal ($P = 0.012$) manifestations. Patients undergoing biologic therapies displayed more frequently SAA levels ‹ 200 mg/L vs. patients who were not undergoing biologic therapies ($P = 0.012$).

Conclusions: Although SAA level does not represent a biomarker for disease activity, it might be a predictor of major organ involvement and ocular disease relapse at certain thresholds in patients with BD.

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KEY WORDS: Behçet's disease, Behçet's Disease Current Activity Form (BDCAF), biomarkers, serum amyloid-A (SAA), uveitis

Behçet's disease is a systemic inflammatory disorder that is clinically defined by oral aphthosis, genital ulcers, and sight-threatening ophthalmologic manifestations. Recurrent episodes of posterior uveitis, panuveitis, and/or retinal vasculitis may severely impair visual function, requiring a careful diagnostic and therapeutic management [1-3]. In addition, skin involvement, such as pseudofolliculitis and erythema nodosum, as well as major vessel disease and central nervous system conditions, may complicate the clinical scenario of these patients [4]. Given the relapsing-remitting nature of Behçet's disease and the diverse phenotype expression, a reliable and easy-to-evaluate marker is desirable to determine disease activity. In this regard, the medical literature has disclosed controversial results regarding traditional inflammatory markers [5-10]. The assessment of disease activity is currently based on the Behçet's Disease Current Activity Form (BDCAF), one of the most used clinimetric scores characterized by good inter-observer reliability [11].

Recent research efforts have focused on the potential role of serum amyloid-A (SAA) as a biomarker of disease activity and predictor of specific organ involvement in patients with Behçet's disease [10,12,13]. SAA is a highly conserved apolipoprotein involved in the acute phase response, which is often increased in different autoinflammatory diseases, both during inflammatory bouts and in the intercritical periods. It occupies a central role in the management of these disorders since close monitoring of SAA levels might confirm treatment efficacy and its normalization avoids reactive AA-amyloidosis, the most severe long-term complication of monogenic autoinflammatory diseases [14].

In relation to the clinical role of SAA in Behçet's disease, our working group recently suggested different associations between increased SAA levels and the occurrence of severe sight- and life-threatening manifestations [12]. In the present study, we investigated the correlation between SAA with disease activity

and specific clinical manifestations in a large cohort of Behçet's disease patients presenting with different systemic clinical signs.

PATIENTS AND METHODS

STUDY POPULATION

Ninety-five serum samples from 64 Behçet's disease patients were collected from January 2016 until June 2017. Samples from the same patients were collected at different moments of disease activity, as assessed by BDCAF. This instrument is based on 12 clinical categories evaluating the presence or absence of clinical manifestations over the 4 weeks prior to the day of the follow-up visit. The BDCAF score ranges from 0–12 and is later transformed into a 0–20 interval scale as described by the BDCAF [11]. All patients enrolled in our study met the International Study Group (ISG) criteria [15] or the International Criteria for Behçet's Disease (ICBD) [16] and were systematically followed in our rheumatologic unit. Exclusion criteria included concomitant inflammatory disorders, positive history of malignancies, ongoing infections, and pregnancy. We also excluded from the study individuals who were obese (defined as a body mass index > 30 kg/m^2). Demographic, clinical, and therapeutic data were collected, including age, gender, human leukocyte antigen (HLA)-B51 positivity, disease duration at the blood sample collection, clinical phenotype at disease onset, disease manifestations at each visit, and all therapies performed including corticosteroids, conventional disease modifying anti-rheumatic drugs (DMARDs), or biologic agents.

STUDY DESIGN

The primary aim of our study was to investigate the correlation between SAA levels and disease activity. Secondary aims were to evaluate disease activity in relationship with different SAA cutoff levels, to examine the association between single organ involvement and the overall major organ involvement with different SAA thresholds, and to assess the influence of biologic therapy on SAA levels.

The study's primary endpoint was evaluation of the correlation between SAA concentrations and status of disease activity at the time of sample collection using the BDCAF. Secondary endpoints were: to identify statistical differences in BDCAF values assessed in patients presenting SAA levels above and below different cutoff values preemptively established at 20 mg/L, 50 mg/L, 100 mg/L, 150 mg/L, and 200 mg/L; to find any statistically significant associations between SAA concentrations in patients with and without specific clinical manifestations and major organ involvement; and to find out any significant associations between predefined SAA concentrations and different therapeutic regimens.

Major organ involvement was defined as the occurrence of disease manifestations in at least one system among intraocular inflamation, vascular, gastrointestinal, or central nervous system.

LABORATORY ASSESSMENT

SAA concentrations were determined with a commercial solid phase sandwich enzyme-linked immunosorbent assay (Human SAA ELISA kit, Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. SAA levels lower than 20 mg/L were considered as normal.

STATISTICAL ANALYSIS

We used the Shapiro–Wilk test to determine the normality of our data. Pearson bivariate correlation or its non-parametric equivalent (as appropriate) were drafted to evaluate linear relationship between variables. Means or medians on different samples were evaluated by independent *t*-test or Mann–Whitney U test as appropriate. Chi-square or Fisher's exact test (as required) were performed to evaluate categorical data. For variables with more than two categories, we used contingency tables with post-hoc analysis and Bonferroni correction for the associated probability values. Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics software, version 24 (SPSS, IBM Corp, Armonk, NY, USA). A 2-sided $P < 0.05$ represented the threshold for statistical significance.

The study was approved by the local ethics committee and adhered to the tenets of the Declaration of Helsinki. A signed informed consent was obtained from all patients involved in our investigation.

RESULTS

Demographic characteristics, clinical phenotype, and therapeutic history from the 64 Behçet's disease patients who were enrolled in the study are shown in Table 1.

We found no correlation between SAA levels and disease activity measured by BDCAF (Spearman rho = 0.085, P = 0.411). Among the established cutoff values, a significant difference was found in the mean BDCAF score between patients presenting with SAA levels < 200 mg/L and those with SAA levels > 200 mg/L (P = 0.027). On the contrary, no differences were found between patients showing SAA levels above and below 20 mg/L (P = 0.42), 50 mg/L (P = 0.80), 100 mg/L (P = 0.38), and 150 mg/L (P = 0.12). SAA levels higher than 200 mg/L were significantly associated with major organ involvement (P = 0.008). With regard to specific organ involvement, significant associations were found between SAA levels > 150 mg/dl and ocular or skin manifestations (P = 0.008 and P = 0.002, respectively). Similarly, mucosal manifestations were significantly more frequent in patients with SAA levels > 150 mg/L (P = 0.012). At post-hoc analysis, this result was specifically verified for bipolar aphthosis ($P < 0.008$ according to Bonferroni correction). Conversely, no significant differences were found regarding gastrointestinal, central nervous system, and vascular involvement in any of the SAA cutoff levels that

Table 1. Demographic, clinical and therapeutic features of the patients enrolled in the study and their serum amyloid A levels

Demographic features	
Age, years, mean ± SD	43.77 ± 14.19
Disease duration, years, mean ± SD	15.69 ± 12.84
Females/Males	31/33
Overall SAA levels, mg/L, mean ± SD	64.84 ± 69.06
Clinical features	**N**
HLA B51 +	41/64
Eye involvement	38/64 (59.4%)
Mucosal involvement	63/64 (98.4%)
Skin involvement	48/64 (75%)
Articular involvement	53/64 (82.8%)
CNS involvement	8/64 (12.5%)
Gut involvement	32/64 (50%)
Vascular involvement	15/64 (23.4%)
ISG criteria fulfillment	49/64 (76.6%)
ICBD criteria fulfillment	64/64 (100%)
BDCAF values, mean ± SD	5.76 ± 3.30
Treatment performed at the sample collection time	
Biologic agents TNF-α blockers IL-1 inhibitors	28/64 (43.8%) 25/64 (39.1%) 3/64 (4.7%)
cDMARDs	29/64 (45.3%)
Corticosteroids	31/64 (48.4%)

BDCAF = Behcet's disease current activity form, cDMARDs = conventional disease modifying anti-rheumatic drugs, CNS = central nervous system, HLA = human leukocyte antigen, ICBD = international criteria for Behcet's disease, IL = Interleukin, ISG = international study group, SAA = serum amyloid A, SD = standard deviation, TNF = tumor necrosis factor

we evaluated ($P > 0.05$ in all cases). Table 2 shows P values referring to frequencies of clinical manifestations for different cutoff levels of SAA serum levels.

Patients undergoing biologic therapies were significantly associated with SAA levels < 200 mg/L compared with patients not treated with biologics ($P = 0.012$). No other significant differences were identified in relation to the concomitant use of corticosteroids and conventional DMARDs ($P > 0.05$ in both cases). SAA levels and mean BDCAF values in accordance to the treatment group are displayed in Table 3.

DISCUSSION

Along with its protean clinical presentations, Behçet's disease is characterized by a complex and still poorly understood etiopathogenesis. The Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides has characterized Behçet's disease as a variable vessel vasculitis [17]. Notwithstanding, in accordance with the lack of high-titer autoantibodies or pathogenetic autoimmune T cell response, the sudden and self-limited nature of inflammatory attacks, and the

Table 2. *P* values obtained by Pearson's chi-square or Fisher's exact test, referring to frequencies of clinical manifestations for different SAA cutoff levels

	SAA cutoff levels*				
Clinical manifestation	**20 mg/L**	**50 mg/L**	**100 mg/L**	**150 mg/L**	**200 mg/L**
Mucosal involvement				0.012	0.005
Skin manifestations				0.002	‹ 0.0001
Musculoskeletal manifestations					
CNS involvement					
Uveitis				0.008	0.008
Gastrointestinal disease					
Large vessel involvement					

*Empty cells indicate not significant results

SAA = serum amyloid-A

Table 3. BDCAF and SAA levels according to the treatment group

Therapy	**BDCAF, mean ± SD**	**SAA levels, mean ± SD**
Number of treatments (22 patients)	6.64 ± 2.65	71.48 ± 82.57
cDMARDs (14 patients)	6.29 ± 3.43	85.6 ± 85.4
Biologic agents in monotherapy (13 patients)	4.23 ± 3.30	37.78 ± 37.23
Combination therapy (15 patients)	5.53 ± 3.66	76.92 ± 72.26

BDCAF = Behçet's disease current activity form, cDMARDs = conventional disease modifying anti-rheumatic drugs, SAA = serum amyloid-A, SD = standard deviationfdxd

therapeutic effectiveness of interleukin (IL)-1 blockade, several experts have classified Behçet's disease as an autoinflammatory disorder [18-20]. Notably, IL-1β, a key proinflammatory cytokine activated by the cytoplasmic multiprotein complex known as *NLRP3* inflammasome, may be upregulated by SAA, which in turn has been found to promote the activation of *NLRP3* inflammasome via P2x7 receptor and cathepsin B-sensitive pathway [21]. On this basis, given the contribution of IL-1 in the complex pathogenesis of Behçet's disease [20], it is tempting to investigate a possible role of SAA in the immune processes involved in this disorder. Specifically, Lopalco and colleagues [22] hypothesized SAA to interact with several proinflammatory cytokines in patients with active Behçet's disease. However, other studies have searched for a practical role of SAA levels as a biomarker of disease activity or a prognostic index, with no definite conclusions [10,12].

In the present study, we did not find a correlation between SAA levels and disease activity, but despite this lack of evidence, we noticed significantly higher mean BDCAF scores in patients with SAA levels > 200 mg/L, which was also the cutoff above which major organ involvement was statistically more frequent. As a consequence, patients presenting SAA levels over 200 mg/L seem to be at higher risk for a complicated Behçet's disease clinical course, with a more likely occurrence of major organ involvement.

Notably, our data showed a significantly higher frequency of eye inflammatory involvement in Behçet's disease patients who had SAA levels above 150 mg/L. In addition, the occurrence of Behçet's disease-related ocular affections was correlated with SAA levels higher than 150 mg/L. These findings are in accordance with previous evidence identifying the same SAA threshold for patients at higher risk for ocular disease activity [12].

Similarly, mucocutaneous manifestations were found to be significantly more frequent in patients showing SAA levels > 150 mg/L. Specifically, among mucous manifestations, bipolar aphthosis was the variable accounting for significance. Looking at past findings, no threshold levels had been found for skin lesions, and a threshold of 30 mg/L had been described for oral aphthosis. However, previous attempts to correlate Behçet's disease mucosal features with different SAA levels had been performed solely on oral aphthosis, while discrepancy in the cutoff levels may be due to differences in the sample size [12].

In contrast to a Korean study [23] reporting upregulated production of SAA in Behçet's disease patients with intestinal involvement, we found no significant association between SAA levels and Behçet's disease-related gastrointestinal disease. However, gut involvement contributed to the aforementioned association with SAA levels when globally considered in the context of major organ involvement. The different genetic background and the distinct epidemiology of intestinal Behçet's disease in the Far East might at least partially explain the discrepancy with previously reported data.

From a therapeutic point of view, in our study SAA levels < 200 mg/L were associated with the use of biologics. As biologic treatments have widely proven to be an effective therapeutic tool for Behçet's disease [24], patients administered with biologics likely present a lower risk for disease relapse. Therefore, this finding is in line with the lower frequency of major organ involvement in patients with SAA levels < 200 mg/L.

Considering the sight- and life-threatening manifestations of Behçet's disease, a proper evaluation of disease activity is warranted to promptly detect potential complications and redirect the otherwise poor prognosis. A reliable biomarker able to detect patients at higher risk of major organ involvement or likely to experience a disease relapse could allow clinicians to modulate treatment and avoid complications. Although acute phase reactants are often used in the clinical practice as diagnostic, prognostic, or therapeutic follow-up markers for many inflammatory diseases, in Behçet's disease patients these markers display a scarce reliability and a poor utility [5-10]. Conversely, although SAA levels do not correlate with disease activity assessed with BDCAF, SAA levels higher than 200 mg/L should suggest to physicians to pay attention to the risk of severe Behçet's disease manifestations. Similarly, SAA levels higher than 150 mg/L may precede ocular disease relapses, potentially leading to visual impairment, as well as to skin and mucosal manifestations potentially impacting on quality of life [25].

Limitations of our study include the retrospective design and the lack of a control group. Nevertheless, our findings represent an additional research effort aimed at shedding light on the actual role of SAA as a potential biomarker in the clinical management of Behçet's disease.

CONCLUSIONS

SAA does not represent a biomarker of disease activity, but might be a useful predictor of major organ involvement and ocular disease relapse at certain SAA thresholds. Based on this finding, the evaluation of SAA levels could be useful in the clinical practice to identify patients at higher risk of life- and sight-threatening complications.

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Capsule

Live-born major congenital heart disease in Denmark

Lyzen et al. investigated whether the occurrence of live-born major congenital heart disease (CHD) changing. In a nationwide, population-based study from 1996 to 2013 in Denmark, the live-born incidence of major CHD decreased from 0.22% to 0.14%. Prenatal detection rate increased, as did the proportion of terminated pregnancies, and when terminated pregnancies were included, the incidence of major CHD remained constant during the study. The increased prenatal detection of major CHD has led to an increased termination of pregnancy rate, with a subsequent decrease in live-birth incidence of major CHD.

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Capsule

Differential methylation affects risk of multiple sclerosis

Specific variants of the human leukocyte antigen (HLA) locus are heritable risk factors for the autoimmune disease multiple sclerosis (MS). However, how these variants confer risk is not well understood. It has been proposed that epigenetic modifications, such as differences in methylation, of noncoding regions near the HLA coding regions may explain why some people are more likely to develop MS. Comparing controls and patients, **Kular** and colleagues identified hypomethylated genomic regions associated with increases in gene expression at the HLA locus that increased the risk of developing MS. Extending this investigation, the authors identified a protective variant that reduces the probability of developing MS that is more highly methylated.

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Eitan Israeli

Capsule

An inhibitor of oxidative phosphorylation exploits cancer vulnerability

Metabolic re-programming is an emerging hallmark of tumor biology and an actively pursued opportunity in discovery of oncology drugs. Extensive efforts have focused on therapeutic targeting of glycolysis, whereas drugging mitochondrial oxidative phosphorylation (OXPHOS) has remained largely unexplored, partly because of an incomplete understanding of tumor contexts in which OXPHOS is essential. **Molina** and co-authors reported the discovery of IACS-010759, a clinical-grade small-molecule inhibitor of complex I of the mitochondrial electron transport chain. Treatment with IACS-010759 robustly inhibited proliferation and induced apoptosis in models of brain cancer and acute myeloid leukemia (AML) reliant on OXPHOS, likely because of a combination of energy depletion and reduced aspartate production that leads to impaired nucleotide biosynthesis. In models of brain cancer and AML, tumor growth was potently inhibited in vivo following IACS-010759 treatment at well-tolerated doses. IACS-010759 is currently being evaluated in phase 1 clinical trials in relapsed/refractory AML and solid tumors.

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Eitan Israeli