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Neutrophil, NETs and Behçet's disease: A review

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ABSTRACT

Behçet's disease (BD) is a chronic systemic vasculitis characterized by recurrent oral and genital ulcers, skin lesions, articular, neurological, vascular and sight-threatening ocular inflammation. BD is thought to share both autoimmune and autoinflammatory disease features. BD is triggered by environmental factors such as infectious agents in genetically predisposed subjects. Neutrophils seem to play an instrumental role in BD and recent works regarding the role of neutrophils extracellular traps (NETs) provides new insight in the pathophysiology of BD and the mechanisms involved in immune thrombosis. This review provides a recent overview on the role of neutrophils and NETs in the pathogenesis of BD.

1. Introduction

Neutrophils and their hyperfunction have been proposed to be involved in the pathogenesis of Behçet's disease (BD) since 1975. Matsumura et al. showed that neutrophil chemotaxis was increased and could be alleviated with colchicine [1]. Since this first publication, other physicians reported the usefulness of colchicine in BD [2,3]. The effect of colchicine on leukocytes count was first observed in 1908 by Dixon et al. [4] and the inhibition of neutrophils migration (as well by hydrocortisone) was evidenced by Mukaide et al. [5]. Many studies have highlighted the increase chemotaxism of BD neutrophil in the 70's [6–8].

Histological studies of different BD specimen revealed the importance of neutrophils infiltrates. In mucosal [9–11], aorta [12], intestinal [13] and conjunctival lesions [14], neutrophils were prominent around the vasa vasorum and expressed the pro-inflammatory cytokines IL-1α, TNF-α and IFN-γ. Neutrophils were more closely adherent to HLA-DR positive endothelial cells. Neurological biopsy or autopsy of neuro-Behçet's patients also revealed perivascular infiltration of neutrophils [15,16]. Histological evaluation of erythema nodosa lesion (ENL) in BD are difficult to distinguishes from ENL of others causes but showed a vasculitis with various degree (mostly venulitis) of neutrophil infiltration [17–20]. Neutrophilic dermatoses are a group of heterogeneous inflammatory disorders characterized by a sterile neutrophilic infiltrate on histopathology. Their management is very similar to that of BD, and neutrophilic dermatoses can be found in BD (including pyoderma gangrenosum). This emphasizes that BD and neutrophilic dermatoses may be part of the same spectrum of disorders [21]. Lastly, histopathology of skin Pathergy test reveals neutrophil infiltration in most studies [22,22,23]. Thus, though vasculitis is often present, the presence of a true necrotizing vasculitis is not found in BD.

From a clinical stand point, neutrophil to lymphocyte ratio has been proposed as a prognostic marker [24,25], as a disease activity marker [26–29], or even as a diagnostic tool of BD [30–32].

In recent years, genetic, immunological and molecular data have largely supported the key role played by neutrophils in this disease.

This review provides a detailed analysis of advances in understanding the role of neutrophil activation and neutrophil extracellular traps (NETs) in the lesional process of BD and their therapeutic implications (Fig. 1).

2. Neutrophil activation in BD

2.1. Chemotaxis

Since 1975, many studies have highlighted the neutrophil

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chemotaxism in BD. Neutrophil migration in patients with active BD disease was demonstrated by many researchers [6–8,33–36]. Neutrophil hypermigration was also demonstrated in pathergy test skin lesions [37] and in in vitro assay on human umbilical vein endothelial cells (HUVECs) monolayer [38]. Some authors found an increased chemotaxis of neutrophils from patients with BD only when incubated with patient's and/or healthy donors (HD) plasma [39] suggesting that some soluble factors may activate these neutrophils. Moreover, IFN-α have been found to decrease neutrophil adhesion [40].

Altogether, these studies suggest that the increase chemotaxis of BD neutrophils may be secondary to soluble factors, bacterial compound or other cell-cell interaction. Increased adhesion molecules both on neutrophils and endothelial cells may facilitate such adhesion and increased chemotaxis [11,17,38,41–44].

2.2. Neutrophil activation markers in BD

Analysis of activation markers by flow cytometry is an easy and effective way to study neutrophil activation and to provide evidence for neutrophil hyperfunction.

Upregulation of CD64 [45], CD11a, CD11b, CD18 [38,46,47] and CD206, CD209 [46], CD66b, CD64 [47] have been found in BD's neutrophils. Conflicting results regarding CD14, and TLR receptors expression exist [39,48].

2.3. Neutrophil granule enzymes

Neutrophil elastase (NE), Myeloperoxydase (MPO) and S100A protein have been found increased in saliva and serum of patients with BD [49–55]. In addition, defensins [56,57] human neutrophil peptide [58] and S100 protein [59] were increased in patients with BD's saliva as compared to healthy donors', patients with endodontic infection and pericoronitis.

2.4. Phagocytosis

Karti et al. and Mizushima et al. showed that neutrophils from BD had higher capacity of phagocytosis than healthy controls [35,40]. However, these results were not confirmed by other groups [48,60–62]. The consistent findings obtained by several groups with an array of microorganisms and by different methods indicate that phagocyte microbicide activity is not primarily disturbed in BD patients.

2.5. Oxidative burst

Besides of chemoattraction, phagocytosis and activation markers, secretion of free radicals is a neutrophils response to fight against pathogens. Reactive oxygen species (ROS) are generated via the action of the NADPH oxidase. This process consumes large amounts of oxygen, which is converted into the highly-reactive superoxide radical O2- and H2O2. Subsequent activation of myeloperoxidase (MPO) generates secondary oxidants that are highly microbiocidal in nature. But, imbalance between oxidant formation and endogenous antioxidant defense is associated with tissue damages (DNA, proteins, membrane lipids, endothelial cells etc) [63]. These events are responsible for the onset of several pathological conditions [64].

The findings of previous studies are congruent: there is an excess in the oxidant system and a decrease of the antioxidant system in BD. Both serum/plasma and neutrophils, stimulated or not, exhibit increased ROS levels [65–76].

High levels of plasma MPO activity have also been reported in Behçet's patients, along with increased levels of plasma nitrate/nitrite, which are substrates for MPO and cause the formation of more ROS [54]. In addition, increased expression of cyclooxygenase-2 in Behçet's

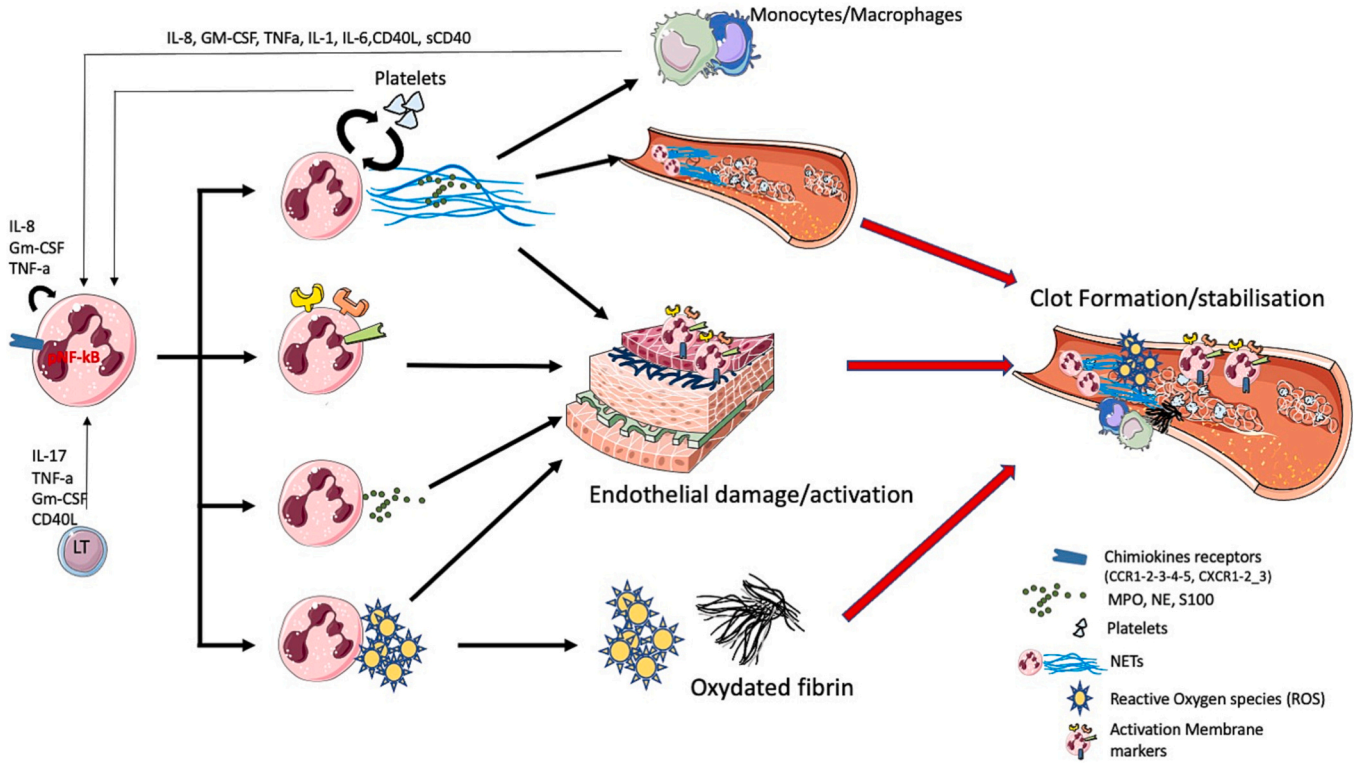

Fig. 1. Schematic representation of the role of neutrophils in Behçet's disease.
TNF-a: Tumor necrosis factor a, Gm-CSF: Granulocyte-macrophage colony stimulation factor, NETs: Neutrophils Extracellular Traps, MPO: Myeloperoxydase, NE: Neutrophil Elastase.
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neutrophils may contribute to increased vascular damage via the synthesis of pro-inflammatory prostaglandins [54].

The consequences of ROS production in BD can be very wide and have not been extensively studied in this condition. Increased ROS production by BD neutrophils may be a direct cause of fibrinogen oxidation, which leads to slower fibrin polymerization and resistance to plasmin-induced lysis. It could therefore be an explanation of the pro thrombotic state in BD [77].

2.6. Genetics

Genes implicated cell chemotaxis (i.e., CCR1-CCR3) were found to be associated with BS and.

rare and low-frequency variants involved in innate immunity have also been found, namely TLR4, NOD2 and MEFV genes. Predisposing variants may participate to neutrophils preactivation state. Moreover, in a transgenic mouse model, Takeno et al. showed that neutrophils from HLA-B51 mice were hyperactivated. They also showed that neutrophils from HLA-B51 positive patients were hyperactivated as compared to HLA-B51 negative patients [78].

2.7. Mechanisms of neutrophil activation

2.7.1. Micro-organisms

Neutrophil activation is a hallmark of bacterial infection. Thus, it was hypothesized that microorganisms or bacterial compounds mays be the cause of neutrophil activation. Indeed, exacerbations of BD have been described following exposure to streptococcal antigens and pneumococcal vaccines [79–81]. Deniz et al. showed that adding streptococcal antigens to the pathergy test increased its sensitivity from 4.8% to 64% [82]. Moreover, some authors showed that monocytes from BD had higher TLR-2 expression that HD. Thus, monocytes could be responsible for the overreaction to streptococcal compounds, and may induce neutrophils hyperactivation [39,83–85]. But this hypothesis needs confirmation.

2.7.2. Cytokines and chimiokines

Many studies showed that neutrophils are activated when incubated with plasma/serum or T lymphocytes medium suggesting a role of one or a cocktail of activation cytokines/molecules.

Yu et al. and Le Joncour et al. also showed that chimiokines receptors (CCR1, CCR3, CCR4, CCR6, CCR7, CXCR1, CXCR2, CXCR3) were enhanced in transcriptomic analysis of BD isolated neutrophils [47,86].

2.7.2.1. IL-8 and GM-CSF. Histological studies revealed that neutrophil infiltration in BD is accompanied by T cell and monocytes that are strongly positive for IL-8. Moreover, skin-derived T-cell clones from BD patients produce large amounts of IL-8 and GM-CSF which are potent activators of neutrophil recruitment and activation [87]. In addition, serum levels of IL-8 [88–93] and GM-GCSF [94] is high in BD especially in the active phase. Thus, cells producing IL-8/GM-CSF cytokines such as monocytes [39] or lymphocytes [95], endothelial cells [96] may be responsible for neutrophil infiltration in BD.

2.7.2.2. Other cytokines: TNF-α, IL-6, IL-1, IL-17. Many inflammatory cytokines such as TNF-α, IL-6 and IL-1 (Kato 2006) are able to activate neutrophils mainly through the activation of the NfkB pathway. These cytokines have widely been studied in BD. They are elevated and corelated with activity [73,97–99]. They can be secreted by a wide range of cells, included neutrophils, and may act as paracrine and/or autocrine activators for neutrophils.

Th17 response yields IL-17A, IL-17F, IL-22 and IL-23 production and neutrophils recruitment. Neutrophils can be recruited either through innate immunity mechanisms or Th17 cells. IL-17 producing cells are able to recruit neutrophils mainly by GM-CSF pathway [100] and endothelial cells [101] but also directly [102]. Interestingly, CD4+ T helper (Th) lymphocytes in BD are predominantly shifted towards Th1 and Th17 [103–106].

2.7.3. Endothelial activation

Endothelial activation has been documented in BD. Indeed, thrombomodulin [107], selectins (P selectin, E selectin etc) [108–110], endothelium derived von Willebrand [74,111] have been outlighted. After activation, endothelial cells express a range of adhesion molecules that promote neutrophil rolling and adhesion. It has been documented that serum from BD patients enhances the adhesion of PMN to endothelial cells in vitro via the upregulation of CD11a and CD18 on neutrophils and the intercellular adhesion molecule-1 (ICAM-1, CD54) on endothelial cells [38]. Neutrophils and others cytokines/molecules cells may activate endothelial cells that can in turn recruit and activate neutrophils these mechanisms underline the important crosstalk between these cells.

2.7.4. Cell-cell interaction

Neutrophil can directly interact with many type of cells. We previously highlighted the critical role of endothelial cell–neutrophil interaction. Besides, platelets (and more importantly activated platelets) induce neutrophil activation which produce oxidants and cytokines: neutrophil-platelet aggregates are involved in the maintenance and the amplification of the systemic inflammatory response. Platelets are activated in BD [112–115]. In addition, activated platelets can induce NEtosis (See paragraph 2) In addition, CD40L and its soluble form (sCD40L) is found mainly on platelets but also in monocytes, lymphocytes, smooth muscle cells, endothelial cells. CD40L and sCD40 have been found in BD: CD40L was upregulated in lymphocytes and monocytes from BD [68] and sCD40L induced NETosis and oxydative burst in neutrophils. High levels of CD40L have been found in BD [116].

2.7.5. Constitutive neutrophil activation

Whether neutrophils are constitutively activated or become activated upon multiple stimuli is not established in BD. In a mouse study, authors suggest that HLA-b51 may predispose neutrophils to be activated [78] but this has not been confirmed elsewhere. Moreover, in BD only 50% of patients carry HLA-B51 [117]. Yavuz et al., found that neutrophils from BD male patients were more activated (showed higher levels of oxidative burst, CD66b, and CD16b) than those from female patients. These findings suggest that the role of testosterone could potentially explain the differences in gender prevalence and severity observed in BD [118].

3. Neutrophil extracellular Traps (NETs)

Researchers have explored the potential role of NETs in BD given that (i) venous thrombosis, neutrophil activation, and ROS generation are hallmarks of the disease, and (ii) ROS generation is one of the major molecular pathways leading to NETosis, which in turn promotes thrombosis and vascular injury. Under inflammatory or infectious conditions, neutrophils are able to generate NETs via a distinct process of cell death named NETosis [119]. NETs consist of extruded cell-free DNA (CfDNA) decorated with histones and granular components that include antimicrobial peptides and proteases. The molecular pathways leading to NETosis include calcium mobilization, generation of ROS, nuclear delobulation involving enzymatic activities of MPO and NE, and chromatin modification via the citrullination of histones by the peptidyl arginine deiminase (PAD4) [120]. Numerous studies have implicated NETs in the etiology of auto inflammatory or autoimmune conditions such as systemic as autoimmune vasculitis [121,122]. Furthermore, NETs are now recognized as a key trigger of thrombus initiation and progression in pathological conditions such as, deep vein thrombosis in mice and humans [123] but also in arterial diseases such as stroke and myocardial infarction [124,125].

To date, 4 studies have explored the role of NETs in BD [68,72,126–128].

Indirect markers of NETs such as MPO-DNA complexes and cf-DNA have been found to be elevated in serum and plasma of BD and was corelated to disease activity and vascular involvement [72]. Besides, neutrophils from BD patients were shown to be more prone to NETosis even in the absence of stimuli in vitro as shown by immunostaining of MPO-Citrulinated histone H3, neutrophil elastase [68,72,126–128]. Moreover, PAD4 mRNA was upregulated in neutrophils from BD [126]. Interestingly, evidence of NETs was shown in vivo in histological specimen of mucocutaneous and vascular lesions from BD [72,126]. Serum/Plasma from BD patients was able to induce NETosis both in BD and HD neutrophils [68,72,126]. As sCD40L levels was increased in BD's plasma, Perazzio et al. hypothesized that it could be a NET inducer. Indeed, NET release was higher after stimulation with sCD40L or BD plasma and decreased after sCD40L blockade [68]. As previously mentioned, CD40L or sCD40L source could be platelets, lymphocytes and/or monocytes. Li et. also showed that high levels of Histone H4 and oxidized DNA in BD NETs might mediate macrophages hyperactivation [128]. Le Joncour et al. and Safi et al. have explored the possible link between these NETs and thrombosis in BD. In an in vitro model of thrombin generation, Le Joncour et al. have shown that NETs axis promotes intravascular coagulation and that the velocity and peak of thrombin generation were positively correlated with NETs markers in serum, and these were decreased with the addition of a NET-disrupting enzyme, DNase [72]. In addition to promoting the thrombin generation, NETs also cause endothelial dysfunction via decreasing cell proliferation and increasing apoptosis [126].

4. Neutrophil/NETs and thrombosis in BD: the concept of immune-thrombosis

Behçet's disease is an immune-inflammatory disorder that involves different vessel types and sizes of the vascular tree and is often complicated by recurrent thrombosis, particularly in the venous compartment [129]. Thrombosis represents an important cause of morbidity and mortality. The understanding of the above pathogenetic mechanisms, together with the clinical experience, has led to consider thrombosis in BS as inflammatory–mediated and consequently, has suggested its treatment with immunosuppression rather than anti-coagulation [130]. It is now widely accepted that there is a strong relationship between inflammation, endothelial dysfunction, oxidative stress, leukocytes and platelets. So far, the concept of thrombo-inflammation and/or immuno- thrombosis has emerge in the field of BD [131,131–133]. Indeed, BD, as an inflammatory and neutrophil mediated disease, represents the spearheads of this concept. Neutrophil activation results in enhanced ROS production. ROS can oxidate fibrinogen altering its general structure which impaired fibrinogen function, both in terms of fibrin polymerization and susceptibility to plasmin-induced lysis, increasing the thrombogenic potential and the resistance to clot lysis [68]. NETs produced by Behçet's neutrophils can activate thrombus formation through various mechanisms, such as activating the intrinsic and extrinsic coagulation pathways [72], enhancing endothelial cell activation [126], and activating platelets. Besides NETs, Neutrophils also activate endothelial cells, platelets via secretion of pro inflammatory cytokines (IL-1, TNF-a, IL-8) [134]. All these phenomena participate to immune thrombosis in Behçet's disease [129].

5. NF-kB and Behçet disease

There is growing evidence of the implication of the NF-κB pathway in BD. The NF-κB pathway is tightly regulated through multiple post-translational mechanisms. Dysfunction of key proteins of NF-κB pathway leading to its upregulation have recently been described in humans in a phenotype very close to BD [135]. The NF-κB pathway could thus represent an interesting target in the pathophysiology of BD but has not yet been widely studied. Phosphodiesterase 4 (PDE4) is an immune and inflammatory cell enzyme which, by degrading the key intracellular signaling messenger cAMP, promotes the NF-κB pathway activation [136–138]. Given the central role of PDE4 in the NF-κB pathway and the clinical effect of PDE4 inhibition [139,140] and the monogenic autoinflammatory diseases involving this pathway, NF-κB pathway seems to be an important field of research in BD [141]. To date, 3 studies suggest the role of Nf-kB pathway in BD's neutrophils. The transcriptomic analysis of mononuclear cells revealed that 5 out of top 10 upregulated biological processes involved leucocyte recruitment to peripheral tissues, especially for neutrophils. Moreover, NF-kB, TNF and IL-1 signaling pathways were prominently enhanced in BD [142]. Yu et al. recently showed in a transcriptomic analysis of isolated neutrophils that the NF-kB pathway was upregulated in BD as compared to HD [86]. Recently, our group showed that PDE4 inhibition tilted down neutrophil activation by decreasing, in vitro and in vivo and at the transcriptomic level, neutrophils activation markers, ROS production and NETs release by BD's neutrophils [47].

6. Therapeutic implications

Steroids, colchicine, anti-TNFa, apremilast are largely used in the therapeutic arsenal of BD. They are known to decrease neutrophil activation and NETs formation. Indeed, colchicine and steroids are the most studied drugs for their impact of neutrophil function both in BD [1,5,75] and other inflammatory conditions [143–145]. Safi et al. and Bettiol et al. and our group showed that colchicine and steroids inhibited the release of NETs by BD neutrophils [47,126,127].

Anti-TNFa have been shown to suppresses proinflammatory activities of mucosal neutrophils in inflammatory bowel disease [146]. Recently, our group showed that apremilast was able to tilt down neutrophil activation by decreasing, in vitro and in vivo and at the transcriptomic level, neutrophils activation markers, ROS production and NETs release by BD's neutrophils [47].

Potential other therapeutic avenue that directly target circulating NETs such as *N*-acetyl-cysteine, PAD4 inhibitor, or DNase I have been efficiently tested in vitro in BD [72,126] and in vitro and in animal models in other inflammatory conditions (such as ischemia reperfusion, SIRS, multiple myeloma, rheumatoid arthritis etc. [147–150]. However, these molecules, although promising, have been tested in vitro and in animal models. Further studies, particularly in humans, will be mandatory before considering their therapeutic use.

7. Conclusion

Understanding the pathogenesis of Behçet's disease is critical for the development of effective and innovative therapies. The presence of multiple unknown environmental factors, together with polygenic inheritance, leads to patient's heterogeneity and makes the awareness of the of its pathogenesis more challenging. It is now clear that innate immunity, and neutrophils in particular, play an instrumental role in the disease, opening up new avenues for research and therapeutics.

Data availability

No data was used for the research described in the article.

References

[1] N. Matsumura, Y. Mizushima, Leucocyte movement and colchicine treatment in Behcet's disease, Lancet. 2 (1975) 813.
[2] P.G. Hazen, B. Michel, Management of necrotizing vasculitis with colchicine. Improvement in patients with cutaneous lesions and Behcet's syndrome, Arch. Dermatol. 115 (1979) 1303–1306.

[3] Y. Mizushima, N. Matsumura, M. Mori, T. Shimizu, B. Fukushima, Y. Mimura, K. Saito, S. Sugiura, Colchicine in Behçet's disease, Lancet. 2 (1977) 1037, https://doi.org/10.1016/s0140-6736(77)92945-2.

[4] W.E. Dixon, W. Malden, Colchicine with special reference to its mode of action and effect on bone-marrow, J. Physiol. 37 (1908) 50–76.

[5] A. Mukaide, T. Kameyama, Effects of drugs on leukocyte migration, Nihon Yakurigaku Zasshi. 72 (1976) 59–61.

[6] J.D. Sobel, S. Haim, N. Obedeanu, T. Meshulam, D. Merzbach, Polymorphonuclear leucocyte function in Behçet's disease, J. Clin. Pathol. 30 (1977) 250–253.

[7] D.W. James, J.R. Walker, M.J. Smith, Abnormal polymorphonuclear leucocyte chemotaxis in Behçet's syndrome, Ann. Rheum. Dis. 38 (1979) 219–221.

[8] Bozkurt, Tuzun, Sayin, Karaozbek, Koksal, Ozer, The Role of Polytetrafluoroethylene Graft Material in Buerger's Disease, Int. J. Angiol. 7 (1998) 188–190.

[9] F. Kaneko, Y. Takahashi, Y. Muramatsu, Y. Miura, Immunological studies on aphthous ulcer and erythema nodosum-like eruptions in Behcet's disease, Br. J. Dermatol. 113 (1985) 303–312, https://doi.org/10.1111/j.1365-2133.1985.tb02082.x.

[10] J.L. Jorizzo, J.L. Abernethy, W.L. White, H.C. Mangelsdorf, C.C. Zouboulis, R. Sarica, K. Gaffney, C. Mat, H. Yazici, Mucocutaneous criteria for the diagnosis of Behçet's disease: an analysis of clinicopathologic data from multiple international centers, J. Am. Acad. Dermatol. 32 (1995) 968–976, https://doi.org/10.1016/0190-9622(95)91333-5.

[11] O. Kose, J. Stewart, A. Waseem, A. Lalli, F. Fortune, Expression of cytokeratins, adhesion and activation molecules in oral ulcers of Behçet's disease, Clin. Exp. Dermatol. 33 (2008) 62–69, https://doi.org/10.1111/j.1365-2230.2007.02558.x.

[12] M. Kobayashi, M. Ito, A. Nakagawa, M. Matsushita, N. Nishikimi, T. Sakurai, Y. Nimura, Neutrophil and endothelial cell activation in the vasa vasorum in vasculo-Behçet disease, Histopathology. 36 (2000) 362–371.

[13] N. Hayasaki, M. Ito, T. Suzuki, K. Ina, T. Ando, K. Kusugami, H. Goto, Neutrophilic phlebitis is characteristic of intestinal Behçet's disease and simple ulcer syndrome, Histopathology. 45 (2004) 377–383, https://doi.org/10.1111/j.1365-2559.2004.01954.x.

[14] T. Matsuo, M. Itami, H. Nakagawa, M. Nagayama, The incidence and pathology of conjunctival ulceration in Behçet's syndrome, Br. J. Ophthalmol. 86 (2002) 140–143, https://doi.org/10.1136/bjo.86.2.140.

[15] A. Borhani Haghighi, H.R. Sharifzad, S. Matin, S. Rezaee, The pathological presentations of neuro-Behçet disease: a case report and review of the literature, Neurologist 13 (2007) 209–214, https://doi.org/10.1097/01.nrl.0000263698.26284.cf.

[16] F. Cohen-Aubart, D. Psimaras, D. Galanaud, F. Bielle, V. Touitou, J. Haroche, Z. Amoura, Cerebral pseudo-tumoral neuro-Behcet: Histological demonstration of an inflammatory and vascular disease, Clin. Neurol. Neurosurg. 161 (2017) 48–50, https://doi.org/10.1016/j.clineuro.2017.08.004.

[17] C. Demirkesen, N. Tüzüner, C. Mat, M. Senocak, N. Büyükbabani, Y. Tüzün, H. Yazici, Clinicopathologic Evaluation of Nodular Cutaneous Lesions of Behçet Syndrome, Am. J. Clin. Pathol. 116 (2001) 341–346, https://doi.org/10.1309/GCTH-0060-55K8-XCTT.

[18] S.I. Chun, W.P. Su, S. Lee, R.S. Rogers, Erythema nodosum-like lesions in Behçet's syndrome: a histopathologic study of 30 cases, J. Cutan. Pathol. 16 (1989) 259–265, https://doi.org/10.1111/j.1600-0560.1989.tb00049.x.

[19] E.J. Kim, B.S. Cho, T.S. Lee, S.J. Kim, J.W. Seo, Morphologic change of the internal elastic lamina in Buerger's disease, J. Korean Med. Sci. 15 (2000) 44–48.

[20] K.R. Chen, Y. Kawahara, S. Miyakawa, T. Nishikawa, Cutaneous vasculitis in Behçet's disease: a clinical and histopathologic study of 20 patients, J. Am. Acad. Dermatol. 36 (1997) 689–696, https://doi.org/10.1016/s0190-9622(97)80318-0.

[21] E.H. Weiss, C.J. Ko, T.H. Leung, R.G. Micheletti, A. Mostaghimi, S. M. Ramachandran, M. Rosenbach, C.A. Nelson, Neutrophilic Dermatoses: a Clinical Update, Curr. Dermatol. Rep. 11 (2022) 89–102, https://doi.org/10.1007/s13671-022-00355-8.

[22] T. Ergun, O. Gürbüz, J. Harvell, J. Jorizzo, W. White, The histopathology of pathergy: a chronologic study of skin hyperreactivity in Behçet's disease, Int. J. Dermatol. 37 (1998) 929–933.

[23] A. Gül, S. Esin, N. Dilsen, M. Koniçe, H. Wigzell, P. Biberfeld, Immunohistology of skin pathergy reaction in Behçet's disease, Br. J. Dermatol. 132 (1995) 901–907, https://doi.org/10.1111/j.1365-2133.1995.tb16946.x.

[24] C.-C. Hou, J.-L. Guan, Risk factors of disease activity in patients with Behçet's syndrome, Clin. Rheumatol. (2020), https://doi.org/10.1007/s10067-020-05386-9.

[25] Y.H. Lee, G.G. Song, Neutrophil-to-lymphocyte ratio, mean platelet volume and platelet-to-lymphocyte ratio in Behçet's disease and their correlation with disease activity: A meta-analysis, Int. J. Rheum. Dis. 21 (2018) 2180–2187, https://doi.org/10.1111/1756-185X.13404.

[26] F. Djaballah-Ider, C. Touil-Boukoffa, Effect of combined colchicine-corticosteroid treatment on neutrophil/lymphocyte ratio: a predictive marker in Behçet disease activity, Inflammopharmacology. 28 (2020) 819–829, https://doi.org/10.1007/s10787-020-00701-x.

[27] Z. Zhang, Q. Su, L. Zhang, Z. Yang, Y. Qiu, W. Mo, Diagnostic value of hemoglobin and neutrophil-to-lymphocyte ratio in Behcet Disease, Medicine (Baltimore) 98 (2019), e18443, https://doi.org/10.1097/MD.0000000000018443.

[28] M. Hammad, O.Z. Shehata, S.M. Abdel-Latif, A.M.M. El-Din, Neutrophil/lymphocyte ratio and platelet/lymphocyte ratio in Behçet's disease: which and when to use? Clin. Rheumatol. 37 (2018) 2811–2817, https://doi.org/10.1007/s10067-018-4194-z.

[29] A. Balkarli, A. Kucuk, H. Babur, F. Erbasan, Neutrophil/lymphocyte ratio and mean platelet volume in Behçet's disease, Eur. Rev. Med. Pharmacol. Sci. 20 (2016) 3045–3050.

[30] S. Alan, S. Tuna, E.B. Türkoğlu, The relation of neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and mean platelet volume with the presence and severity of Behçet's syndrome, Kaohsiung J. Med. Sci. 31 (2015) 626–631, https://doi.org/10.1016/j.kjms.2015.10.010.

[31] E.N. Rifaioglu, B. Bülbül Şen, Ö. Ekiz, A. Cigdem Dogramaci, Neutrophil to lymphocyte ratio in Behçet's disease as a marker of disease activity, Acta Dermatovenerol Alp Pannonica Adriat. 23 (2014) 65–67.

[32] M. Yuksel, A. Yildiz, M. Oylumlu, F.M. Turkcu, M.Z. Bilik, A. Ekinci, B. Elbey, E. Tekbas, S. Alan, Novel markers of endothelial dysfunction and inflammation in Behçet's disease patients with ocular involvement: epicardial fat thickness, carotid intima media thickness, serum ADMA level, and neutrophil-to-lymphocyte ratio, Clin. Rheumatol. 35 (2016) 701–708, https://doi.org/10.1007/s10067-015-2907-0.

[33] A. Carletto, M.L. Pacor, D. Biasi, P. Caramaschi, S. Zeminian, P. Bellavite, L. M. Bambara, Changes of neutrophil migration without modification of in vitro metabolism and adhesion in Behçet's disease, J. Rheumatol. 24 (1997) 1332–1336.

[34] J.N. Fordham, P.G. Davies, A. Kirk, H.L.F. Currey, Polymorphonuclear function in Behçet's syndrome, 2023.

[35] Y. Mizushima, Recent research into Behçet's disease in Japan, Int. J. Tissue React. 10 (1988) 59–65.

[36] J. Efthimiou, I.E. Addison, B.V. Johnson, In vivo leucocyte migration in Behçet's syndrome, Ann. Rheum. Dis. 48 (1989) 206–210, https://doi.org/10.1136/ard.48.3.206.

[37] T. Ergun, O. Gürbüz, J. Harvell, J. Jorizzo, W. White, The histopathology of pathergy: a chronologic study of skin hyperreactivity in Behçet's disease, Int. J. Dermatol. 37 (1998) 929–933, https://doi.org/10.1046/j.1365-4362.1998.00474.x.

[38] S. Sahin, T. Akoğlu, H. Direskeneli, L.S. Sen, R. Lawrence, Neutrophil adhesion to endothelial cells and factors affecting adhesion in patients with Behçet's disease, Ann. Rheum. Dis. 55 (1996) 128–133.

[39] F.S. Neves, S. Carrasco, C. Goldenstein-Schainberg, C.R. Gonçalves, S.B.V. de Mello, Neutrophil hyperchemotaxis in Behçet's disease: a possible role for monocytes orchestrating bacterial-induced innate immune responses, Clin. Rheumatol. 28 (2009) 1403–1410, https://doi.org/10.1007/s10067-009-1261-5.

[40] S.S. Kartı, E. Ovalı, S. Ratip, M. Çetiner, H. Direskeneli, M. Bayık, T. Akoğlu, Effect of interferon-α2a on neutrophil adhesion and phagocytosis in chronic myeloid leukemia and Behçet's disease, Clin. Rheumatol. 21 (2002) 211–214, https://doi.org/10.1007/s10067-002-8288-1.

[41] A. Hamzaoui, K. Hamzaoui, A. Chabbou, K. Ayed, Circulating intercellular adhesion molecules in blood and bronchoalveolar lavage in Behçet's disease, Mediat. Inflamm. 4 (1995) 355–358, https://doi.org/10.1155/S0962935195000573.

[42] H. Kaku, H. Mizukawa, I. Kishi, T. Yanagida, G. Inaba, Peripheral leukocyte adhesion molecules in patients of Behçet's disease associated with active ocular lesions, Ryumachi. 34 (1994) 608–615.

[43] N.V. Butta, I. Fernández-Bello, F.J. López-Longo, V. Jiménez-Yuste, Endothelial Dysfunction and Altered Coagulation As Mediators of Thromboembolism in Behçet Disease, Semin. Thromb. Hemost. 41 (2015) 621–628, https://doi.org/10.1055/s-0035-1556727.

[44] H.S. Inaloz, C. Evereklioglu, B. Unal, N. Kirtak, A. Eralp, S.S. Inaloz, The significance of immunohistochemistry in the skin pathergy reaction of patients with Behçet's syndrome, J. Eur. Acad. Dermatol. Venereol. 18 (2004) 56–61, https://doi.org/10.1111/j.1468-3083.2004.00547.x.

[45] K. Ureten, I. Ertenli, M.A. Oztürk, S. Kiraz, A.M. Onat, M. Tuncer, H. Okur, A. Akdogan, S. Apras, M. Calgüneri, Neutrophil CD64 expression in Behçet's disease, J. Rheumatol. 32 (2005) 849–852.

[46] B. Choi, C.-H. Suh, H.-A. Kim, H.M. Sayeed, S. Sohn, The Correlation of CD206, CD209, and Disease Severity in Behçet's Disease with Arthritis, Mediat. Inflamm. 2017 (2017) 7539529, https://doi.org/10.1155/2017/7539529.

[47] A. Le Joncour, P. Régnier, A. Maciejewski-Duval, E. Charles, S. Barete, P. Fouret, M. Rosenzwajg, D. Klatzmann, P. Cacoub, D. Saadoun, Type-4 phosphodiesterase (PDE4) blockade reduces neutrophil activation in Behçet's disease, Arthritis Rheum. (2023), https://doi.org/10.1002/art.42486.

[48] E. Eksioglu-Demiralp, H. Direskeneli, A. Kibaroglu, S. Yavuz, T. Ergun, T. Akoglu, Neutrophil activation in Behçet's disease, Clin. Exp. Rheumatol. 19 (2001) S19–S24.

[49] T. Novak, F. Fortune, L. Bergmeier, I. Khan, E. Hagi-Pavli, Neutrophil elastase and endogenous inhibitors in Behçet's disease saliva, Clin. Exp. Immunol. 202 (2020) 93–105, https://doi.org/10.1111/cei.13483.

[50] O. Değer, A. Orem, N. Akyol, S. Bahadir, S. Yildirmis, Polymorphonuclear leukocyte elastase levels in patients with Behçet's disease, Clin. Chim. Acta 236 (1995) 129–134, https://doi.org/10.1016/0009-8981(95)06033-a.

[51] R. Oliveira, P. Napoleão, J. Banha, E. Paixão, A. Bettencourt, B.M. da Silva, D. Pereira, F. Barcelos, A. Teixeira, J.V. Patto, A.M. Viegas-Crespo, L. Costa, Crosstalk between inflammation, iron metabolism and endothelial function in Behçet's disease, Clin. Hemorheol. Microcirc. 56 (2014) 175–185, https://doi.org/10.3233/CH-131725.

[52] Y.D. Akcay, F.G. Sagin, K. Aksu, G. Keser, E. Taylor, I. Knight, P.G. Winyard, E. Y. Sozmen, A panel of oxidative stress assays does not provide supplementary diagnostic information in Behcet's disease patients, J. Inflamm. (Lond). 9 (2012) 13, https://doi.org/10.1186/1476-9255-9-13.

[53] C. Yazici, K. Köse, M. Caliş, M. DemIr, M. Kirnap, F. Ateş, Increased advanced oxidation protein products in Behçet's disease: a new activity marker? Br. J. Dermatol. 151 (2004) 105–111, https://doi.org/10.1111/j.1365-2133.2004.06003.x.

[54] M.A. Melikoglu, C. Kacar, A. Cort, G. Yucel, M. Aslan, Oxidases and oxygenases in regulation of neutrophil redox pathways in Behçet's disease patients, Journal of Enzyme Inhibition and Medicinal, Chemistry. 27 (2012) 12–17, https://doi.org/10.3109/14756366.2011.572281.

[55] E.C. Han, S.B. Cho, K.J. Ahn, S.H. Oh, J. Kim, D.S. Kim, K.H. Lee, D. Bang, Expression of Pro-inflammatory Protein S100A12 (EN-RAGE) in Behçet's Disease and Its Association with Disease Activity: A Pilot Study, Ann. Dermatol. 23 (2011) 313–320, https://doi.org/10.5021/ad.2011.23.3.313.

[56] H. Kucukkolbasi, S. Kucukkolbasi, H.F. Ayyildiz, R. Dursun, H. Kara, Evaluation of hbetaD-1 and hbetaD-2 levels in saliva of patients with oral mucosal diseases, West Indian Med. J. 62 (2013) 230–238.

[57] J.K. Ahn, J.-W. Hwang, J.-M. Oh, E.-K. Bae, J. Lee, Y.S. Lee, E.-M. Koh, H.-S. Cha, Increased α-defensin-1 expression in Korean patients with Behcet's disease, Joint Bone Spine 78 (2011) 593–597, https://doi.org/10.1016/j.jbspin.2011.01.012.

[58] G. Mumcu, H. Cimilli, Ü. Karacayli, N. Inanc, F. Türe-Özdemir, E. Eksioglu-Demiralp, T. Ergun, H. Direskeneli, Salivary levels of HNP 1–3 are related to oral ulcer activity in Behçet's disease, Int. J. Dermatol. 52 (2013) 1198–1201, https://doi.org/10.1111/j.1365-4632.2012.05504.x.

[59] G. Mumcu, H. Cimilli, U. Karacayli, N. Inanc, F. Ture-Ozdemir, E. Eksioglu-Demiralp, T. Ergun, H. Direskeneli, Salivary levels of antimicrobial peptides Hnp 1–3, Ll-37 and S100 in Behcet's disease, Arch. Oral Biol. 57 (2012) 642–646, https://doi.org/10.1016/j.archoralbio.2011.11.003.

[60] S.F. Perazzio, P.V. Soeiro-Pereira, A.W.S. de Souza, A. Condino-Neto, L.E. C. Andrade, Behçet's disease heterogeneity: cytokine production and oxidative burst of phagocytes are altered in patients with severe manifestations, Clin. Exp. Rheumatol. 33 (2015) S85–S95.

[61] G. Atalay, E. Eksioglu-Demiralp, T. Akoglu, H. Direskeneli, The effects of nitric oxide donors and inhibitors on neutrophil functions in Behçet's disease, Clin. Exp. Rheumatol. 20 (2002) S17–S20.

[62] A. Tekeli, O. Tekeli, O. Ozdemir, In vitro Candidacidal effect of polymorphonuclear neutrophils of Behçet's patients with or without ocular involvement, APMIS. 109 (2001) 108–112, https://doi.org/10.1034/j.1600-0463.2001.d01–111.x.

[63] L. Glennon-Alty, A.P. Hackett, E.A. Chapman, H.L. Wright, Neutrophils and redox stress in the pathogenesis of autoimmune disease, Free Radic. Biol. Med. 125 (2018) 25–35, https://doi.org/10.1016/j.freeradbiomed.2018.03.049.

[64] M. Mittal, M.R. Siddiqui, K. Tran, S.P. Reddy, A.B. Malik, Reactive Oxygen Species in Inflammation and Tissue Injury, Antioxid. Redox Signal. 20 (2014) 1126–1167, https://doi.org/10.1089/ars.2012.5149.

[65] K. Saglam, A.F. Serce, M.I. Yilmaz, F. Bulucu, A. Aydin, C. Akay, A. Sayal, Trace elements and antioxidant enzymes in Behçet's disease, Rheumatol. Int. 22 (2002) 93–96, https://doi.org/10.1007/s00296-002-0195-x.

[66] S. Taysi, B. Demircan, N. Akdeniz, M. Atasoy, R.A. Sari, Oxidant/antioxidant status in men with Behçet's disease, Clin. Rheumatol. 26 (2007) 418–422, https://doi.org/10.1007/s10067-006-0513-x.

[67] T. Yoshida, M. Tanaka, A. Sotomatsu, K. Okamoto, S. Hirai, Serum of Behçet's disease enhances superoxide production of normal neutrophils, Free Radic. Res. 28 (1998) 39–44, https://doi.org/10.3109/10715769809097874.

[68] S.F. Perazzio, P.V. Soeiro-Pereira, V.C. Dos Santos, M.V. de Brito, B. Salu, M.L. V. Oliva, A.M. Stevens, A.W.S. de Souza, H.D. Ochs, T.R. Torgerson, A. Condino-Neto, L.E.C. Andrade, Soluble CD40L is associated with increased oxidative burst and neutrophil extracellular trap release in Behçet's disease, Arthritis Res. Ther. 19 (2017) 235, https://doi.org/10.1186/s13075-017-1443-5.

[69] O. Harzallah, A. Kerkeni, T. Baati, S. Mahjoub, Oxidative stress: correlation with Behçet's disease duration, activity and severity, Eur. J. Intern. Med. 19 (2008) 541–547, https://doi.org/10.1016/j.ejim.2008.08.001.

[70] R.A. Najim, K.E. Sharquie, A.R. Abu-Raghif, Oxidative stress in patients with Behcet's disease: I correlation with severity and clinical parameters, The, J. Dermatol. 34 (2007) 308–314, https://doi.org/10.1111/j.1346-8138.2007.00278.x.

[71] J.P. Freitas, P. Filipe, A. Yousefi, I. Emerit, F.G. Rodrigo, Oxidative stress in adamantiades-Behçet's disease, DRM. 197 (1998) 343–348, https://doi.org/10.1159/000018029.

[72] A. Le Joncour, R. Martos, S. Loyau, N. Lelay, A. Dossier, A. Cazes, P. Fouret, F. Domont, T. Papo, M. Jandrot-Perrus, M.-C. Bouton, P. Cacoub, N. Ajzenberg, D. Saadoun, Y. Boulaftali, Critical role of neutrophil extracellular traps (NETs) in patients with Behcet's disease, Ann. Rheum. Dis. (2019), https://doi.org/10.1136/annrheumdis-2018-214335.

[73] N. Akdeniz, M. Esrefoglu, M.S. Keleş, A. Karakuzu, M. Atasoy, Serum interleukin-2, interleukin-6, tumour necrosis factor-alpha and nitric oxide levels in patients with Behcet's disease, Ann. Acad. Med. Singap. 33 (2004) 596–599.

[74] J.C. Chambers, D.O. Haskard, J.S. Kooner, Vascular endothelial function and oxidative stress mechanisms in patients with Behçet's syndrome, J. Am. Coll. Cardiol. 37 (2001) 517–520, https://doi.org/10.1016/s0735-1097(00)01137-2.

[75] S. Korkmaz, I. Erturan, M. Nazıroğlu, A.C. Uğuz, B. Ciğ, I.S. Övey, Colchicine modulates oxidative stress in serum and neutrophil of patients with Behçet disease through regulation of Ca^{2+} release and antioxidant system, J. Membr. Biol. 244 (2011) 113–120, https://doi.org/10.1007/s00232-011-9404-4.

[76] J.K. Ahn, S. Kim, J. Kim, J. Hwang, K.H. Kim, H.-S. Cha, A comparative metabolomic evaluation of Behcet's disease with arthritis and seronegative arthritis using synovial fluid, PLoS One 10 (2015), e0135856, https://doi.org/10.1371/journal.pone.0135856.

[77] M. Becatti, G. Emmi, E. Silvestri, G. Bruschi, L. Ciucciarelli, D. Squatrito, A. Vaglio, N. Taddei, R. Abbate, L. Emmi, M. Goldoni, C. Fiorillo, D. Prisco, Neutrophil Activation Promotes Fibrinogen Oxidation and Thrombus Formation in Behcet Disease, Circulation. 133 (2016) 302–311, https://doi.org/10.1161/CIRCULATIONAHA.115.017738.

[78] M. Takeno, A. Kariyone, N. Yamashita, M. Takiguchi, Y. Mizushima, H. Kaneoka, T. Sakane, Excessive function of peripheral blood neutrophils from patients with Behçet's disease and from HLA-B51 transgenic mice, Arthritis Rheum. 38 (1995) 426–433, https://doi.org/10.1002/art.1780380321.

[79] Y. Mizushima, T. Matsuda, K. Hoshi, S. Ohno, Induction of Behçet's disease symptoms after dental treatment and streptococcal antigen skin test, J. Rheumatol. 15 (1988) 1029–1030.

[80] M. Saeidinejad, S. Kardash, L. Connell, Behcet's disease and severe inflammatory reaction to 23-valent pneumococcal polysaccharide vaccine: a case report and review of literature, Scott. Med. J. 63 (2018) 119–121, https://doi.org/10.1177/0036933018801215.

[81] T. Hügle, A. Bircher, U.A. Walker, Streptococcal hypersensitivity reloaded: severe inflammatory syndrome in Behcet's disease following 23-valent polysaccharide *Streptococcus pneumoniae* vaccine, Rheumatology (Oxford) 51 (2012) 761–762, https://doi.org/10.1093/rheumatology/ker388.

[82] R. Deniz, Z. Emrence, Y. Yalçınkaya, B.A. Esen, M. İnanç, M.L. Öcal, A. Gül, Improved sensitivity of skin pathergy test with polysaccharide pneumococcal vaccine antigens in the diagnosis of Behçet disease, Rheumatology (Oxford) (2022) keac543, https://doi.org/10.1093/rheumatology/keac543.

[83] J.E. Do, S.Y. Kwon, S. Park, E.-S. Lee, Effects of vitamin D on expression of Toll-like receptors of monocytes from patients with Behcet's disease, Rheumatology (Oxford) 47 (2008) 840–848, https://doi.org/10.1093/rheumatology/ken109.

[84] E. Isogai, H. Isogai, N. Fujii, K. Oguma, Macrophage activation by leptospiral lipopolysaccharide, Zentralblatt Fur Bakteriologie, Inte. J. Med. Microbiol. 273 (1990) 200–208.

[85] S. Yavuz, Y. Elbir, A. Tulunay, E. Eksioglu-Demiralp, H. Direskeneli, Differential expression of toll-like receptor 6 on granulocytes and monocytes implicates the role of microorganisms in Behcet's disease etiopathogenesis, Rheumatol. Int. 28 (2008) 401–406, https://doi.org/10.1007/s00296-007-0470-y.

[86] X. Yu, L. Li, M. Zhang, J. Liu, H. Chen, F. Zhang, W. Zheng, Transcriptional analysis of neutrophils from patients with Behçet's disease reveals activation and chemotaxis of neutrophils, Clin. Immunol. 245 (2022), 109161, https://doi.org/10.1016/j.clim.2022.109161.

[87] M. Keller, Z. Spanou, P. Schaerli, M. Britschgi, N. Yawalkar, M. Seitz, P. M. Villiger, W.J. Pichler, T Cell-Regulated Neutrophilic Inflammation in Autoinflammatory Diseases, J. Immunol. 175 (2005) 7678–7686, https://doi.org/10.4049/jimmunol.175.11.7678.

[88] S.P.K. Durmazlar, G.B. Ulkar, F. Eskioglu, S. Tatlican, A. Mert, A. Akgul, Significance of serum interleukin-8 levels in patients with Behcet's disease: high levels may indicate vascular involvement, Int. J. Dermatol. 48 (2009) 259–264, https://doi.org/10.1111/j.1365-4632.2009.03905.x.

[89] G. Gür-Toy, N. Lenk, B. Yalcin, S. Aksaray, N. Alli, Serum interleukin-8 as a serologic marker of activity in Behçet's disease, Int. J. Dermatol. 44 (2005) 657–660, https://doi.org/10.1111/j.1365-4632.2004.02184.x.

[90] H. Yin, Y. Song, M. Zheng, J. Han, J. Tang, Behcet's Disease With Cerebral Artery Infarction Caused by Cerebral Arteritis as an Early Symptom Only With Elevated Interleukin-8, Front. Neurol. 10 (2019) 1102, https://doi.org/10.3389/fneur.2019.01102.

[91] R. Itoh, T. Takenaka, S. Okitsu-Negishi, K. Matsushima, M. Mizoguchi, Interleukin 8 in Behçet's disease, J. Dermatol. 21 (1994) 397–404, https://doi.org/10.1111/j.1346-8138.1994.tb01762.x.

[92] J. Katsantonis, Y. Adler, C.E. Orfanos, C.C. Zouboulis, Adamantiades-Behçet's disease: serum IL-8 is a more reliable marker for disease activity than C-reactive protein and erythrocyte sedimentation rate, Dermatology. 201 (2000) 37–39, https://doi.org/10.1159/000018426.

[93] Z.M. Akkurt, M. Bozkurt, D. Uçmak, H. Yüksel, H. Uçak, B. Sula, Z. Gürsel Özkurt, M. Yildiz, D. Akdeniz, M. Arica, Serum Cytokine Levels in Behçet's Disease, J. Clin. Lab. Anal. 29 (2015) 317–320, https://doi.org/10.1002/jcla.21772.

[94] T. Kawakami, S. Ohashi, Y. Kawa, H. Takahama, M. Ito, Y. Soma, M. Mizoguchi, Elevated serum granulocyte colony-stimulating factor levels in patients with active phase of sweet syndrome and patients with active behcet disease: implication in neutrophil apoptosis dysfunction, Arch. Dermatol. 140 (2004) 570–574, https://doi.org/10.1001/archderm.140.5.570.

[95] G. Parlakgul, E. Guney, B. Erer, Z. Kılıcaslan, H. Direskeneli, A. Gul, G. Saruhan-Direskeneli, Expression of regulatory receptors on γδ T cells and their cytokine production in Behcet's disease, Arthritis Res Ther. 15 (2013) R15, https://doi.org/10.1186/ar4147.

[96] C.C. Zouboulis, J. Katsantonis, R. Ketteler, R. Treudler, E. Kaklamani, S. Hornemann, P. Kaklamanis, C.E. Orfanos, Adamantiades-Behçet's disease: interleukin-8 is increased in serum of patients with active oral and neurological manifestations and is secreted by small vessel endothelial cells, Arch. Dermatol. Res. 292 (2000) 279–284, https://doi.org/10.1007/s004030000128.

[97] B. Adam, E. Calikoglu, Serum interleukin-6, procalcitonin and C-reactive protein levels in subjects with active Behçet's disease, J. Eur. Acad. Dermatol. Venereol. 18 (2004) 318–320, https://doi.org/10.1111/j.1468-3083.2004.00907.x.

[98] B. Tong, X. Liu, J. Xiao, G. Su, Immunopathogenesis of Behcet's Disease, Front. Immunol. 10 (2019), https://doi.org/10.3389/fimmu.2019.00665.

[99] S. Pay, T. Abbasov, H. Erdem, U. Musabak, I. Simsek, A. Pekel, A. Akdogan, A. Sengul, A. Dinc, Serum MMP-2 and MMP-9 in patients with Behçet's disease: do their higher levels correlate to vasculo-Behçet's disease associated with aneurysm formation? Clin. Exp. Rheumatol. 25 (2007) S70–S75.

[100] K.H.G. Mills, Induction, function and regulation of IL-17-producing T cells, Eur. J. Immunol. 38 (2008) 2636–2649, https://doi.org/10.1002/eji.200838535.

[101] L. Roussel, F. Houle, C. Chan, Y. Yao, J. Bérubé, R. Olivenstein, J.G. Martin, J. Huot, Q. Hamid, L. Ferri, S. Rousseau, IL-17 promotes p38 MAPK-dependent endothelial activation enhancing neutrophil recruitment to sites of inflammation, J. Immunol. 184 (2010) 4531–4537, https://doi.org/10.4049/jimmunol.0903162.

[102] K.L. Flannigan, V.L. Ngo, D. Geem, A. Harusato, S.A. Hirota, C.A. Parkos, N. W. Lukacs, A. Nusrat, V. Gaboriau-Routhiau, N. Cerf-Bensussan, A.T. Gewirtz, T. L. Denning, IL-17A-mediated neutrophil recruitment limits expansion of segmented filamentous bacteria, Mucosal Immunol. 10 (2017) 673–684, https://doi.org/10.1038/mi.2016.80.

[103] G. Geri, B. Terrier, M. Rosenzwajg, B. Wechsler, M. Touzot, D. Seilhean, T.-A. Tran, B. Bodaghi, L. Musset, V. Soumelis, D. Klatzmann, P. Cacoub, D. Saadoun, Critical role of IL-21 in modulating TH17 and regulatory T cells in Behçet disease, J. Allergy Clin. Immunol. 128 (2011) 655–664, https://doi.org/10.1016/j.jaci.2011.05.029.

[104] H. Direskeneli, H. Fujita, C.A. Akdis, Regulation of TH17 and regulatory T cells in patients with Behçet disease, J. Allergy Clin. Immunol. 128 (2011) 665–666, https://doi.org/10.1016/j.jaci.2011.07.008.

[105] W. Chi, S. Zhou, P. Yang, L. Chen, $CD4^+$ T cells from behcet patients produce high levels of IL-17, Eye Sci. 26 (2011) 65–69, https://doi.org/10.3969/j.issn.1000-4432.2011.02.013.

[106] K. Hamzaoui, A. Hamzaoui, F. Guemira, M. Bessioud, M. Hamza, K. Ayed, Cytokine profile in Behçet's disease patients. Relationship with disease activity, Scand. J. Rheumatol. 31 (2002) 205–210.

[107] A. Gürgey, A. Gurler, A.F. Oner, L. Mesci, S. Kirazli, Thrombomodulin levels in Behçet's disease with and without the factor V Leiden mutation, Clin. Rheumatol. 17 (1998) 186–188, https://doi.org/10.1007/BF01451044.

[108] G. Triolo, A. Accardo-Palumbo, G. Triolo, M.C. Carbone, A. Ferrante, E. Giardina, Enhancement of endothelial cell E-selectin expression by sera from patients with active Behçet's disease: moderate correlation with anti-endothelial cell antibodies and serum myeloperoxidase levels, Clin. Immunol. 91 (1999) 330–337, https://doi.org/10.1006/clim.1999.4687.

[109] Y. Turkoz, C. Evereklioglu, A. Ozkiriş, S. Mistik, M. Borlu, I.H. Ozerol, F. Duygulu, O. Ilhan, Serum levels of soluble P-selectin are increased and associated with disease activity in patients with Behçet's syndrome, Mediat. Inflamm. 2005 (2005) 237–241, https://doi.org/10.1155/MI.2005.237.

[110] R.A. Sari, A. Kiziltunç, S. Tays'y, S. Akdem'yr, M. Gündoğdu, Levels of soluble E-selectin in patients with active Behçet's disease, Clin. Rheumatol. 24 (2005) 55–59, https://doi.org/10.1007/s10067-004-0982-8.

[111] M. Caliskan, S. Yilmaz, E. Yildirim, H. Gullu, D. Erdogan, O. Ciftci, G. Kaynar, E. Yucel, H. Muderrisoglu, Endothelial functions are more severely impaired during active disease period in patients with Behcet's disease, Clin. Rheumatol. 26 (2007) 1074–1078, https://doi.org/10.1007/s10067-006-0449-1.

[112] H. Yapişlar, S. Aydogan, M. Borlu, O. Ascioglu, Decreased nitric oxide and increased platelet aggregation levels in patients with Behçet's disease, Thromb. Res. 119 (2007) 461–465, https://doi.org/10.1016/j.thromres.2006.02.008.

[113] S. Akar, M.A. Ozcan, H. Ateş, O. Gürler, I. Alacacioglu, G.H. Ozsan, N. Akkoç, S. Ozkan, F. Demirkan, F. Onen, Circulated activated platelets and increased platelet reactivity in patients with Behçet's disease, Clin. Appl. Thromb. Hemost. 12 (2006) 451–457, https://doi.org/10.1177/1076029606293430.

[114] M. Macey, E. Hagi-Pavli, J. Stewart, G.R. Wallace, M. Stanford, P. Shirlaw, F. Fortune, Age, gender and disease-related platelet and neutrophil activation ex vivo in whole blood samples from patients with Behçet's disease, Rheumatology (Oxford) 50 (2011) 1849–1859, https://doi.org/10.1093/rheumatology/ker177.

[115] A.P. Wilson, J. Efthimiou, D.J. Betteridge, Decreased prostacyclin sensitivity of platelets in patients with Behçet's syndrome, Eur. J. Clin. Investig. 18 (1988) 410–414, https://doi.org/10.1111/j.1365-2362.1988.tb01032.x.

[116] I. Fernández Bello, M.T. Álvarez, F.J. López-Longo, E.G. Arias-Salgado, M. Martín, V. Jiménez-Yuste, A. Rodríguez de la Rúa, N.V. Butta, Platelet soluble CD40L and matrix metalloproteinase 9 activity are proinflammatory mediators in Behçet disease patients, Thromb. Haemost. 107 (2012) 88–98, https://doi.org/10.1160/TH11-08-0556.

[117] M. Takeno, The association of Behçet's syndrome with HLA-B51 as understood in 2021, Curr. Opin. Rheumatol. 34 (2022) 4–9, https://doi.org/10.1097/BOR.0000000000000846.

[118] S. Yavuz, G. Ozilhan, Y. Elbir, A. Tolunay, E. Eksioglu-Demiralp, H. Direskeneli, Activation of neutrophils by testosterone in Behçet's disease, Clin. Exp. Rheumatol. 25 (2007) S46–S51.

[119] V. Brinkmann, U. Reichard, C. Goosmann, B. Fauler, Y. Uhlemann, D.S. Weiss, Y. Weinrauch, A. Zychlinsky, Neutrophil extracellular traps kill bacteria, Science. 303 (2004) 1532–1535, https://doi.org/10.1126/science.1092385.

[120] O.E. Sorensen, N. Borregaard, Neutrophil extracellular traps - the dark side of neutrophils, J. Clin. Invest. 126 (2016) 1612–1620, https://doi.org/10.1172/JCI84538.

[121] K. Kessenbrock, M. Krumbholz, U. Schönermarck, W. Back, W.L. Gross, Z. Werb, H.-J. Gröne, V. Brinkmann, D.E. Jenne, Netting neutrophils in autoimmune small-vessel vasculitis, Nat. Med. 15 (2009) 623–625, https://doi.org/10.1038/nm.1959.

[122] A.M. Abreu-Velez, J.G. Smith, M.S. Howard, Presence of neutrophil extracellular traps and antineutrophil cytoplasmic antibodies associated with vasculitides, N. Am. J. Med. Sci. 1 (2009) 309–313.

[123] K. Martinod, D.D. Wagner, Thrombosis: tangled up in NETs, Blood. 123 (2014) 2768–2776.

[124] C. Ducroux, L. Di Meglio, S. Loyau, S. Delbosc, W. Boisseau, C. Deschildre, M. Ben Maacha, R. Blanc, H. Redjem, G. Ciccio, S. Smajda, R. Fahed, J.-B. Michel, M. Piotin, L. Salomon, M. Mazighi, B. Ho-Tin-Noe, J.-P. Desilles, Thrombus neutrophil extracellular traps content impair tPA-induced thrombolysis in acute ischemic stroke, Stroke 49 (2018) 754–757, https://doi.org/10.1161/STROKEAHA.117.019896.

[125] E. Laridan, F. Denorme, L. Desender, O. François, T. Andersson, H. Deckmyn, K. Vanhoorelbeke, S.F. De Meyer, Neutrophil extracellular traps in ischemic stroke thrombi, Ann. Neurol. 82 (2017) 223–232, https://doi.org/10.1002/ana.24993.

[126] R. Safi, R. Kallas, T. Bardawil, C.J. Mehanna, O. Abbas, R. Hamam, I. Uthman, A.-G. Kibbi, D. Nassar, Neutrophils contribute to vasculitis by increased release of neutrophil extracellular traps in Behçet's disease, J. Dermatol. Sci. 92 (2018) 143–150, https://doi.org/10.1016/j.jdermsci.2018.08.010.

[127] A. Bettiol, M. Becatti, E. Silvestri, F.R. Argento, E. Fini, A. Mannucci, S. Galora, I. Mattioli, M.L. Urban, D. Malandrino, A. Palermo, N. Taddei, G. Emmi, D. Prisco, C. Fiorillo, Neutrophil-mediated mechanisms of damage and in-vitro protective effect of colchicine in non-vascular Behçet's syndrome, Clin. Exp. Immunol. (2021), https://doi.org/10.1111/cei.13664.

[128] L. Li, X. Yu, J. Liu, Z. Wang, C. Li, J. Shi, L. Sun, Y. Liu, F. Zhang, H. Chen, W. Zheng, Neutrophil extracellular traps promote aberrant macrophages activation in Behçet's disease, Front. Immunol. 11 (2020), 590622, https://doi.org/10.3389/fimmu.2020.590622.

[129] A. Bettiol, F. Alibaz-Oner, H. Direskeneli, G. Hatemi, D. Saadoun, E. Seyahi, D. Prisco, G. Emmi, Vascular Behçet syndrome: from pathogenesis to treatment, Nat. Rev. Rheumatol. 19 (2023) 111–126, https://doi.org/10.1038/s41584-022-00880-7.

[130] G. Hatemi, R. Christensen, D. Bang, B. Bodaghi, A.F. Celik, F. Fortune, J. Gaudric, A. Gul, I. Kötter, P. Leccese, A. Mahr, R. Moots, Y. Ozguler, J. Richter, D. Saadoun, C. Salvarani, F. Scuderi, P.P. Sfikakis, A. Siva, M. Stanford, I. Tugal-Tutkun, R. West, S. Yurdakul, I. Olivieri, H. Yazici, update of the EULAR recommendations for the management of Behçet's syndrome, Ann. Rheum. Dis. 77 (2018) (2018) 808–818, https://doi.org/10.1136/annrheumdis-2018-213225.

[131] G. Emmi, M. Becatti, A. Bettiol, G. Hatemi, D. Prisco, C. Fiorillo, Behçet's syndrome as a model of thrombo-inflammation: the role of neutrophils, Front. Immunol. 10 (2019) 1085, https://doi.org/10.3389/fimmu.2019.01085.

[132] R. Palankar, A. Greinacher, Challenging the concept of immunothrombosis, Blood. 133 (2019) 508–509, https://doi.org/10.1182/blood-2018-11-886267.

[133] M. Becatti, G. Emmi, A. Bettiol, E. Silvestri, G. Di Scala, N. Taddei, D. Prisco, C. Fiorillo, Behçet's syndrome as a tool to dissect the mechanisms of thrombo-inflammation: clinical and pathogenetic aspects, Clin. Exp. Immunol. 195 (2019) 322–333, https://doi.org/10.1111/cei.13243.

[134] H. Zhang, Y. Wang, M. Qu, W. Li, D. Wu, J.P. Cata, C. Miao, Neutrophil, neutrophil extracellular traps and endothelial cell dysfunction in sepsis, Clin. Transl. Med. 13 (2023), e1170, https://doi.org/10.1002/ctm2.1170.

[135] F. Berteau, B. Rouviere, A. Delluc, A. Nau, R. Le Berre, G. Sarrabay, I. Touitou, C. de Moreuil, Autosomic dominant familial Behçet disease and haploinsufficiency A20: A review of the literature, Autoimmun. Rev. 17 (2018) 809–815, https://doi.org/10.1016/j.autrev.2018.02.012.

[136] J.L. Jimenez, C. Punzón, J. Navarro, M.A. Muñoz-Fernández, M. Fresno, Phosphodiesterase 4 inhibitors prevent cytokine secretion by T lymphocytes by inhibiting nuclear factor-kappaB and nuclear factor of activated T cells activation, J. Pharmacol. Exp. Ther. 299 (2001) 753–759.

[137] E.D. Deeks, Apremilast: a review in oral ulcers of Behçet's disease, Drugs. 80 (2020) 181–188, https://doi.org/10.1007/s40265-019-01253-3.

[138] P.H. Schafer, A. Parton, L. Capone, D. Cedzik, H. Brady, J.F. Evans, H.-W. Man, G. W. Muller, D.I. Stirling, R. Chopra, Apremilast is a selective PDE4 inhibitor with regulatory effects on innate immunity, Cell. Signal. 26 (2014) 2016–2029, https://doi.org/10.1016/j.cellsig.2014.05.014.

[139] G. Hatemi, M. Melikoglu, R. Tunc, C. Korkmaz, B. Turgut Ozturk, C. Mat, P. A. Merkel, K.T. Calamia, Z. Liu, L. Pineda, R.M. Stevens, H. Yazici, Y. Yazici, Apremilast for Behçet's syndrome–a phase 2, placebo-controlled study, N. Engl. J. Med. 372 (2015) 1510–1518, https://doi.org/10.1056/NEJMoa1408684.

[140] G. Hatemi, A. Mahr, Y. Ishigatsubo, Y.-W. Song, M. Takeno, D. Kim, M. Melikoğlu, S. Cheng, S. McCue, M. Paris, M. Chen, Y. Yazici, Trial of Apremilast for Oral Ulcers in Behçet's Syndrome, N. Engl. J. Med. 381 (2019) 1918–1928, https://doi.org/10.1056/NEJMoa1816594.

[141] S.F. Perazzio, E.J. Allenspach, K.K. Eklund, M. Varjosalo, M.M. Shinohara, T. R. Torgerson, M.R.J. Seppänen, Behçet disease (BD) and BD-like clinical phenotypes: NF-kB pathway in mucosal ulcerating diseases, Scand. J. Immunol. (2020), e12973, https://doi.org/10.1111/sji.12973.

[142] V. Km, V. Ni, Giannis Ampatziadis-Michailidis, Panagiotis Moulos, Georgios A. Pavlopoulos, Pantelis Hatzis, George Kollias, Petros P. Sfikakis, Distinct transcriptional profile of blood mononuclear cells in Behçet's disease: insights into the central role of neutrophil chemotaxis, Rheumatology (Oxford, England) 60 (2021), https://doi.org/10.1093/rheumatology/keab052.

[143] C. Angelidis, Z. Kotsialou, C. Kossyvakis, A.-R. Vrettou, A. Zacharoulis, F. Kolokathis, V. Kekeris, G. Giannopoulos, Colchicine pharmacokinetics and mechanism of action, Curr. Pharm. Des. 24 (2018) 659–663, https://doi.org/10.2174/1381612824666180123110042.

[144] S. Ronchetti, E. Ricci, G. Migliorati, M. Gentili, C. Riccardi, How glucocorticoids affect the neutrophil life, Int. J. Mol. Sci. 19 (2018), https://doi.org/10.3390/ijms19124090.

[145] F.J. van Overveld, U.A. Demkow, D. Górecka, J. Zielinski, W.A. De Backer, Inhibitory capacity of different steroids on neutrophil migration across a bilayer

of endothelial and bronchial epithelial cells, Eur. J. Pharmacol. 477 (2003) 261–267, https://doi.org/10.1016/s0014-2999(03)02153-8.

[146] C. Zhang, W. Shu, G. Zhou, J. Lin, F. Chu, H. Wu, Z. Liu, Anti-TNF-α therapy suppresses proinflammatory activities of mucosal neutrophils in inflammatory bowel disease, Mediat. Inflamm. 2018 (2018) 3021863, https://doi.org/10.1155/2018/3021863.

[147] H.M. Al-Kuraishy, A.I. Al-Gareeb, H.A. Al-hussaniy, N.A.H. Al-Harcan, A. Alexiou, G.E.-S. Batiha, Neutrophil Extracellular Traps (NETs) and Covid-19: A new frontiers for therapeutic modality, Int. Immunopharmacol. 104 (2022), 108516, https://doi.org/10.1016/j.intimp.2021.108516.

[148] Z.W. Lai, R. Hanczko, E. Bonilla, T.N. Caza, B. Clair, A. Bartos, G. Miklossy, J. Jimah, E. Doherty, H. Tily, L. Francis, R. Garcia, M. Dawood, J. Yu, I. Ramos, I. Coman, S.V. Faraone, P.E. Phillips, A. Perl, N-acetylcysteine reduces disease activity by blocking mammalian target of rapamycin in T cells from systemic lupus erythematosus patients: a randomized, double-blind, placebo-controlled trial, Arthritis Rheum. 64 (2012) 2937–2946, https://doi.org/10.1002/art.34502.

[149] V.C. Willis, A.M. Gizinski, N.K. Banda, C.P. Causey, B. Knuckley, K.N. Cordova, Y. Luo, B. Levitt, M. Glogowska, P. Chandra, L. Kulik, W.H. Robinson, W.P. Arend, P.R. Thompson, V.M. Holers, N-alpha-benzoyl-N5-(2-chloro-1-iminoethyl)-L-ornithine amide, a protein arginine deiminase inhibitor, reduces the severity of murine collagen-induced arthritis, J. Immunol. 186 (2011) 4396–4404, https://doi.org/10.4049/jimmunol.1001620.

[150] S. Gupta, M.J. Kaplan, The role of neutrophils and NETosis in autoimmune and renal diseases, Nat. Rev. Nephrol. 12 (2016) 402–413, https://doi.org/10.1038/nrneph.2016.71.