Interleukin-1 Inhibition in Behçet's disease

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ABSTRACT: Behçet's disease (BD) is a systemic inflammatory disorder characterized by a protean clinical spectrum and an enigmatic pathogenesis. After being classified as an autoimmune disorder, spondyloarthritis and vasculitis, today BD is considered at the crossroad between autoimmune and auto-inflammatory syndromes. Many pathogenetic, clinical and therapeutic clues support this recent interpretation, enabling novel treatment choices such as interleukin (IL)-1 inhibition. Thus, in the last decade the IL-1 receptor antagonist anakinra and the anti-IL-1β monoclonal antibody canakinumab were increasingly administered in BD patients resistant to standard therapies, leading to interesting results and intriguing new pathogenetic implications. However, further studies are essential to both establish how the innate and acquired immune systems interact in BD patients and identify the best way of administering anti-IL-1 agents with regard to dosage, interval of administration, and organ response.

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Behçet's disease (BD) is a systemic inflammatory disorder affecting a wide range of organs and tissues. In particular, skin lesions, arthritis, venous thrombosis, arterial aneurysms, intestinal ulcers, pulmonary lesions, and central nervous system manifestations represent potential clinical signs of BD, in addition to the originally described triad of recurrent oral aphthosis, genital ulcers and uveitis [1]. Although BD is generally not a life-threatening condition, mortality can be associated with vascular-thrombotic and neurological manifestations [2].

To date, the pathogenesis of BD is mostly unknown. However, after being repeatedly classified, BD is now regarded at the crossroad between autoimmune and auto-inflammatory syndromes. The current definition of auto-inflammatory diseases identifies a wide number of conditions manifesting with seemingly unprovoked episodes of recurrent inflammation [3].

This notion was first proposed on the basis of clinical observations identifying BD as an inflammatory disorder characterized by a relapsing-remitting disease course with a lack of autoantibodies or autoreactive antigen-specific T cells, as in monogenic auto-inflammatory syndromes (MAIS) [4,5]. Many efforts have been made in the last decade to discover pathogenetic connections and overlapping pathways between BD and MAIS, thus considerably expanding the treatment options for BD. With regard to children, while management recommendations are available for other vasculitic syndromes, such as Kawasaki disease [6], neither therapeutic paradigms nor evidence-based guidelines are available for BD occurring in childhood. However, on the basis of a new pathogenetic interpretation of BD, further treatment tools beyond anti-tumor necrosis factor (TNF) therapies have recently been suggested [7]. In particular, interleukin (IL)-1β inhibition, representing the cornerstone of therapy in adult and pediatric patients diagnosed with different MAIS, was recently investigated as a potential option for BD resistant to standard treatment [3].

This review will present current evidence on the role of innate immunity in BD, including pathogenetic and clinical findings along with the newest findings on IL-1 inhibition as an effective treatment approach for BD patients.

PATHOGENETIC EVIDENCE

Although BD pathogenesis is still not completely known, neutrophils are deemed to be central in BD, and increased production of IL-8, TNFα and IL-1 from lymphomononuclear and/or endothelial cells contribute to neutrophil activation in these patients. More specifically, neutrophils seem to be activated by a particular subset of IL-8-producing T cells that secrete TNFα, which in turn induces the production of other pro-inflammatory cytokines such as IL-1 [8]. As a result, the role of IL-1 as a pivotal cytokine in BD pathogenesis is considered a crucial clue for classifying BD among polygenic auto-inflammatory disorders. In particular, IL-1 was found to be significantly higher in patients with both active and inactive BD compared to healthy controls. Notably, lipopolysaccharide-induced production of IL-1 by monocytes was even higher in BD patients compared to patients with familial Mediterranean fever (FMF),

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the most important among MAIS [9]. Furthermore, IL-33, a recently identified member of the IL-1 family involved in innate immunity, was increased in the sera of BD patients along with its soluble ligand sST2. Moreover, serum sST2 level significantly correlated with BD activity, evaluated with the BD currently active form (BDCAF), Iranian BD dynamic activity measure (IBDDAM), erythrocyte sedimentation rate and C-reactive protein. In addition, both IL-33 and sST2 were highly expressed in the epidermis and dermis of patients with BD compared to healthy controls [10].

Noteworthy, single nuclear polymorphisms of IL-1 gene were also found to be significantly more represented in patients with BD in different ethnic groups. In particular, susceptibility to BD was increased in individuals carrying the IL-1β+3953 T allele and TT genotype, the IL-1α -889C and IL-1β +5887T haplotypes as well as the IL-1β-511 and IL-1β + 3962C alleles [11].

In order to identify any relationship with innate immunity in BD pathogenesis, the NACHT, LRR and PYD domains containing protein 3 (NLRP3) inflammasome was recently investigated in BD patients. NLRP3 inflammasome is an intracellular multiprotein complex leading to the secretion of inflammatory cytokines via caspase-1 activation. In particular, the NLRP3 inflammasome leads to the active secretion of IL-1β and is closely involved in the pathogenesis of MAIS, in particular FMF and cryopyrin-associated periodic syndrome (CAPS) [12]. Regarding BD, mRNA and protein levels of NLRP3 inflammasome components were recently found to be significantly increased in peripheral blood mononuclear cells of BD patients with skin manifestations, compared to healthy controls, before and after lipopolysaccharide stimulation. Similarly, increased expression of the NLRP3 inflammasome was observed in BD skin lesions compared to tissues obtained from patients with erythema nodosum. Noteworthy, in the same study IL-1β secretion in BD patients appeared to be related to NLRP3 inflammasome activation [13]. In this regard, another interesting study suggested IL-1β production by monocyte-derived macrophages through activation of the NLRP3 inflammasome by reactive oxygen species (ROS) resulting from mitochondria [14].

Interestingly, toll-like receptors (TLRs) 2, 4 and 9 were shown to be involved in the pathogenesis of BD [14,15]. These findings are intriguing, as TLRs represent a family of pattern recognition receptors involved in both innate and acquired immunity. In particular, TLR2 and TLR4, acting as signaling receptors activated by bacterial wall components, seem to up-regulate IL-1β production through a ROS-NLRP3 inflammasome pathway in active BD patients, implicating innate immunity mechanisms in BD pathogenesis [14,16]. In addition, genotype analysis revealed that some TLR-4 and TLR-9 gene polymorphisms are significantly more frequent in BD patients than in healthy controls in Japanese and/or Turkish populations. Notably TLR-9, recognizing a component of bacterial DNA, was previously reported to be involved in other autoimmune/auto-inflammatory disorders, such as poly-dermatomyositis and Crohn's disease [15].

A further important clue to the auto-inflammatory hypothesis is based on the expression of the P2X7 receptor (P2X7r) in monocytes from BD patients. The P2X7r is an ion channel triggered by adenosine triphosphate released by damaged cells. It plays a key role in promoting the release of pro-inflammatory cytokines, especially IL-1β, by inducing a massive potassium efflux that in turn accelerates the assembling of the NLRP3 inflammasome, and then the maturation of pro-IL-1β. With regard to P2X7r, we found that BD patients display a higher monocyte surface expression and a higher sensitivity to stimulation in terms of calcium permeability, when compared with healthy controls. In addition, monocytes from BD patients showed increased P2X7r-dependent IL-1β secretion. These results suggest a possible role of the innate immunity activation-dependent inflammatory response in BD, supporting the view that IL-1 may be of pivotal pathogenic relevance [17].

Interestingly, some researchers recently suggested that decreased expression of C-type lectin domain family 12, member A (CLEC12A), is a common denominator in the hyperinflammatory responses in both BD and gout, another established polygenic auto-inflammatory disorder. In particular, CLEC12A is a pattern-recognition receptor of innate immunity working as a negative regulator of inflammation by inhibiting granulocyte and monocyte/macrophage functions. Specifically, CLEC12A is capable of recognizing monosodium urate crystals, a well-known stimulus for NLRP3-inflammasome. In this regard, beyond gout, BD is currently the only inflammatory condition exhibiting an increased response to monosodium urate crystal. However, further studies are needed to better establish the role of CLEC12A receptor in BD [18].

Table 1 summarizes current knowledge on the innate immune system in BD pathogenesis, distinguishing immune components in cells, cytokines, intracellular components, and surface receptors.

CLINICAL EVIDENCE

The auto-inflammatory hypothesis was first proposed on the basis of clinical observations. In particular, many similarities have been highlighted between BD and MAIS, including FMF, hyper-immunoglobulinemia D syndrome (HIDS), TNF receptor-associated periodic syndrome (TRAPS), CAPS, Blau syndrome, pyogenic arthritis, and pyoderma gangrenosum and acne (PAPA) syndrome. Specifically, bipolar aphthosis is often found in patients with HIDS, uveitis is also described in patients with Blau syndrome and severe CAPS, and BD skin manifestations are similar to those of PAPA syndrome. Furthermore, an association between acneiform skin lesions and arthritis has been documented in BD. Notably, the pathergy phenomenon has been described for both BD and PAPA syndrome [5].

A further clue suggesting a relationship between BD and

Table 1. Current pathogenetic evidence on the innate immune system involvement in Behçet's disease

Innate immunity components	Players	Involvement in BD
Cells	Neutrophils	Activation by a subset of IL-8-producing T cells [8]
	Macrophages	Production of IL-1β through activation of the NLRP3 inflammasome by ROS [14]
	Monocytes	Increased P2X7r-dependent IL-1β secretion in BD [17]
Cytokines	IL-1	Significantly higher in patients with both active and inactive BD compared to healthy controls [9]
		Polymorphism of IL-1 gene significantly more represented in patients with BD [11]
	IL-33	Increased in serum from BD patients along with its soluble ligand sST2 [10]
		Soluble ligand correlates with BD activity [10]
		Highly expressed in the epidermis and dermis of patients with BD [10]
Intracellular proteins	NLRP3 inflammasome	NLRP3 components significantly increased in peripheral blood mononuclear cells from BD patients and in BD skin lesions, compared to healthy controls and erythema nodosum patients, respectively [13]
		IL-1β secretion in BD related to NLRP3 inflammasome activation [13,14]
		IL-1β production by monocyte-derived macrophages via *NLRP3* inflammasome induced by ROS [14]
Surface receptors	TLR	TLR-2 and -4 upregulate IL-1β production through a ROS-*NLRP3* inflammasome pathway [14,16]
		TLR-4 and TLR-9 gene polymorphisms significantly more frequent in BD patients than healthy controls [15]
	P2X7r	Higher monocyte surface expression in BD than healthy controls [17]
		Higher sensitivity to stimulation when compared to healthy controls [17]
		Increased P2X7r-dependent IL-1β secretion in BD [17]

BD = Behçet's disease, IL = interleukin, P2X7r = P2X7 receptor, ROS = reactive oxygen species, TLR = Toll-like receptors, NLRP3 = NACHT, LRR, and PYD domains-containing protein 3

MAIS is the coexistence of BD and FMF in some patients [4,19]. Notably, a retrospective study of 4000 FMF patients found that 16 patients also met the International Study Group (ISG) diagnostic criteria for BD. The relatively high prevalence of BD among FMF patients suggests that FMF and BD occur together more commonly than expected [4]. In addition, the hypothesis that FMF may be induced in subjects with a FMF-related mutation and coexistent inflammatory conditions, such as BD, was also proposed [19]. These exciting observations seem to be corroborated by epidemiologic data showing an overlapping geographic distribution for both clinical entities [1-3]. Interestingly, frequencies of FMF-related mutations in BD patients were found in up to 40.4% of cases, and positivity of single mutations seems to be more frequent in BD patients with vascular involvement and thrombosis. However, other authors did not find consistent results and suggested that BD and FMF merely represent two separate entities with no mutual effect and a mild trend toward a higher than expected association. Thus, the question remains unresolved [20].

Regarding TRAPS, the low penetrance mutation R92Q has been associated with an increased risk of extracranial venous thrombosis in patients diagnosed with BD [21]. Similarly, mutations responsible for HIDS have been described in patients with typical BD features, but no significant increase of HIDS, CAPS or PAPA syndrome-related mutations was found in patients with BD when compared with controls [22]. Whether BD susceptibility genes are involved in the MAIS inflammatory pathways is still a matter of debate and research.

THERAPEUTIC EVIDENCE

The recently reported impressive clinical response to IL-1 inhibition in BD patients is a further clue supporting the concept that IL-1 plays a pathological role in this disease [23-39]. To date, three anti-IL-1 agents have been tested in BD: the IL-1 receptor antagonist anakinra, the anti-IL-1β monoclonal antibody canakinumab, and the recombinant humanized anti-IL-1β gevokizumab. Anakinra was first used by Botsios and colleagues [23] in 2008 as a monotherapy in a resistant BD patient, leading to a prompt and sustained response [23]. Two years later, anakinra proved beneficial in a teenage patient with FMF and BD complicated by secondary amyloidosis. In this case, treatment was effective in arresting clinical and laboratory inflammation, though proteinuria continued to deteriorate [24]. In the subsequent years an increasing number of case reports described the efficacy of anakinra in patients with protean BD clinical manifestations [25-28]. More recently, a survey on BD patients resistant to standard therapies showed that eight of nine patients responded to anakinra within 1–2 weeks. However, most patients experienced a clinical relapse over time, and only one patient showed a complete response to anakinra treatment. However, responses to anakinra were on the whole effective, and anakinra was therefore continued in

Table 2. Overview of studies reporting patients with Behçet's disease treated with anakinra

Autors [ref]	No. of patients	Main clinical involvement	Dosage	Results
Botsios et al. [23]	1	Mucocutaneous, gastrointestinal, fever	100 mg/day	Complete remission in 7–10 days
Bilginer et al. [24]	1	Mucocutaneous, articular, fever, amyloidosis	1 mg/kg/day	Complete remission during 18 months follow-up; amyloidosis-related proteinuria increased
Emmi et al. [25]	1	Mucocutaneous, ocular, gastrointestinal, articular	100 mg/day	Complete remission during 12 months follow-up
Caso et al. [26]	1	Mucocutaneous, ocular, articular	100 mg/day	Complete remission in a few days
Emmi et al. [28]	2	Mucocutaneous, gastrointestinal, articular (1/2)	100 mg/day	First patient: complete remission during 8 months follow-up; second patient: data not yet available
Cantarini et al. [29]	9	Ocular (5/9), cutaneous (4/9), articular (6/9), fever (5/9)	Up to 150 mg/day (adults), up to 2.5 mg/kg/day (children)	Eight of 9 patients showed prompt improvement after starting anakinra; 7 patients had a relapse in a mean period of 29 weeks
Caso et al. [30]	1	Mucocutaneous, musculoskeletal, ocular; the patient complained also of pemphigus	100 mg/day	Partial improvement lasting for only 20 days
Vitale et al. [31]	1	Mucocutaneous, ocular, articular, neurological, fever	100 mg/day	Complete remission during 18 months follow-up; ocular relapse after 18 months
Pagnini et al. [35]	1	Mucocutaneous, ocular, fever	4 mg/kg/day	Partial response; a switch to canakinumab was needed after 19 months of therapy
Emmi et al. [36]	27	Mucocutaneous, ocular, neurological, gastroenteric, vascular, articular	100 mg/day	The median time of response to anakinra was 6 weeks; adherence to treatment in 75th percentile of 2.0 months and median 6.0 months, with overall cumulative treatment survival of 26.3% at 24 months. No adverse events recorded. Six patients shifted from anakinra to canakinumab

most patients for a mean period of 13 months. Notably, increasing the dose of anakinra led to good clinical responses when the effective standard dose was unsuccessful. Conversely, a less remarkable response was observed for mucocutaneous manifestations [29]. Nonetheless, anakinra ineffectiveness was also described in BD patients [29-31]. For such patients the anti-IL-1β agent canakinumab can be a useful therapeutic choice [29,32-37]. In 2012 two case reports described canakinumab as an effective treatment in counteracting BD stigmata and normalizing all markers of inflammation at a dosage of 150 mg every 8 weeks [32,33]. In 2014, we reported three additional patients with complete remission during a 6–12 month follow-up. However, canakinumab led to better results when administered every 6 weeks [34]. Moreover, canakinumab at a dose of 4 mg/kg every 28 days was proven useful and safe also when administered in children with BD [35].

More recently, we performed a multicenter retrospective study aimed at evaluating the efficacy of both anakinra and canakinumab in a cohort of 30 BD patients. In accordance with previously reported data, our results showed that BD patients with an initial low response to anakinra recovered completely following an increase in the drug dose. Alternatively, switching to canakinumab after anakinra failure represented another therapeutic opportunity for refractory patients. Furthermore, patients experiencing a disease relapse while on canakinumab administered at 150 mg every 8 weeks could benefit by shortening the interval between administrations to 150 mg every 6 weeks. The median time of response to therapy was 6 weeks for anakinra and 3 weeks for canakinumab. In addition, the

drug survival was acceptable for both IL-1 inhibitors evaluated and no serious adverse events were reported [36]. The excellent safety profile of anti-IL1 agents in BD has been confirmed by another recent multicenter observational cohort study specifically evaluating biologic agents [37].

A third anti-IL-1 agent, gevokizumab, has also been assessed in BD patients with refractory multiresistant and sight-threatening uveitis: a single intravenous infusion of gevokizumab (dosage 0.3 mg/kg) led to rapid and complete resolution of intraocular inflammation with marked improvement of visual acuity in 21 days. Moreover, five patients re-treated with gevokizumab for recurrent uveitis responded to a second dose, maintaining their response for several months and despite any discontinuation of immunosuppressive agents or increase in the corticosteroid dosage [38].

Notably, the importance of IL-1 inhibition in BD patients also derives from an epidemiologic observation: unlike anti-TNFα agents, IL-1 antagonists do not seem to interfere significantly with the risk of tuberculosis, suggesting that anti-IL-1 agents are a feasible treatment in areas where tuberculosis is endemic [39].

Tables 2 and 3 summarize published clinical studies on the efficacy of IL-1 inhibitors in patients diagnosed with BD.

CONCLUSIONS

An increasing volume of evidence places BD at the crossroad between autoimmune and auto-inflammatory disorders. In the present review we have considered pathogenetic, clinical and therapeutic elements supporting the auto-inflammatory

Table 3. Overview of studies reporting patients with Behçet's disease treated with canakinumab

Authors [ref]	No. of patients	Main clinical involvement	Dosage	Results
Ugurlu et al. [32]	1	Mucocutaneous, ocular	Single dose of 150 mg	Complete remission during 8 weeks follow-up
Cantarini et al. [33]	1	Mucocutaneous, articular, abdominal, fever	150 mg every 8 weeks	Complete remission during 6 months follow-up
Vitale et al. [34]	3	Mucocutaneous (2/3), articular (3/3), abdominal (2/3), fever (3/3), vascular (2/3)	150 mg every 6 weeks	Complete remission during 6–12 months follow-up. A vascular relapse occurred (with canakinumab at dose of 150 mg every 8 weeks)
Cantarini et al. [29]	1	Gastrointestinal, ocular, articular, fever	150 mg every 8 weeks	Complete remission within 2 weeks; partial efficacy on the oral aphthosis
Emmi et al. [28]	1	Mucocutaneous, ocular	150 mg every 6 weeks	Good control of eye signs during 6 months follow-up
Vitale et al. [31]	1	Mucocutaneous, ocular, articular, neurological, fever	150 mg every 6 weeks	Good control of eye signs during 6 months follow-up, but additional brain lesions identified on brain MRI after 6 months
Pagnini et al. [35]	1	Mucocutaneous, ocular, fever	4 mg/kg every 28 days	Complete clinical and laboratory remission in 4 weeks; 6 months after the first dose the boy was completely asymptomatic
Emmi et al. [36]	9	Mucocutaneous, ocular, neurological, gastroenteric, vascular, articular	150 mg every 6–8 weeks	Median time of response to canakinumab 3 weeks; adherence to initial treatment at 75th percentile of 8.0 months and median 13.0 months, with overall cumulative survival of 40.6% at 23 months. No adverse events or severe adverse events

hypothesis. However, other clues highlight the autoimmune component at the basis of BD, such as class I major histocompatibility complex associations and response to treatment with immunosuppressive agents. In addition, the therapeutic benefit observed with interferon-α supports the hypothesis of a Th1-driven disease [40]. On the whole, these observations confirm the complexity of BD due to the extremely protean clinical picture and the difficult management. Of note, anti-IL-1 agents are proving to be a valid treatment tool in patients resistant to standard therapies; by bolstering TNF inhibition, they could be the treatment of choice when anti-TNF agents are not advisable. In this context, looking far into the future, investigating predictive factors of response to therapy and identifying the optimal dosages or intervals of administration are interesting challenges.

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References
1. Gül A. Behçet's disease: an update on the pathogenesis. *Clin Exp Rheumatol* 2001; 19: S6-12.
2. Koné-Paut I, Darce-Bello M, Shahram F, et al. Registries in rheumatological and musculoskeletal conditions. Paediatric Behçet's disease: an international cohort study of 110 patients. One-year follow-up data. *Rheumatology* (Oxford) 2011; 50: 184-8.
3. Lopalco G, Cantarini L, Vitale A, et al. Interleukin-1 as a common denominator from autoinflammatory to autoimmune disorders: premises, perils, and perspectives. *Mediators Inflamm* 2015; 2015: 194864.
4. Rigante D. The protean visage of systemic autoinflammatory syndromes: a challenge for inter-professional collaboration. *Eur Rev Med Pharmacol Sci* 2010; 14: 1-18.
5. Gül A. Behçet's disease as an autoinflammatory disorder. *Curr Drug Targets Inflamm Allergy* 2005; 4: 81-3.
6. De Rosa G, Pardeo M, Rigante D. Current recommendations for the pharma-
cologic therapy in Kawasaki syndrome and management of its cardiovascular complications. *Eur Rev Med Pharmacol Sci* 2007; 11: 301-8.
7. Caso F, Costa L, Rigante D, et al. Biological treatments in Behçet's disease: beyond anti-TNF therapy. *Mediators Inflamm* 2014; 2014: 107421.
8. Keller M, Spanou Z, Schaerli P, et al. T cell-regulated neutrophilic inflammation in autoinflammatory diseases. *J Immunol* 2005; 175: 7678-86.
9. Mege JL, Dilsen N, Sanguedolce V, et al. Overproduction of monocyte derived tumor necrosis factor alpha, interleukin (IL) 6, IL-8 and increased neutrophil superoxide generation in Behçet's disease. A comparative study with familial Mediterranean fever and healthy subjects. *J Rheumatol* 1993; 20: 1544-9.
10. Kim DJ, Baek SY, Park MK, et al. Serum level of interleukin-33 and soluble ST2 and their association with disease activity in patients with Behçet's disease. *J Korean Med Sci* 2013; 28: 1145-53.
11. Liang Y, Xu WD, Zhang M, et al. Meta-analysis of association between cytokine gene polymorphisms and Behcet's disease risk. *Int J Rheum Dis* 2013; 16: 616-24.
12. Rigante D, Vitale A, Lucherini OM, Cantarini L. The hereditary autoinflammatory disorders uncovered. *Autoimmun Rev* 2014; 13: 892-900.
13. Kim EH, Park MJ, Park S, Lee ES. Increased expression of the NLRP3 inflammasome components in patients with Behçet's disease. *J Inflamm* (Lond) 2015; 12: 41.
14. Liang L, Tan X, Zhou Q, et al. IL-1β triggered by peptidoglycan and lipopolysaccharide through TLR2/4 and ROS-NLRP3 inflammasome-dependent pathways is involved in ocular Behçet's disease. *Invest Ophthalmol Vis Sci* 2013; 54: 402-14.
15. Sakamoto N, Sekine H, Kobayashi H, Sato Y, Ohira H. Association of the toll-like receptor 9 gene polymorphisms with Behcet's disease in a Japanese population. *Fukushima J Med Sci* 2012; 58: 127-35.
16. Kirino Y, Zhou Q, Ishigatsubo Y, et al. Targeted resequencing implicates the familial Mediterranean fever gene *MEFV* and the toll-like receptor 4 gene *TLR4* in Behçet disease. *Proc Natl Acad Sci USA* 2013; 110: 8134-9.
17. Castrichini M, Lazzerini PE, Gamberucci A, et al. The purinergic P2×7 receptor is expressed on monocytes in Behçet's disease and is modulated by TNF-α. *Eur J Immunol* 2014; 44: 227-38.
18. Oğuz AK, Yılmaz S, Akar N, et al. C-type lectin domain family 12, member A: a common denominator in Behçet's syndrome and acute gouty arthritis. *Med Hypotheses* 2015; 85:186-91.
19. Livneh A, Aksentijevich I, Langevitz P, et al. A single mutated *MEFV* allele in Israeli patients suffering from familial Mediterranean fever and Behçet's disease (FMF-BD). *Eur J Hum Genet* 2001; 9: 191-6.
20. Aksu K, Keser G. Coexistence of vasculitides with familial Mediterranean fever. *Rheumatol Int* 2011; 31: 1263-74.
21. Amoura Z, Dodé C, Hue S, et al. Association of the R92Q *TNFRSF1A* mutation

and extracranial deep vein thrombosis in patients with Behçet's disease. *Arthritis Rheum* 2005; 52: 608-11.

22. Koné-Paut I, Sanchez E, Le Quellec A, Manna R, Touitou I. Autoinflammatory gene mutations in Behçet's disease. *Ann Rheum Dis* 2007; 66: 832-4.

23. Botsios C, Sfriso P, Furlan A, Punzi L, Dinarello CA. Resistant Behçet disease responsive to anakinra. *Ann Intern Med* 2008; 149: 284-6.

24. Bilginer Y, Ayaz NA, Ozen S. Anti-IL-1 treatment for secondary amyloidosis in an adolescent with FMF and Behçet's disease. *Clin Rheumatol* 2010; 29: 209-10.

25. Emmi G, Silvestri E, Cameli AM, et al. Anakinra for resistant Behçet uveitis: why not? *Clin Exp Rheumatol* 2013; 31: 152-3.

26. Caso F, Rigante D, Vitale A, Lucherini OM, Cantarini L. Efficacy of anakinra in refractory Behçet's disease sacroiliitis. *Clin Exp Rheumatol* 2014; 32: S171.

27. Comarmond C, Wechsler B, Bodaghi B, Cacoub P, Saadoun D. Biotherapies in Behçet's disease. *Autoimmun Rev* 2014; 13: 762-9.

28. Emmi G, Silvestri E, Ciucciarelli L, Squatrito D, Emmi L. Reply: anti-IL1 blocking agents in drug-resistant Behçet's syndrome: our little case series. *Clin Exp Rheumatol* 2014; 32: S172.

29. Cantarini L, Vitale A, Scalini P, et al. Anakinra treatment in drug-resistant Behçet's disease: a case series. *Clin Rheumatol* 2015; 34: 1293-301.

30. Caso F, Iaccarino L, Bettio S, et al. Refractory pemphigus foliaceus and Behçet's disease successfully treated with tocilizumab. *Immunol Res* 2013; 56: 390-7.

31. Vitale A, Rigante D, Caso F, Franceschini R, Cantarini L. Interleukin-1 blockade in neuro-Behçet's disease: a case-based reflection. *Int J Rheum Dis* 2015 - Epub ahead of print. doi: 10.1111/1756-185X.12680.

32. Ugurlu S, Ucar D, Seyahi E, Hatemi G, Yurdakul S. Canakinumab in a patient with juvenile Behçet's syndrome with refractory eye disease. *Ann Rheum Dis* 2012; 71: 1589-91.

33. Cantarini L, Vitale A, Borri M, Galeazzi M, Franceschini R. Successful use of canakinumab in a patient with resistant Behçet's disease. *Clin Exp Rheumatol* 2012; 30: S115.

34. Vitale A, Rigante D, Caso F, et al. Inhibition of interleukin-1 by canakinumab as a successful mono-drug strategy for the treatment of refractory Behçet's disease: a case series. *Dermatology* 2014; 228: 211-14.

35. Pagnini I, Bondi T, Simonini G, Giani T, Marino A, Cimaz R. Successful treatment with canakinumab of a paediatric patient with resistant Behçet's disease. *Rheumatology* (Oxford) 2015; 54: 1327-8.

36. Emmi G, Talarico R, Lopalco G, et al. Efficacy and safety profile of anti-interleukin-1 treatment in Behçet's disease: a multicenter retrospective study. *Clin Rheumatol* 2015 (Epub ahead of print. doi: 10.1007/s10067-015-3004-0).

37. Cantarini L, Talarico R, Generali E, et al. Safety profile of biologic agents for Behçet's disease in a multicenter observational color study. *Int J Rheum Dis* 2015 (Epub ahead of print. doi: 10.1111/1756-185X.12732).

38. Gül A, Tugal-Tutkun I, Dinarello CA, et al. Interleukin-1β-regulating antibody XOMA 052 (gevokizumab) in the treatment of acute exacerbations of resistant uveitis of Behçet's disease: an open-label pilot study. *Ann Rheum Dis* 2012; 71: 563-6.

39. Cantarini L, Lopalco G, Caso F, et al. Effectiveness and tuberculosis-related safety profile of interleukin-1 blocking agents in the management of Behçet's disease. *Autoimmun Rev* 2015; 14: 1-9.

40. Kotter I, Hamuryudan V, Ozturk ZE, Yazici H. Interferon therapy in rheumatic diseases: state of the art 2010. *Curr Opin Rheumatol* 2010; 22: 278-83.