Review

Non-invasive vascular biomarkers in patients with Behçet's disease: review of the data and future perspectives

A.D. Protogerou[1,2], E.G. Nasothimiou[1], P.P. Sfikakis[2,3], A.G. Tzioufas[1,2]

[1]*Department of Pathophysiology;*
[2]*Joint Academic Rheumatology Program;*
[3]*1st Dept. of Propaedeutic Internal Medicine, Medical School of the National & Kapodistrian University of Athens, Greece.*

Athanase D. Protogerou, MD, PhD
Efthimia G. Nasothimiou, MD, PhD
Petros P. Sfikakis, MD, PhD
Athanasios G. Tzioufas, MD, PhD

Please address correspondence to:
Prof. Athanase D. Protogerou,
Cardiovascular Prevention and Research Unit,
Department of Pathophysiology,
Medical School,
National and Kapodistrian University of Athens,
75, Mikras Asias Street (Building 16, 3rd floor),
115 27 Athens, Greece.
E-mail: aprotog@med.uoa.gr

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ABSTRACT

Vascular inflammation in small to large veins and arteries contributes substantially to mortality above that of the general population in Behçet's disease. Recent data verified also the presence of accelerated classical subclinical arterial damage (atheromatosis, arteriosclerosis, arterial hypertrophy) even in patients free of overt vascular complications, and may be complementary to that of vasculitis. Early detection of such vascular damage might provide helpful pathophysiological insight and potentially even guide treatment management. Herein, we review the existing literature for each one of the most widely applied non-invasive vascular biomarkers (assessing endothelial dysfunction, atheromatosis/hypertrophy, arteriosclerosis and central haemodynamic parameters) that are clinically used in primary cardiovascular prevention. We aim to: (i) identify early pathophysiological vascular pathways, complementary to vasculitis, in the development of vascular complications and (ii) identify gaps in knowledge and suggest future research topics. We identified evidence of proof of concept for some of the widely applied non-invasive vascular biomarkers (carotid plaques, pulse wave velocity, flow mediated dilatation). Yet, several steps in their clinical validation process are lacking. Extensive vascular phenotyping of a large prospective observational patient cohort with the application of these easy-to-use, low-cost, free of any adverse effect, non-invasive methods should be performed in order to test their ability to provide clinically meaningful guidance regarding the prognosis and treatment of Behçet's disease.

Introduction

Behçet's disease (BD) is a systemic disease, with still unknown aetiology(s) and pathogenesis and varying disease presentations (1), characterised by recurrent oral aphthae, followed by genital ulcers, arthritis, variable skin and ocular lesions, gastrointestinal and central nervous system involvement, as well as, vascular disease (2, 3). Inflammation and vasculitis involving vessels of all sizes is the dominant underlying pathological characteristic of all kind of lesions (3). Vascular involvement or disease (4), a term used to indicate the involvement of small to large vessels (predominantly of veins) (4), but very often of arteries as well (5)), is observed in the 40% of patients with ABD (5).

Mortality in BD, as measured by standardised mortality ratios, is significantly increased - especially in young males - and tends to decrease significantly with disease duration (5). Similarly, "disease burden" from mucocutaneous and articular manifestations, as well as from eye involvement, in BD is usually confined to the early years of the disease's course, suggesting that in many patients these traits of the disease/syndrome "burn out" with time. On the contrary, mortality and morbidity due to central nervous system involvement and vascular disease are exceptions and may appear later, five to ten years after the onset of the disease (5). This time-window is critical in order to apply life saving prediction and prevention strategies, since in the largest prospective 20-year outcome cohort of patients with BD (262 males and 125 females, 9.8% deaths) vascular disease (aneurysm of the pulmonary artery and of the aorta) complications and neurologic involvement related to central nervous system recurrent vasculitis attacks, were the main causes of death (5).

As outlined in the recent 2008 European League Against Rheumatism (EULAR) recommendations for the management of BD (6), the aim of

treatment is twofold: (i) to prevent irreversible damage which leads to increased mortality or permanent disability, and (ii) to prevent exacerbations of the disease (*e.g.* mucocutaneous and skin involvement), which even if they do not lead to increased mortality, they limit the quality of life (6). However, as stated in these recommendations (6) there are no evidence to guide the management of major complications, such as vascular disease in BD (6). Moreover, no evidence on prognostic biomarkers (*i.e.* of parameters that might objectively measure and evaluate the pathogenic processes or pharmacologic responses (7)) in BD exists so far (2). In particular no vascular biomarker exists that might help clinicians to identify those BD patients at risk to develop vascular involvement during the course of the disease in order to optimise vascular prevention strategies.

A recent meta-analysis of the data in BD on the classical pathway of atheromatosis (8) verified the presence of accelerated subclinical arterial plaque presence and reappraised this pathology as a potential complementary to vasculitis cause leading to increased mortality. Evidence also suggest that other classical pathways of arterial damage (arteriosclerosis, arterial hypertrophy) are activated in parallel and might participate in the development of vascular complication in BD (9). The contribution of these classical pathways in the development of vascular complication in BD may be complementary to that of vasculitis. It is therefore relevant to review the literature in BD regarding the current state-of-the-art non-invasive vascular biomarkers that are currently widely applied in the general population, as well as, in other chronic diseases, in order to optimise primary cardiovascular disease (CVD) prevention strategies.

To this end, after briefly (a) discussing the rational of individualised CVD prevention strategies on the basis of non-invasive vascular biomarkers and (b) addressing the effect of inflammation/chronic inflammatory diseases on the vascular properties, the present study reviewed the existing literature in BD for each one of the most widely applied non-invasive vascular biomarkers that are currently used in clinical research and/or practice for the evaluation of endothelial function, arterial hypertrophy/remodeling, arterial stiffness (arteriosclerosis) and subclinical atheromatosis in high CVD risk populations. The aim was twofold: (i) to identify early pathophysiological vascular pathways in the development of vascular complications and (ii) to identify gaps in knowledge and suggest future research topics regarding the potential role of vascular biomarkers in the clinical management of vascular involvement in BD.

The role of non-invasive vascular biomarkers in modern individualised CVD prevention strategies

All international scientific societies (10) recommend the use of CVD risk estimation scores as the first step in the algorithm for the design of individualised CVD prevention strategies. However, these scores need substantial improvement (11, 12). This fact is particularly relevant in the presence of chronic inflammatory diseases such as rheumatoid arthritis (13). In this line of action the modern CVD prevention strategies attempt to provide individualised tailor-made CVD risk prediction by the incorporation of non-invasively acquired vascular biomarkers. In individuals with usual CVD risk factors (hypertension (14), hypercholesterolemia (15), diabetes (16)) in asymptomatic individuals (17-19), as well as in patients with rheumatoid arthritis (20), non-invasively assessed vascular biomarkers are increasingly recommended by the respective scientific societies, as tools that refine the risk/prognosis and guide the management of vascular complications in clinical practice.

A large variety of non-invasively assessed vascular biomarkers have been developed during the past thirty years. In a recent position paper of the European Society of Cardiology we extensively reviewed the most widely applied vascular biomarkers and provided clinical recommendations for primary and secondary CVD prevention (21). Each of these vascular biomarkers provides insightful information on different aspects of the pathology of the vascular damage (endothelial function, arterial remodeling, hypertrophy, arteriosclerosis/stiffness, atheromatosis) in different vascular beds (21). These biomarkers reflect satisfactory early functional or morphological changes before overt disease manifests (21). They provide insight in the pathophysiology and CVD mechanics of each chronic disease and may open a window of opportunity to prevent the occurrence of overt CVD disease by timely treatment (21). Most importantly, specific vascular biomarkers have provided conceiving evidence regarding their ability to refine CVR risk assessment by reclassifying a clinically meaningful number of individuals in the correct CVR risk category (21). Moreover, vascular biomarkers might be used as future surrogate endpoints, instead of clinical endpoints, in order to reduce the cost of clinical trials and to provide evidence on therapeutic strategies in specific populations that cannot be attained with classical clinical endpoints (*e.g.* in adolescents with elevated blood pressure) (21, 22).

According to the American Heart Association (23), each novel risk biomarker (and thus each vascular biomarker) should be evaluated for/by (table): (i) proof of concept (presence of difference between subjects with and without an outcome); (ii) prospective validation (i.e. the ability to predict the development of future outcomes in a prospective cohort or nested case-cohort study); (iii) incremental value (i.e. the ability to provide additive predictive information over and above established/standard risk markers/scores); (iv) clinical utility (i.e. the ability to improve the predicted risk sufficiently to change recommended therapy); (v) clinical outcomes (*i.e.* the ability to improve clinical outcomes, especially when tested in a randomised clinical trial); (vi) cost-effectiveness. This meticulous evaluation should be performed separately in each chronic disease. Of note, in each chronic disease different pathophysiological mechanisms may lead to similar vascular damage, therefore similar vascular responses to different therapeutic intervention are expected.

Inflammation, chronic inflammatory diseases, vascular damage/dysfunction and vascular biomarkers

Since the late 80s when the etiopathogenic role of inflammation on vascular damage appeared (24, 25), compelling evidence on the topic has been gathered. Even a mild acute inflammatory response exerts a detrimental effect on endothelial function (26) and arterial stiffening (27); of note this effect can be prevented in the presence of anti-inflammatory treatment (27). Moreover, early rupture of unstable (*i.e.* vulnerable) plaques in the presence of inflammation might an important cause of acute cardiovascular events (28). It is therefore suggested that these mechanisms might represent a trigger for acute CVD events.

Evidence also support the notion that chronic low-grade inflammation even in healthy individuals (29) or chronic-intermittent high-grade inflammation, such as rheumatoid arthritis (30-33), systemic lupus erythematosus (30), systemic vasculitis (34, 35) leads to endothelial dysfunction and arterial stiffening (*i.e.* arteriosclerosis). Of note, both endothelial dysfunction and arterial stiffening seems to be reversed by chronic anti-inflammatory treatment (33, 35-37).

Moreover and beyond the very early initiating step of endothelial dysfunction, evidence indicate that in inflammation multiple independent pathways (including both the innate and adoptive immune systems) are key regulatory processes that link classical CVD risk factors with plaque development (atheromatosis) and atherothrombosis (38). Therefore, it comes as no surprise that numerous studies revealed the acceleration of the atheromatic processes in chronic inflammatory diseases, such as rheumatoid arthritis (30, 32, 33, 39-41) and in systemic lupus erythematosus (42, 43).

The clinical translation of early vascular damage requires the meticulous step-by-step evaluation as described above. Evidence regarding the prospective validation of subclinical atheromatosis (plaque presence) exist in rheumatoid arthritis (44), systemic sclerosis (45) and systemic lupus erythematosus (45), suggesting that this pathway may participate in the increase of CVD morbidity/mortality observed in these diseases. Similar data regarding the prospective validation of arteriosclerosis (stiffness) biomarkers exist only in rheumatoid arthritis (46). However, data regarding the incremental value and clinical utility of all the vascular biomarkers in chronic inflammatory joint and/or autoimmune diseases are missing. What is more the elucidation of the exact pathophysiological pathways and the quantification of their contribution in vascular disease in each inflammatory disease has not been achieved yet, even for rheumatoid arthritis the most extensively studied chronic inflammatory disease in terms of CVD risk (47).

Non-invasive vascular biomarkers of endothelial function in BD

The endothelium holds a pivotal role in cardiovascular heath and disease. During the last 20 years several methodologies have been developed for the non-invasive in vivo assessment of endothelial function in clinical research at the level of both the micro- and macrocirculation (48). These methods, as reviewed in a recent position statement by the European Society of Cardiology (48), are mainly based on the monitoring of vasomotion (by changes in diameter, volume or flow) after stimulation of the endothelium. Although these methods provide useful insight regarding the pathophysiology of vascular disease and effect of pharmacological and non-pharmacological treatments on the endothelium, none of them have been considered useful so far for clinical practice by any international recommendation board, mainly due to reproducibility, methodological issues, lacking of reference values (21). However, their usefulness as clinical research tools is widely accepted.

Endothelial dysfunction of the macrocirculation, as assessed by flow-mediated dilatation (FMD) at the level of the brachial artery in a cross-sectional study, was reported to be impaired 15 years ago in patients with active BD (49). In the same study the investigators demonstrated that endothelial dysfunction was acutely reversible by ascorbic acid underlying the cardinal role of increased oxidative stress in the impairment of endothelial function. The impairment in FMD was verified by subsequent studies (40-52). FMD is predominantly impaired in BD patients with vascular involvement (51) and it is modulated by the interaction of disease activity with corticosteroid use (52). After careful dissection of observational cross-sectional and longitudinal data analysis (48) it was revealed that FMD is impaired in the presence of active disease and that this effect is reversed by corticosteroid treatment. This finding generated the hypothesis that increased inflammatory burden during the relapse of BD is associated with impaired endothelial function that can be reversed by the anti-inflammatory effect of corticosteroid treatment. On the contrary, in patients with inactive BD (*i.e.* with low or normal inflammatory burden) who receive (even low dose) chronic corticosteroid treatment, the endothelial function was impaired. These findings suggest that the prolonged continuation of chronic corticosteroid use in the absence of active disease has a detrimental effect on FMD (52). This effect might be partly related either to the direct effect of corticosteroids on the arterial wall (52).

A very recent meta-analysis (8), that included most of the available studies (51, 53-59), verified that endothelial function is impaired at the level of the macrocirculation, as assessed by FMD. Of note, in line with our previous finding (52), FMD was more profoundly impaired in the presence of active (during a relapse) disease but still found impaired in inactive BD patients, *i.e.* during remission.

Data reporting impaired endothelial function at the level of the microcirculation on the basis of vasoactive vascular methodology are currently very limited. Gullu *et al.* (60) reported that coronary flow reserve - an index of coronary endothelial dysfunction - is impaired in BD. Of note, during the active disease period (61) coronary microvascular function more prominently impaired; therefore it is possible that BD patients are more vulnerable to

manifest CVD events during relapses of disease.

Data on the ability of FDM or any other vascular endothelium biomarker to predict clinical outcomes, to reclassify vascular risk and to guide treatment in BD are not available. Overall, the current data provide proof of concept regarding the presence of impaired endothelial function in BD, especially during active phases suggesting potentially increased CVD risk during relapses of BD, but even during inactive phases suggesting residually increased CVD risk due to treatment effects or other underlying pathology. However, no evidence is available on the ability of any biomarker that describes endothelial dysfunction to predict the incidence of vascular complications, CVD mortality or overall mortality in BD.

Non-invasive vascular biomarkers of atheromatosis and arterial hypertrophy in BD

In the absence of clinically overt CVD the subclinical atheromatosis is widely studied both in clinical practice and research by the use of high-resolution ultrasound and the measurement of common carotid intimal-medial thickness (cIMT) and/or carotid plaque presence (62). Although cIMT and carotid plaque cannot be used always interchangeably – because the former may represent either carotid wall hypertrophy or atheromatosis whereas carotid plaques represent per se atheromatosis (21, 63) – these two vascular biomarkers are not always clearly discriminated in the literature especially in early studies. Carotid ultrasound is relative easy in use after reasonable training on the basis of existing methodological consensus and reference values (21). It is officially recommended – especially in intermediate CVD risk populations (21) including rheumatoid arthritis (20) for CVD risk optimisation.

A recent meta-analysis (8) that included all the available studies (51, 55, 57, 59, 64-73) showed that cIMT is increased in BD compared to controls. Similarly, the same meta-analysis (8) showed that carotid plaques are 3 times more prevalent in BD compared to the control group (65-70, 72). These results verify the presence of accelerate subclinical atheromatosis in BD, suggesting that this pathology might explain the presence of increased CVD mortality in this population. The previously described endothelial dysfunction in BD as well as the intermittent inflammation (vasculitis), autoimmune mechanisms and finally drugs may all contribute to the acceleration of atheromatosis in BD.

However, no data are at present available in BD regarding the ability of cIMT or carotid plaques to predict clinical outcomes, to reclassify vascular risk and to guide treatment. Moreover, cIMT in other arterial beds beyond the carotid arteries has not been investigated in BD. Given that fact that evidence suggests the predilection of chronic inflammatory disease (74) to accelerate atheromatosis in specific arterial bed, such studies are of interest in order to identify the overall atheromatosis burden and CVD risk.

Non-invasive vascular biomarkers of arterial stiffness (arteriosclerosis) in BD

Arterial stiffness can be easily and reproducible measured in clinical practice by several non-invasive methods and reference values are available for carotid to femoral pulse wave method, which is currently proposed as the gold-standard method to measure arteriosclerosis (75). This method is officially recommended for CVD risk assessment optimisation mainly in intermediate CVD risk populations (21).

Arterial stiffness, most often assessed by carotid to femoral pulse wave velocity, has been evaluated in eleven studies from seven different BD cohorts (64, 67, 73, 76-82). Independently from the evaluated arterial bed (ascending aorta, abdominal aorta or carotid artery), all studies but one (78), concluded that arterial stiffness is increased in patients with BD compared to controls. Of note, arterial stiffness was found to be equally increased in BD when compared to patients with rheumatoid arthritis and systemic lupus erythematosus (81), *i.e.* with two chronic inflammatory diseases with well-described increased incidence of CVD mortality. The previously described endothelial dysfunction found in BD, the intermittent vessel wall inflammation, as well as autoimmune mechanisms and BD-related drugs may all contribute to the acceleration of arterial stiffening in BD. Whether vasa vasorum subclinical vasculitis is valid mechanism, as suggested for rheumatoid arthritis (83), related to large arteries stiffening has to be investigated in BD.

Although the available studies have several limitations, mainly related to their small sample size and beside the fact that the available data have not been so far meta-analysed, the overall evidence suggest that accelerated arterial stiffening is present in BD. Increased arterial stiffness might therefore represent – as previously proposed by in vitro and clinical data from other clinical conditions – an additional mechanical trigger leading to arterial aneurysm formation/rupture (84, 85), vascular thrombosis (86-90), as well as, in central nervous system involvement in BD.

Non-invasive biomarkers of aortic blood pressure and pressure wave reflections in BD

Several non-invasive techniques and methods permit the reproducible and accurate non-invasive assessment of aortic blood pressure as well as of pressure wave reflections (91) even in ambulatory conditions (92). The use of both aortic blood pressure and pressure wave reflections is recommended for CVD risk assessment optimisation (21); easy to use techniques do exits, with reasonable methodological consensus and European population based references (21).

Scarce data on aortic blood pressure and pressure wave reflections (as assessed by augmentation index) are available in BD, using pulse wave analysis methods and techniques. We have previously shown that, in patients free of corticosteroids and inactive BD, aortic systolic blood pressure and pressure wave reflections were increased compared to the control group (93). Of note, the brachial systolic blood pressure was similar between the two groups suggesting that the conventional brachial cuff based blood pressure meas-

urement fails to detect haemodynamic changes in this population. On the contrary, in active BD both aortic systolic blood pressure wave reflections were found decreased, even though aortic stiffness was further increased compared to controls and BD with inactive disease (93). Although there are no solid evidence to explain these haemodynamic alterations in active and inactive BD we might speculate the following mechanisms: (i) the well-described increase in arterial stiffness present in BD is a major cause for increased pressure wave reflections and aortic blood pressure (93) (ii) a permanent vasculitis-associated distraction of the microcirculation might increase pressure reflection coefficients from distal arterial sites (*e.g.* at the level of the very small arteries) leading to increased pressure wave reflections and increase predominantly in aortic systolic blood pressure; (iii) on the other hand, during relapse and increased activity of BD a transient decrease in distal reflection coefficients may take place due to peripheral vasodilation. A recent study using a novel 24-hour ambulatory methodology to assess pressure wave reflections and aortic blood pressure (82) showed similar trends in pressure wave reflections and aortic blood pressure between active and inactive ABD patients, but the differences did not reach statistical significance. The confounding effect of corticosteroid treatment may explain this discrepancy since patients under corticosteroids were not excluded from this study (82).

Limitations

As already partly discussed, there are limitations in this attempt of ours to review and dissect the association BD with early vascular dysfunction/damage, as assessed by the most widely applied non-invasive vascular biomarkers. The detailed study of the molecular pathways leading to arterial atheromatosis and arteriosclerosis in BD was beyond the scope of the present review. Moreover, the exact mechanisms remain largely unknown and difficult to dissect. The main reasons for that are: (i) to the multifactorial nature of the vascular disease, including potential difference in genetic predisposition in each inflammatory disease and particularly in BD (ii) the presence of CVD comorbidities (iii) the direct effect on the arterial wall by drugs used for BD treatment (e.g. corticosteroids), (iv) the intermittent and fluctuating nature of the inflammation during disease duration, which can not be easily monitored either due to the lack of proper study design methodology or due to the lack of inflammatory biomarkers that can monitor effectively BD relapses/activity (94) and last but not least, (v) the interaction between age, disease duration and all the aforementioned factors. All these factors (underlying molecular pathways, CVD comorbidities or drugs) might be regarded as BD "intrinsic" characteristics that contribute to the development of vascular damage, beyond the effect of systemic or local inflammation and vasculitis.

Other methodological issues should be also addressed. Most of the data presented derive form small cohorts, due to the absence of an international network dedicated to the study of vascular properties. Moreover, several studies were without appropriate control group or appropriate statistical adjustments for potential confounders. Finally, the interpretation of findings was often misleading due to the limited experience on vascular biomarkers by the authors.

Finally, it should be acknowledged that the currently available and herein discussed non-invasive vascular biomarkers are dedicated tools for the study of arterial disease. On the other hand, in BD vascular disease involvement of small to large vessels affects predominantly the veins (4) (although very often the arteries as well (5)). However, many of the arterial biomarkers described in this review have close associations with venous complications and not only arterial pathologies (86, 90), potentially due to the systemic nature of the vascular diseases.

Unanswered questions and future research

The present review identified numerous unanswered questions and topics for future research in BD related to the discussed vascular biomarkers. The main ones are shortlisted below:

- Do they associate with the presence of vascular disease and complications in BD?
- To what extend BD-related treatment can deteriorate or improve them?
- Can they be used to monitor BD activity/replaces?
- Can they identify patients at higher risk to develop vascular events and vascular death?
- Can they effectively reclassify BD patients at higher CVD risk beyond the current widely applied risk models and be used to guide treatment?

Conclusion

The present review showed evidence of potential future clinical value in BD for some of the discussed widely applied non-invasive vascular biomarkers. However none of them can be currently recommended for clinical use since several steps in their clinical validation process are still lacking in patients with BD (Table I). In the future research part, follow-up studies for the determination of the value of the vascular biomarkers in patients with BD are needed. Endothelial dysfunction, mainly assessed by FMD, has valid proof of concept in BD; however it remains of limited clinical value in BD, as well as in the general population, due to methodological drawbacks. Subclinical atheromatosis, measured by carotid ultrasonography, has also proof of concept in BD; it is currently the most promising vascular tool for future use in order to reclassify vascular risk in patients with BD. Arterial stiffness, as assessed by carotid to femoral pulse wave velocity has also potentially proof of concept but further evidence and meta-analysis of the data is needed. Other vascular biomarkers such as aortic blood pressure and pressure wave reflections have been scarcely investigated at the moment.

In a clinical research setting extensive vascular phenotyping of a large prospective observational cohort of BD patients with the application of the discussed easy-to-use, low-cost, free of any adverse effect, non-invasive methods (applanation tonometry and ultrasound) should be performed. Such a

Table I. Summary of the evidence on major vascular biomarkers in Behçet's disease (*meta-analysis available).

	Proof of concept	Prospective validation	Incremental value	Clinical utility	Clinical outcomes	Ease of use	Methodological consensus	Reference values
Carotid ultrasonography	+++*	-	-	-	-	++	++	Yes, for cIMT measured with the echotracking method.
Arterial stiffness Carotid-femoral pulse wave velocity	++	-	-	-	-	+++	+++	Yes
Aortic blood pressure / wave reflections	+	-	-	-	-	+++	+++	Yes for aortic blood pressure
Endothelial function Flow mediated dilatation	+++*	-	-	-	-	+	++	No

study will provide the needed information about the predictive value of the discussed biomarkers and will test their ability to provide clinically meaningful guidance regarding the prognosis and treatment of patients with BD.

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