Vascular involvement in Behçet's disease: ultrasound assessment of femoral vein intima-media thickness, nailfold capillaroscopy and endothelial progenitor cells in a national referral centre

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Abstract

Objective

To assess vascular involvement at different levels in patients with Behçet's disease (BD).

Methods

We conducted an observational study of unselected consecutive patients with BD assessed in a national referral centre. Demographic and clinical variables were collected. Common femoral vein (CFV) intima-media thickness (IMT) was measured with a high-resolution Doppler ultrasound (US). Microvascular involvement was evaluated by performing a nailfold capillaroscopy (NFC). Endothelial progenitor cells (EPC) were measured in peripheral blood of patients and healthy controls (HC) by flow cytometry.

Results

A total of 42 patients with BD were evaluated. Clinical vascular manifestations were present in 17 of them. Arterial hypertension was associated with a higher frequency of vascular manifestations (p=0.003). The median [IQR] value of the CFV IMT was significantly higher in patients with vascular manifestations (0.65 [0.45–0.82] vs. 0.49 [0.39–0.55]; p=0.028). The NFC examination was abnormal in 54.8% of the patients, being the most common findings: capillary loop dilation (45.2%), mega capillaries (21.4%) and micro haemorrhages (16.7%). A significant increase in EPC frequency was observed in patients with BD when compared with HC (p=0.011).

Conclusion

The assessment of CFV IMT with Doppler US constitutes a useful technique to evaluate clinical vascular involvement in BD patients. Microvascular involvement is not uncommon in BD and can be easily assessed by NFC. Furthermore, EPC may be a useful blood biomarker of the disease.

Key words

Behçet's disease, vascular Behçet, intima-media thickness, Doppler ultrasound, nailfold capillaroscopy, endothelial progenitor cells

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Introduction

Behçet's disease (BD) is a chronic, relapsing, multisystemic inflammatory disorder characterised by mucocutaneous, articular, ocular, vascular, gastrointestinal and neurological manifestations. It is considered a systemic vasculitis, included in the "variable-vessel vasculitis" group, following the 2012 Chapel-Hill classification (1, 2).

Ocular and mucocutaneous manifestations of BD are globally recognised and widely studied (3-5). However, vascular lesions are also frequent in BD, but less explored (6). Clinical vascular involvement is present in up to 40% of patients with BD. Superficial venous thrombosis (SVT) and deep vein thrombosis (DVT) are the most frequent vascular manifestations. Arterial involvement, although present in only 3–5% of patients, represents a unique feature of BD, with aneurysms located in peripheral, visceral or pulmonary arteries (6). Venous wall thickness (VWT) assessed with Doppler ultrasound (US) has recently been suggested as an indirect sonographic marker of the degree of vascular end organ damage, including venous disease. Multiple studies have proven that increased VWT, especially in femoral and popliteal veins, is a clinical feature of great interest in BD (7). However, it remains unclear if US findings correlate with vascular involvement in BD. The common femoral vein (CFV) has been chosen as the best location to assess VWT through US. The cut-off point of ≥0.5 mm in VWT for the diagnosis of BD has reported a sensitivity of 81–82% and a specificity of 78.4-81.1%. Therefore, it has been proposed as an efficient and non-invasive tool for BD diagnosis (8).

Microvascular involvement may be clinical (as Raynaud's phenomenon) or subclinical. Nailfold capillaroscopy (NFC) is a simple, non-invasive imaging technique that allows in vivo assessment of microvasculature through direct observation of the capillary network (9). NFC has been reported to be useful in the non-invasive diagnosis and follow-up of several autoimmune systemic diseases, especially for systemic sclerosis (10, 11). Capillary abnormalities such as dilated loops and mega capillaries, microaneurysms and microhaemorrhages have been observed in patients with BD (12).

Endothelial dysfunction has been associated with the development of atherosclerosis and cardiovascular diseases. Moreover, it has been observed as a first step of accelerated atherosclerosis in diverse autoimmune diseases, especially in rheumatoid arthritis (RA) (13, 14). Endothelial progenitor cells (EPC) are involved in vasculogenesis and endothelial tissue repair and have been implied in the pathogenesis of multiple vascular disorders, including autoimmune and interstitial lung diseases (15, 16). In this context, vascular endothelial dysfunction has also been reported in patients with BD (17). Nevertheless, little is known about the contribution of EPC in BD vasculitis (18).

Currently, vascular assessment of patients with BD is not considered a daily routine practice. Moreover, there is still no valid laboratory markers for BD diagnosis. Taking all of this into consideration, the aims of the present study including BD patients with and without clinical vascular manifestations were: a) to evaluate the CFV IMT, b) to determine NFC abnormalities, and c) to establish the role of EPC in this disease.

Patients and methods

Patients, enrolment criteria and study design

We conducted an observational study of unselected consecutive patients with BD assessed in a national referral centre from March to May 2021. The study protocol was approved by the local Clinical Research Ethics Committee (Internal code: 2020.453) and was performed in accordance with the principles of the Declaration of Helsinki. All patients fulfilled the 2014 ICBD criteria (19). They were evaluated sequentially with a scheduled clinic visit after signing an informed consent. Exclusion criteria included: patients with other associated rheumatic disease, patients under the age of 18 and patients who did not sign the informed consent. Demographic and clinical variables were collected. Patients were considered to have vascular involvement if they had history of DVT, pulmonary thrombo-

embolism, SVT, arterial thrombosis (peripheral arterial thrombosis, stroke, and transient ischaemic attack), aneurysms, acute myocardial infarction or secondary Raynaud's phenomenon.

Doppler ultrasound examination

Bilateral carotid artery and CFV Doppler US was performed in 42 BD patients by an experienced rheumatologist. High-resolution Doppler US system LOGIQ S8XDclear equipped with a high-resolution linear transducer (8-12 MHz) was used.

We assessed the intima-media thickness (IMT) in the carotid arteries and the CFV. In the case of common carotid artery, measurement was made on the posterior wall of the vessels, 1 cm from the carotid bifurcation. The presence of carotid artery plaques was also assessed. In the CFV, measurement was made on the posterior wall, 2 cm before the CFV bifurcation. Posterior wall is always preferred because the anterior wall may cause reverberation artifacts and imperfect delineation. Each measurement was made two times and the medium value was registered.

Nailfold capillaroscopy evaluation

NFC was performed jointly by two observers in eight fingers (excluding the thumbs) with a 500x magnification capillaroscope (Dino-Lite CapillaryScope 500 (MEDL4N5) in 42 BD patients. To better visualize the capillaries, immersion oil was applied in each nailfold. Patients were informed to protect their hands from trauma prior to the capillaroscopy examination. The room temperature of the examination room was kept at ~24ºC. All patients were asked about their history of Raynaud's phenomenon, smoking habits, ischaemic ulcers, and history of hypertension, dyslipidaemia and diabetes.

The NFC system was evaluated in terms of capillary distribution, capillary density, and capillary morphology. The following features were considered abnormal: diffuse enlarged capillaries (≥2 capillaries with a diameter of 20–50 μm in at least 2 different fingers), mega capillaries (>50 μm in diameter), reduced vascular density (<9 capillaries/mm or avascular areas), diffuse microhaemorrhages (≥2 haemorrhages in at least 2 different fingers) and granular/slow flow.

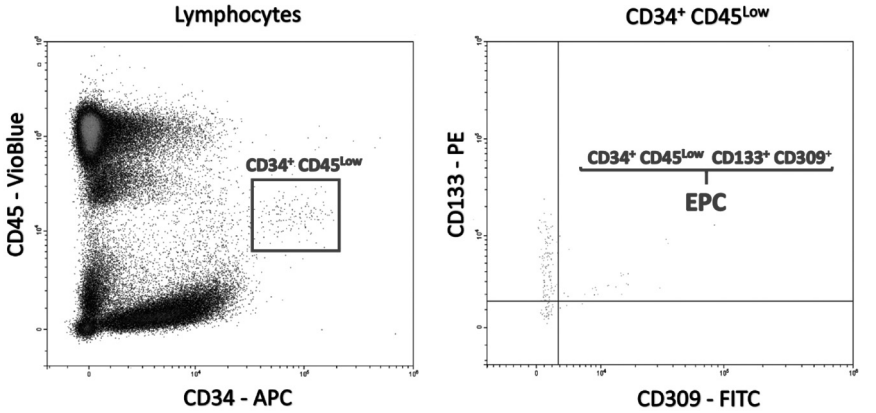

Fig. 1. Gating strategy used to identify EPC.

Endothelial progenitor cell quantification

In this study, EPC from peripheral venous blood were characterised by simultaneous expression of cell surface markers that reflect stemness (CD34), immaturity (CD133), endothelial commitment [CD309 or vascular endothelial growth factor receptor 2 (VEGFR-2)], and a low expression of the pan-leukocyte marker (CD45) (15).

EPC quantification was analysed by direct flow cytometry. Briefly, 200 μL of peripheral blood was pre-incubated with Fc receptors blocking reagent (Miltenyi Biotech, Madrid, Spain). Then, cells were labelled with APC-conjugated anti-CD34 (Miltenyi Biotech, Madrid, Spain), VioBright FITC-conjugated anti-CD309 (VEGFR-2) (Miltenyi Biotech, Madrid, Spain), PE-conjugated anti-CD133/2(293C3) (Miltenyi Biotech, Madrid, Spain), Vioblue-conjugated anti-CD45 (Miltenyi Biotech, Madrid, Spain) monoclonal antibodies, or with isotype-matched antibodies (Miltenyi Biotech, Madrid, Spain). After conjugation, red blood cells were lysed by incubating in fluorescence-activated cell sorting (FACS) lysing solution (BD Bioscience, San Jose, CA, USA) and white blood cell pellets were then washed once with phosphate-buffered saline (PBS). Labelled cells were analysed in a CytoFLEX flow cytometer (Beckman Coulter, Brea, CA, USA) using a Cytexpert 2.3 analyser (Beckman Coulter, Brea, CA, USA), acquiring approximately 1 × 10^5 events per sample. First, $CD34^+$ and $CD45^{low}$ were gated and then assayed for expression of CD133 and CD309 in the lymphocyte gate. Thus, EPC were considered as $CD34^+$, $CD45^{low}$, $CD133^+$ and $CD309^+$ cells (Fig. 1). EPC quantification was expressed as the percentage of cells in the lymphocyte gate (15, 20-22).

Statistical analysis

The data obtained were analysed using SPSS (Statistical Package for Social Sciences) software v. 26.0. Continuous variables were analysed with Kolmogorov-Simirnov or Shapiro-Wilk's normality tests and variables were shown as the mean ± standard deviation or as median [interquartile range] according to their distribution being normal or not. *p*-values <0.05 were considered as statistically significant. Student's t test or Mann-Whitney U-test was used to compare continuous variables, and Chi-squared test or Fisher's exact test for categorical variables, as appropriate. For non-continuous variables a descriptive study was performed, showing in percentages the clinical findings obtained.

Results

We evaluated 17 BD patients with vascular manifestations and 25 BD patients without vascular manifestations. Main clinical and demographic characteristic are described in Table I. The vascular manifestations were DVT (n=4), SVT (n=1), arterial aneurysms (n=2), acute myocardial infarction (n=3), arterial thrombosis (n=1) and Raynaud's phenomenon (n=10). A significant in-

Table I. Demographic and clinical characteristics of patients with Behçet's disease with or without clinical vascular manifestations.

	Patients with vascular manifestations (n=17)	Patients without vascular manifestations (n=25)	*p*-value
Sex *(men)*, n (%)	10 (41.2)	13 (48)	0.663
Age *(years)*, mean ± SD	51.24 ± 12.8	45.56 ± 12.88	0.133
HLA B51 positive, n/tested cases	4/17	14/25	
Evolution time (years) from diagnosis, mean ± SD	13.35 ± 10.45	13.04 ± 8.52	0.891
Cardiovascular risk factors			
Hypertension, n (%)	7 (41.2)	1 (4)	**0.003**
Diabetes mellitus, n (%)	1 (5.9)	2 (8)	0.794
Dyslipidaemia, n (%)	6 (35.3)	5 (20)	0.268
Smoking habit *(current or former smokers)*, n (%)	10 (58.8)	12 (48)	0.067
Clinical manifestations			
Oral ulcers, n (%)	16 (94.1)	25 (100)	0.220
Genital ulcers, n (%)	12 (70.6)	16 (64)	0.657
Erythema nodosum like, n (%)	9 (52.9)	6 (24)	0.055
Pseudo folliculitis, n (%)	9 (52.9)	19 (76)	0.120
Uveitis, n (%)	7 (41.2)	9 (36)	0.735
Arthralgia, n (%)	14 (82.4)	17 (68)	0.299
Neurological manifestations, n (%)	2 (11.8)	2 (8)	0.683
Ultrasound findings			
Femoral vein IMT (mm), median [IQR]	0.6 [0.45-0.82]	0.49 [0.39-0.55]	**0.028**
Common carotid artery IMT (mm), median [IQR]	0.52 [0.44-0.64]	0.55 [0.47-0.61]	0.848

IMT: intima-media thickness. IQR: interquartile range.

crease in vascular manifestations was observed in patients with arterial hypertension (p=0.003). HLA B51 presence was more frequent in patients with no vascular manifestations. No significant increase in C-reactive protein (CRP) nor erythrocyte sedimentation rate (ESR) was found in BD patients with vascular manifestations compared with patients without vascular involvement.

Doppler ultrasound findings

The median [IQR] value of the CFV IMT was significantly higher in patients with vascular manifestations (0.6 [0.45-0.82] *vs*. 0.49 [0.39-0.55]; p=0.028) (Fig. 2). In contrast, no differences were observed in the carotid artery IMT or in the presence of carotid plaques between patients with or without vascular involvement. A total of 4 patients had carotid plaques but none of them had critical stenosis.

Nailfold capillaroscopy findings

NFC was performed in all BD patients, being abnormal in 54.8% of them. The most common findings were capillary loop dilation (45.2%), capillary tortuosity (31%), mega capillaries (21.4%) and microhaemorrhages (16.7%) (Table II and Fig. 3). A comparison between vascular with non-vascular BD and their abnormal NFC examinations provided no significant differences between both groups (p=0.663).

Endothelial progenitor cell findings

Instant blood samples to study EPC levels were available in 18 BD patients and 12 Healthy controls (HC). The median [IQR] frequency of EPC was higher in BD patients than in HC (0.0234% [0.0165- 0.030825] *vs*. 0.00905% [0.006525- 0.026575], p=0.011) (Fig. 4). Within the BD patients, no statistically significant differences were obtained when comparing vascular with non-vascular BD patients (p=0.285).

Discussion

Vascular involvement in BD is one of the mayor clinical phenotypes of this disease and is included in the newest diagnostic criteria. Early assessment of vascular involvement in BD may result in an improvement of morbi-mortality (23).

In our study we did not observe differences between the vascular Behçet's group and the non-vascular Behçet's group regarding age, sex, disease duration, risk factors and other clinical manifestations, except the presence of arterial hypertension which was clearly increased in the vascular Behçet's group (p=0.003). In contrast, we found a statistically significant difference between vascular and non-vascular patients regarding CFV IMT (0.6 [0.45-0.82] vs 0.49 [0.39-0.55]; p=0.028). In addition, we report an abnormal NFC examination in 54.8% of the patients, with presence of mega capillaries in 21.4% and microhaemorrhages in 16.7% of them, demonstrating microvascular involvement through this technique. Finally, we report that the median [IQR] frequency of EPC levels was higher in BD than in HC (p=0.011), which reflects subclinical endothelial damage in these patients.

Venous inflammation is one of the key features of BD. Venous macrovascular involvement in BD was first studied with magnetic resonance imaging (24). Later, Boulon *et al*. (25) studied the first BD case with venous Doppler US. This group discovered an increase of wall thickness in the right great saphenous vein. Since then, US in BD has gained some attention for its possible diagnostic value.

VWT is proposed to be a surrogate marker of venous disease. Increased VWT is reported in BD patients with or without clinical vascular manifestations, thus, making US examination a promising tool for BD diagnosis. US is a non-invasive, cost-effective and easily accessible radiological modality with a high patient compliance. (7, 8, 26). Various studies have shown the potential role of VWT measurement in BD diagnosis, demonstrating its increased value in comparison with healthy controls (7, 8, 24, 25, 27). However, only a few reports compared the US findings between vascular Behçet patients and non-vascular Behçet patients. In a study by Seyahi *et al*. (26) BD patients with DVT had an increased femoral VWT than those without vascular involvement, and both BD groups had an increased VWT than HC. Kaymaz *et al*. (28) compared carotid IMT, jugular vein wall thickness, portal vein wall thickness and femoral vein wall thickness in vascular and non-vascular BD

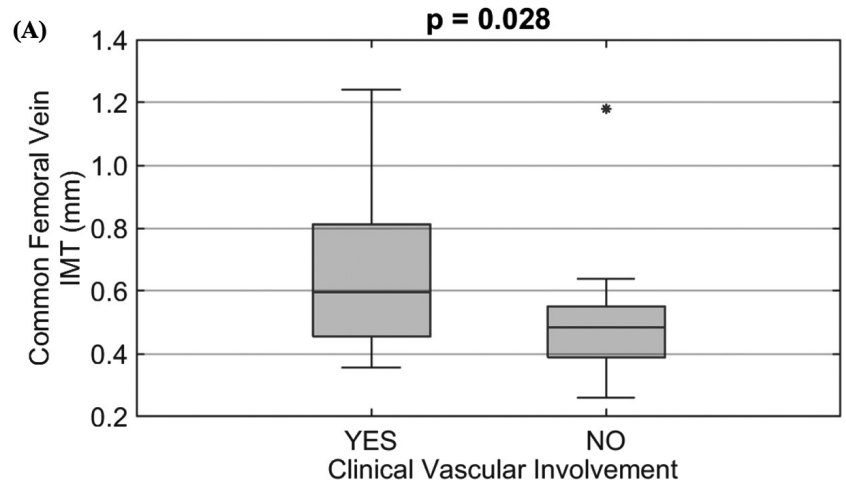

Fig. 2. Ultrasound Intima-media thickness measurements.
A: Results represented as a box-plot graphic in both Behçet's disease with and without clinical vascular manifestations. Mann-Whitney U-test.
B: Doppler ultrasound image of right femoral vein in longitudinal plane of one of our patients. In red we can identify the femoral artery and beneath the common femoral vein and its two main branches. The IMT of this vein is remarkably increased (white arrows, IMT of 1.18 mm). The standardised measurement point is approximately 2 cm prior to the division of the common femoral vein.

patients and a HC group. They did not find a statistical difference between patients with or without vascular involvement in terms of bilateral jugular, CFV wall thickness and carotid IMT. In their study, portal VWT was higher in BD patients with vascular involvement compared to those without vascular involvement and HC. Nevertheless, all VWT were found to be higher between non-vascular BD and HC, which offers more evidence to the value of US as a diagnostic technique.

In our study, we decided to measure the IMT of the CFV instead of the total VWT, as other researchers have performed. Our decision was made based on the challenging measure of the complete VWT in some patients, with irregularities or no clear delimitation. We agreed to establish an easier method for clinicians to put into practice and thus focused on the IMT with the clear hypoechoic media layer easier to identify.

Up to date, there is only one previous abstract on CFV IMT in BD patients by Sevik *et al*. 19th ICBD Congress-P086. 42 BD patients and 35 HC were enrolled for this study and both IMT and whole vein thickness was measured by Doppler ultrasound. They found that IMT in BD was increased in both legs compared with HC ($p<0.0001$) and a significant positive correlation between IMT and whole vein thickness. Nonetheless, they found no significant difference between BD with or without major organ involvement (defined as vascular, ocular and neurological). Their results suggest that during active vasculitis not only adventitia and media are infiltrated by inflammatory cells, but also the intima layer, producing a full layer inflammation in BD regardless of vascular manifestations. The different results may be in part due to different definitions of vascular involvement. Nevertheless, this study also provides further evidence that Doppler US may have a promising role in the diagnosis of BD.

Some studies have also explored subclinical atherosclerosis in BD by assessing the carotid IMT. Endothelial dysfunction is regarded as the foundation of the atherosclerotic process and the increase of the carotid IMT is widely accepted as subclinical evidence of it. It has been proven that BD patients have a higher carotid intima media thickness than HC in various studies (29,30). The above-mentioned study by Kaymaz *et al*. (28) compared vascular and non-vascular BD patients with HC in a series of Doppler measurements including carotid IMT. They did not find any significant difference between both groups. In our study, we got similar results, with no statistically significant differences when comparing both groups regarding mean carotid IMT ($p=0.848$). The prevalence of carotid plaques was also similar in both groups.

The disparities between studies as well as the cut off values proposed by many of them may be explained by various factors. First, the sample size is not the same and at least one of them used only male patients (27). Other reason may be the criteria for defining a vascular BD patient. Some studies only included DVT patients (26), and others did not take into account arterial aneurysms, cardiac infarction and strokes. In our case, we also included secondary Raynaud's phenomenon as a vascular manifestation. These disparities between BD groups may vary the results obtained. Finally, US is unfor-

Table II. Clinical vascular and nailfold capillaroscopy data of 42 patients with Behçet's disease.

Demographic data	**n (%)**
Women/Men	19 (45.2)/ 23 (54.8)
Age (years), mean ± SD	47.86 ± 12.99
Clinical vascular involvement	**n (%)**
Deep vein thrombosis	4 (9.52)
Superficial thrombophlebitis	1 (2.38)
Arterial aneurysms	2 (4.76)
Acute myocardial infarction	3 (7.14)
Arterial thrombosis	1 (2.38)
Raynaud's phenomenon	10 (23.81)
Nailfold capillaroscopy findings	**n (%)**
Abnormal	23 (54.8)
Dilated loops (20-50 µm) ≥2 in at least 2 fingers	19 (45.2)
Mega capillaries (>50 µm)	9 (21.4)
≥2 in at least 2 fingers	3 (7.1)
Capillary tortuosity	13 31)
Micro haemorrhages	7 (16.7)
≥2 in at least 2 fingers	1 (2.4)
Reduced vascular density	3 (7.1)
Granular flow	1 (2.4)

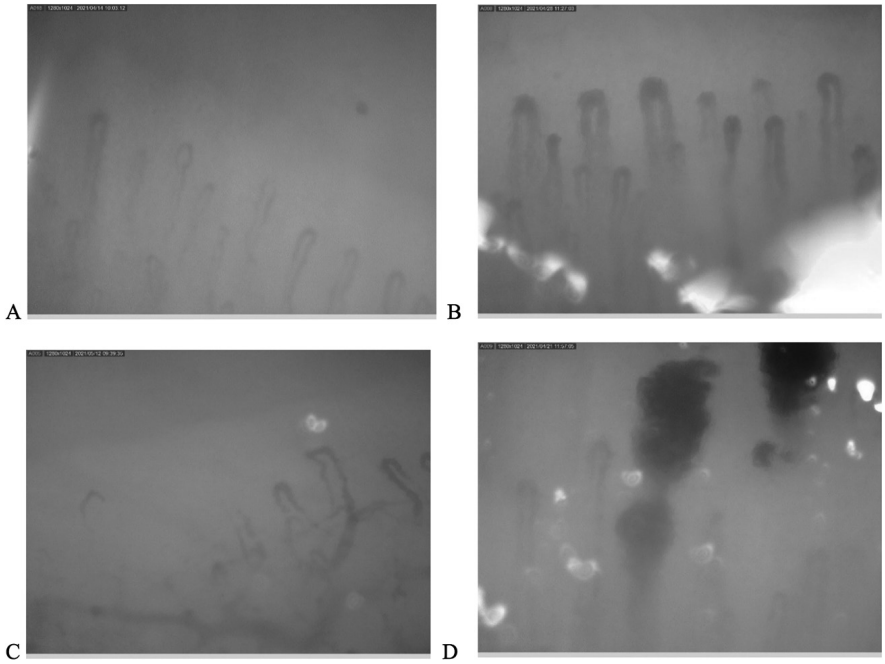

Fig. 3. Nailfold capillaroscopy images of patients with Behçet's disease.
A: normal pattern; **B**: dilated loops; **C**: reduced vascular density; **D**: micro haemorrhages.

tunately an operator dependent technique, which is a major limitation for homogenous results.

NFC is widely used for assessing Raynaud's phenomenon and connective tissue diseases, especially systemic sclerosis. By contrast, not much attention has been paid to its role in vasculitides such as BD. It is also a simple, non-invasive and inexpensive technique, which can be easily repeated for a continuous evaluation of the patient's state. In the largest study with NFC and BD performed by Movasat *et al*. (12) with 128 BD patients, NFC was abnormal in 51 patients (40%). Enlarged capillaries were seen in 33 patients (26%), haemorrhages in 21 (16%), and capillary loss only in one patient (0.8%). Although there are some differences in the technique, their results are very similar compared to ours.

Another study by *Ibrahim et al*. (31) with 50 patients of BD found capillary dilation in 24% of the patients, microhaemorrhages in 54% and branching/tortuosity in 64% in them. In comparison with a HC group of 30 people these findings were statistically significant (p<0.001). The frequency of microhaemorrhages is considerably higher in this study and not consistent with others including Movasat *et al*. and ours. This may be due to a different technique and diverse devices. In another study by Alan *et al*. (11) 82 BD patients were examined with 200x magnification NFC. This group considered abnormal tortuosity, defined as two or more cross capillaries over each 1 mm length and thus 91.5% of the patients had and abnormal NFC examination. Bizarre capillaries (capillaries outside normal view) accounted for 9.8% of the findings, microhaemorrhages were found in 4.9% of the total and only 2 patients had mega capillaries (2.4%). These results also differ with the others, reporting less microhaemorrhages and mega capillaries in BD patients. Nevertheless, all studies have their own limitations with different equipment, magnification and interpretation of abnormality, which is probably the cause of such disparities. It is also necessary to elucidate if ethnic factors influence NFC findings. Our study is the first to explore the comparison between vascular and non-vascular BD patients regarding NFC. However, no statistical significance differences were obtained when performing the appropriate tests, with the exception of capillary tortuosity, which was more frequent in patients with vascular involvement (p=0.011).

Subclinical vascular damage at the cellular level can be studied through EPC, which appear to play an important role in endothelium repair and neovascularisation. In response to vascular inflammation, injury, or tissue ischaemia, they migrate to the peripheral circulation and convert into mature endothelial cells to re-establish vascular integrity and blood supply (32). The main objective of the EPC assessment in BD is to confirm our hypothesis that, due to subclinical vascular damage, the levels of this type of cells are increased and may become

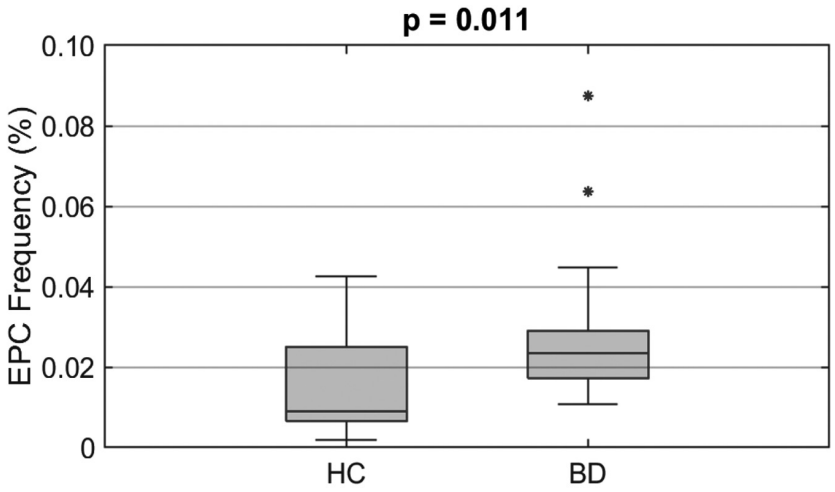

Fig. 4. Quantification of EPC population by flow cytometry of 18 BD patients and 12 HC. EPC were considered as $CD34^+$, CD45low, $CD309^+$ and CD133+ cells in the lymphocyte gate and were expressed as the percentage of cells in this gate. Mann-Whitney U-test.
EPC: endothelial progenitor cells; HC: healthy controls. BD: Behçet's disease.

a useful biomarker of the disease in the future. BD being the prototype of systemic inflammatory disease that causes thrombosis, it is crucial to further comprehend EPC's role in this disease.

Our results are encouraging and clearly demonstrate an increase in the frequency of EPC in BD in comparison with HC. Our criteria for defining EPC were CD34+, CD45low, CD309+ (sometimes referred as KDR+) and CD133+. This definition is extremely important because the univocal interpretation of data is limited by the extremely low frequency of the analysed cell populations and by the lack of consensus of the utilised markers and EPC definition. Unfortunately, no statistically significant differences were obtained when comparing vascular with non-vascular BD and their respective EPC frequencies (p=0.285). Further studies are needed to address this issue.

Up to date, EPC are not widely studied and only a few articles regarding BD and EPC have been published, which show different results. Floris *et al*. (33) in a cohort of 32 BD patients and 12 controls found no significant association between EPC levels in both groups (p=0.316). Similarly, Bozkirli *et al*. (34) observed that EPC levels were comparable in patients with BD and HC (p=0.849) and they did not correlate with disease activity or other clinical features, with the exception of thrombosis. Another study by Fadini *et al*. (35) showed that BD patients had significantly lower levels of CD34+KDR+ and CD34+CD133+KDR+ EPC than controls. Furthermore, negative correlations between EPC phenotypes and BD duration, and positive correlations between CD34+KDR+ EPC and both BD activity scores and CRP levels were also found. This showed a progressive decline in EPC in BD patients. This study concluded that EPC mobilisation might occur during bursts of vascular inflammation and in the long-term chronic inflammation might be followed by progressive EPC reduction.

On the other hand, Arica *et al*. (18) studied a total of 45 BD patients and 28 HC. EPC were defined as CD34+CD133+KDR+ for early endothelial progenitor cells and CD34+KDR+ as late endothelial progenitor cells. This group found that the mean plasma level of EPC and circulating endothelial cells (CECs, CD34+CD133+), VEGF, matrix metalloproteinase-9, CRP, and ESR were significantly higher in patients with BD. All of these parameters, except circulating endothelial cells, were also found to be higher in patients with active disease than in patients with inactive disease. Early EPC showed significant correlations with CRP and CECs. This study showed no correlation between EPC levels and disease duration, which may be due to shorter disease durations in this group of patients.

Contradictory results have also been reported in other diseases like systemic lupus erythematosus (SLE) and RA. Some studies have proposed lower EPC levels in both SLE and RA, while others have reported higher levels or no difference. Lack of standardised procedures for quantitation of EPC and different characteristics of study groups like disease activity/severity are probably the main reasons for inconsistencies in the studies' results (36-39).

We realise that our study has several limitations, the most important being the small sample size of patients and the absence of a HC group for IMT comparison. Hence, the results should be interpreted with caution. Secondly, the treatment received by some patients might influence the levels of EPC, particularly those with vascular manifestations which received a more intense treatment.

Further prospective and larger studies are needed to provide more confident results about vascular assessment in patients with BD.

In conclusion, our study confirms the utility of the assessment of CFV IMT with Doppler US to evaluate clinical vascular involvement in BD patients. Microvascular involvement is not uncommon in BD and can be easily assessed by NFC. Furthermore, EPC may be a useful blood biomarker of the disease.

Take home messages

What is already known about this subject?

- Macrovascular involvement in BD can be assessed through Doppler ultrasound.
- Microvascular involvement in BD is not uncommon, however, few studies report nailfold capillaroscopy (NFC) and endothelial progenitor cell (EPC) evaluation.

What does this study add?

- We found that intima-media thickness of common femoral vein is increased in vascular BD patients in comparison with non-vascular BD patients.
- NFC reports an abnormal examination in more than half of BD patients. Moreover, EPC levels are increased in comparison with healthy controls.

How might this impact on clinical practice or further developments?

- Doppler ultrasound examination may become a helpful resource to assess vascular involvement of patients with BD in clinical practice.
- NFC can evaluate microvascular involvement in these patients and EPC may become a future biomarker of the disease.

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