The role of endothelial dysfunction and inflammation in chronic venous disease

Ricardo Castro-Ferreira, MD[1,2]

Rita Cardoso[1]

Adelino Leite-Moreira, MD, PhD[1]

Armando Mansilha, MD, PhD[2]

[1] Departamento de Cirurgia e Fisiologia, Unidade de Investigação Cardiovascular, Faculdade de Medicina da Universidade do Porto, Portugal

[2] Serviço de Angiologia e Cirurgia Vascular, Centro Hospitalar São João, Portugal

Corresponding Author:

Armando Mansilha, MD, PhD

Departamento de Cirurgia e Fisiologia, Faculdade de Medicina da Universidade do Porto, Alameda Prof. Hernâni Monteiro, 4200–319 Porto, Portugal

E-mail: vascular.mansilha@gmail.com

Key Words

Chronic Venous Disease, varicose veins, endothelial dysfunction, inflammation

Abstract

Chronic venous disease is a potentially prevalent and debilitating condition affecting millions of individuals, mostly in western world. Predisposing genetic and environmental factors contribute to its development. However, the main etiology remains to be elucidated. An extensive literature search was conducted in Medline using the following key words algorithm: ("Chronic venous disease" OR "Chronic venous insufficiency" OR "varicose veins") AND ("endothelial dysfunction" OR "inflammation"). Besides being a multifactorial disease, it is now recognized that the hallmark of chronic venous disease pathophysiology likely remains in inflammation, possibly triggered by sustained venous hypertension and valvular incompetence. Shear stress changes are directly sensed by endothelial cells, leading to its activation and subsequent recruitment of leukocytes and release of pro-inflammatory agents. Dysfunctional endothelium has a pivotal role perpetuating the inflammatory cascade, with consequent pathological venous changes and chronic venous disease worsening. Endothelial dysfunction may be the central player in the link between varicose veins and deep vein thrombosis. In this article we aim to analyze the crucial role of endothelial activation in the everlasting inflammatory cycle that characterizes chronic venous disease.

1. Introduction

Chronic venous disease (CVD) is a common condition with a wide spectrum of clinical presentations[1]. Structural and functional alterations in healthy veins lead to symptoms and signs usually seen in CVD. Symptoms often include aching, heaviness, a feeling of swelling, cramps, burning, tingling, and restless legs, while signs include telangiectases, varicose veins, edema, and skin changes or venous ulcers[1]. CVD has been reported to have a prevalence of up to 40% in females and up to 17% in males, depending on the geographic region and the disease classification[2]. Since its prevalence is higher in developed countries than in underdeveloped ones, CVD has been recognized as a Western disease. At these regions, its high prevalence has subsequent important costs in investigations, treatment, morbidity and associated loss of working days, all contributing to the socioeconomic impact of CVD[3].

Between risk factors are increasing age, female gender, pregnancy, family history, obesity and prolonged standing position[2]. Besides environmental factors predisposing to CVD, including varicose veins (VV), several genetic conditions have been associated with its development. Those include, chromosomal abnormalities, genetic mutations and nucleotide polymorphisms[4]. Trissomie of chromosome 7, 12 and 18 and monossomie of 14; congenital disorders such as Klippel-Trenaunay syndrome (translocation 8:14 or 5:11), cerebral autossomic dominant arteriopathy with subcortical infarcts and leukoencephalopaty (CADASIL, associated with Notch3 mutations),

lymphoedema distichiasis (FOXC2 gene mutation), Ehlers-Danlos syndrome (COL3A1 gene mutation) and Chuvash polycythemia (VHL gene mutation) are related to CVD[4]. Hemocromatosis gene mutation and factor XIII gene variants have been found in patients with VV, possibly predisposing to more severe forms of CVD[4]. Moreover, several other genetic abnormalities have been described, including polymorphisms of MMP-9, TIMP-2 and COL1A2 genes, thrombomodulin gene mutations and others[4]. However, the majority of patients with CVD do not have any of the aforementioned alterations and a specific gene responsible for VV development has never been described.

Last two decades has been rich in investigation about CVD and its development mechanisms[2]. In order to improve communication among physicians, decision making, reporting and diagnosis, the Clinical–Etiology–Anatomy–Pathophysiology (CEAP) classification system was developed and is often used to classify clinical presentation of CVD in C_0 to C_6 stages[3]. The burden of CVD also motivated development of instruments useful in evaluation of quality of life (QoL) impairment. One of the most popular is a 20-item self-reported instrument, the Chronic Venous Disease Questionnaire (CIVIQ-20), firstly designed and validated by Launois in 1996[5], and recently simplified on a 14-item format combining social dimension with three key parameters: pain, physical and psychological (CIVIQ-14)[6]. CIVIQ is widely accepted as

a sensitive instrument detecting the pivotal dimensions of QoL impaired by disease and changes with treatment. It has already been validated in seventeen linguistic versions[7].

The main etiology of CVD has not yet been clarified. Despite of a variety of mechanisms being proposed, it is well established that valve reflux is a key determinant of CVD with an ongoing discussion about whether valve dysfunction is an initial event triggering venous disease or if it is a secondary event to venous wall remodeling[8].

Actually, recent studies support venous wall and valvular damage as one of the major players, with evidence suggesting that primary vein wall changes may precede valvular incompetence. Further investigations focusing these subject has shed lights on the role of abnormal distensiblity of the connective tissue as the basis of venous dilation and insufficiency, with an altered synthesis and increased removal of collagen in the vein wall, secondary to an MMP/TIMP imbalance[9-11].

More recently, research has unearthed the role of inflammation and subsequent localized endothelial activation/dysfunction, by reducing the synthesis of anti-inflammatory agents while enhances the expression of pro-inflammatory and pro-thrombotic molecules[12-15]. Venous reflux is thought to be the cause of venous hypertension[3]. Its consequence is the reduction of shear stress (SS), a key regulator of endothelial activation state[3, 163(3, 16(16)3(3, 16(16)](3, 16)(3, 16)(3, 16), which promotes vein wall and venous valve pathological changes[17-19]. In fact, SS reduction triggers activation of endothelial cells (EC) and leukocytes, enhancing the expression of adhesion

molecules and the infiltration of inflammatory cells into venous wall and leaflets, thus establishing an environment that promotes local inflammation[18, 19]. Changes in EC normal signaling through different pathways culminate in production of inflammatory mediators such as chemokines, cytokines, growth factors, proteases, and others that further worsens and perpetuates inflammation[4].

A review centered in endothelial dysfunction in CVD not only clarifies its key role in venous disease pathophysiology but also enlightens the more obscure connection of VV to other vascular diseases. This article aims to summarize the current evidence regarding the pivotal role of endothelial activation in the everlasting inflammatory cycle that characterizes CVD.

2. Evidence in favor of a primary role of venous wall changes in CVD pathogenesis

Recent studies on CVD pathogenesis have focused on the structural and biomechanical changes in the vein wall[11]. In fact, despite the investigators agreement that valve reflux and venous hypertension are major players in development of VV, there is no consensus as to whether primary valve insufficiency is the triggering event or the insufficiency is secondary to vein wall remodeling and dilation (Fig. 1)[10, 11].

Actually, there is evidence indicating that venous reflux and hypertension is likely due to pathological changes of vein walls and subsequent venous weakening and

dilation resulting in incompetence of the valves[8]. Supporting this hypothesis, varicose veins are often observed below competent valves, and commonly precede valve incompetence[10]. Venous dilation is also more frequently seen distal to a valve rather than proximal, which would be more likely if valvular dysfunction is the initial event[10]. Moreover, a study found that dilated segments were proximal to competent venous valves and adjacent to a normal appearing distal vein segment. Notably, in VV patients, normal appearing segments of vein just adjacent to the varicose vein had the same biochemical profile as that of the diseased segment[20].

The trigger for such structural and functional changes in the vein wall and valves remains unclear, although inflammatory events triggered by hemodynamic abnormalities may play an important role in venous disease etiology[10, 21]. The underlying hypothesis is that CVD results from alterations in cellular and extracellular matrix components, causing weakness and altered venous tone[10]. Since these vein wall changes were demonstrated to be associated with inflammation and subsequent endothelial activation[1], one may think that endothelial dysfunction is an early and key event in CVD pathogenesis (Fig.1).

3. The role of endothelial activation / dysfunction in CVD pathogenesis

The vascular endothelium has paracrine, endocrine and autocrine actions with a central role in maintenance of cardiovascular homeostasis[12]. EC are capable of detect minimal changes in their biomechanical environment, including alterations in SS, vessel distension or stiffness of extracellular matrix (ECM) and then produce a variety of signals and factors in order to maintain vascular integrity[12, 13].

Healthy endothelium has an anti-inflammatory action, being able to sense changes in hemodynamic forces and react by synthesizing and releasing vasoactive substances such has nitric oxide (NO) or prostacyclin (PGI2)[13]. However, when diseased, production of anti-inflammatory molecules decreases, whereas the expression of pro-inflammatory and pro-thrombotic molecules is enhanced. Damaged endothelium is also characterized by an imbalance in bioavailability of vasodilators and endothelium-derived contracting factors (Figure 1)[12].

In fact, persistently elevated venous pressure is associated with maladaptive remodeling of venous wall, with the increase in wall stress contributing to the development of enlarged and tortuous veins[22-24]. Moreover, there is a proliferation of EC and smooth muscle cells (SMC) and also increased expression of MMP–2, observations already made in human samples of varicose veins[24]. Activation of the

transcription factor activator protein 1 (AP-1) seems to be necessary for venous remodeling and MMP–2 expression in this context, as its blockade abolished all these processes[24]. It is known that stretch-stimulated EC or SMC increase the expression and activity of some NADPH oxidases, increasing the production of ROS, such as hydrogen peroxide, which activates AP-1[25]. Thus, alterations in biomechanical forces occurring in CVD could trigger endothelial activation, thus promoting the production of ROS and subsequent maladaptive venous remodeling.

Indeed, the role of biomechanical forces in venous remodeling and whether such hemodynamic modifications are cause or consequence of CVD has been extensively discussed[11]. The increased diameter and remodeling of ECM observed in VV may likely result from reduction in SS. Thus, similarly to other several cardiovascular diseases, the maintained alteration in SS appears to underlie CVD development[25].

Healthy endothelium is influenced by laminar SS which enhances the expression and activity of endothelial nitric oxide synthase (NOS), promoting NO production. NO inhibits proliferation of SMC and stimulates their relaxation by promoting cGMP production, thus leading to vasodilatation. NO also have recognized anti-inflammatory effects, as it is capable of inactivating ROS, agents with well characterized pro-inflammatory actions[23].

Consequently, in areas of disturbed blood flow, such as varicosities, a decreased SS acts on EC, producing a variety of pro-inflammatory responses and contributing to

maladaptive remodeling of the vessel wall. Notably, EC of veins are exposed to an overall level of SS that is much lower than EC of arteries, which was proposed to limit the venous capability to release NO and thus to counteract pathophysiological remodeling processes[23].

Furthermore, the endothelium regulates venous tone by releasing several vasoactive substances, some constricting and others relaxing the veins[12]. Imbalance of these vasoactive substances may contribute to the overall relaxation of varicose veins[26]. A reduction in factors contributing to vasoconstriction such as the noradrenaline, endothelin-1 binding and endothelin-B receptor density, probably due to endothelial injury, has been shown in varicose compared with non-varicose veins[10]. Moreover, factors that induce vasodilatation, such as NO, PGI2, endothelium-derived hyperpolarizing factor, were found to be upregulated in VV[11, 26]. In fact, in normal tissue, the inducible NOS (iNOS) is produced only at low levels. However, the expression of iNOS is increased in VV tissue, particularly at the tortuous segments, perhaps contributing to enhanced vasodilatation observed in VV[1].

Carrasco et al.[14] have investigated the endothelial function in human segments of VV, by evaluating the relaxation response induced by acetylcholine (Ach). Endothelium-dependent relaxation in response to Ach in VV was lower when compared with control segments (Fig.2). Furthermore, they demonstrated that lower concentrations of Ach are capable of a progressive endothelium-mediated relaxation,

which is likely due to release of endothelial relaxing factors[14]. However, at higher concentrations, the relaxing response of the diseased endothelium was replaced by SMC contractile responses, through activation of muscarinic receptors. Therefore, these findings support the presence of endothelial dysfunction in VV, by demonstrating impairment in endothelium-induced relaxation. In addition, they found that escin, an established antivaricose agent, also displays venous endothelial protection, unearthing novel mechanisms by which venoactive drugs might improve CVD symptoms[14].Studies demonstrating an abnormal increase in circulating EC suggest the presence of, not only local endothelial damage, but the extent of the disease to a systemic level[27]. Cesarone et al.[28], evaluated the levels of circulating EC before and after a 4-week treatment with a venoactive drug acting on capillary filtration and endothelium. They found a significant decrease in the number of circulating EC in patients with CVD, after treatment[28]. Thus, besides providing evidence about endothelial dysfunction in CVD, these investigations also demonstrate novel drugs protective function of the damaged endothelium.

On the other hand, when diseased or activated, endothelium promotes the release of agents that stimulate thrombosis, such as von Willebrand Factor[29], plasminogen activator inhibitor-1[30] and FVIII[31] as well as inflammation, as C reactive protein[29] and interleukin-6 (Fig. 2)[29]. These agents are biomarkers of endothelial dysfunction[15], and their levels correlate with a higher cardiovascular risk[32]. It has also been described that normal venous endothelium can become dysfunctional and release pro-

thrombotic/inflammatory factors when exposed to increased endoluminal pressure[33]. Since CVD is a condition characterized by a sustained increased in venous pressure, with stasis or reversal of blood flow in affected vessels[10], it gather conditions that may promote a pro-thrombotic state of the endothelium. The release of pro-thrombotic agents by activated endothelium might be related to the recent correlation established between VV and deep vein thrombosis[34]. In that way, since arterial disease, such as atherosclerosis, also relates with a pro-thrombotic and pro-inflammatory environment, with accumulating evidence on the role of endothelial dysfunction[35], a parallelism between arterial and venous disease could be hypothesized. In fact, one may assume that endothelial dysfunction is a major player in both CVD and atherosclerosis pathophysiology. However, in veins, instead of an atherosclerotic plaque formation, venous wall dilation and insufficiency would be promoted.

4. The role of inflammation and leukocyte-endothelium interaction

Recent advances in the understanding of CVD pathogenesis have also focused on leukocyte-endothelium interactions and progression to inflammation, highlighting their role in vein wall remodeling and valve failure[11, 22].

The trigger for the inflammatory process is thought to be an abnormal venous flow[21]. In fact, it is likely that sustained venous hypertension and subsequent stasis lead to distension of vein wall and distortion of venous valves, causing venous flow reversal

and regions of low SS. The exposure of EC to flow reversal triggers their activation, whereas leukocytes are activated by reduced SS. The subsequent leukocyte-endothelium interaction is the starting point of the inflammatory cascade(Fig. 3)[21].

The endothelium activation induces the expression of inflammatory mediators, such as cytokines as well as leukocyte rolling, adhesion and migration through the endothelium of vein wall and valve is enhanced[4]. Cytokines are present in all inflammatory processes and a shift in their expression pattern was already described in CVD. Transforming growth factor β1 (TGF-β1), tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) were found to be increased in VV, being important in triggering the inflammatory cascade responsible for vascular remodeling[4]. It was also demonstrated that TGF-β1 is increased in the tortuous segments of varicose veins, as compared with non-tortuous segments[1]. Additionally, increased VEGF was already described in patients with CVD[1]. These findings indicate that cytokines released during inflammation response may have a role in structural changes occurred in wall of VV.

Moreover, a recent study analyzed the expression pattern of circulating levels of several cytokines/chemokines in CVD patients[36]. It was found that EGF, PDGF and RANTES blood levels were increased in VV, in comparison with blood from general circulation in CVD patients, indicating that these cytokines may be sensitive biomarkers. Since RANTES has chemotactic functions for leukocytes, it may represent a link between the endothelial activation and the inflammatory process[36].

The activation of endothelium during inflammatory response also leads to enhanced production of several adhesion molecules that contribute to leukocyte activation and recruitment. Basal plasma levels of intercellular adhesion molecule 1 (ICAM-1), endothelial leukocyte-adhesion molecule 1 (ELAM-1), and vascular-cell adhesion molecule 1 (VCAM-1) were found to be elevated in patients with CVD when compared with healthy subjects. The levels increased significantly in response to venous hypertension induced by standing[1].

Additionally, the intense leukocyte infiltration is observed in CVD patients, mainly in response to the elevation of blood pressure[37]. Ono et al[22] found infiltration of valve leaflets and venous wall by monocytes and macrophages in all vein specimens from patients with CVD and in no specimens from healthy patients. Infiltration was associated with areas of endothelium that expressed ICAM-1[22]. Saharay et al.[38] demonstrated that, after venous hypertension induction in patients with CVD, levels of L-selectin and the integrin CD11b on circulating neutrophils and monocytes decreased, reflecting the sequestration of these cells in the microcirculation[38]. Simultaneously, plasma levels of soluble L-selectin increased, reflecting enhanced leukocyte adhesion to endothelium[38]. In addition, it has been demonstrated that plasma from patients with CVD induces more leukocyte activation when compared with healthy patients[19].

Thus, these data provide evidence about the enhanced adhesion of leukocytes and its sequestration in tissues of patients with CVD submitted to increased levels of

venous pressure. The leukocyte trapping and extensive leukocyte activation in CVD patients is likely responsible for production and releasing of cytotoxic substances which lead to venous wall reconstruction and valvular damage[19]. This inflammatory process secondarily leads to progressive valvular incompetence, reflux and venous hypertension which further promote the maintenance of an inflammatory reaction on the venous endothelium[21]. This sequence of events induces chronic and recurring injury of the vein wall and perpetuates disease progression.

Takase et al.[39] found enhanced markers of inflammation such as granulocytes, monocytes, macrophages and lymphocytes and an increased expression of P-selectin and ICAM-1 in a rat model of venous hypertension. MMP-2 and MMP-9 levels were also elevated[39]. Morphologic changes in the valves such as reduction in leaflet height and width, and disappearance of some valves were observed, as well as dilatation of the venous wall[39]. These alterations compromised valvular function to the point of incomplete valve closure and subsequent reflux. They proposed that the chronic elevation of venous pressure possibly triggers an inflammatory response in venous valves, in which granulocytes, monocyte/macrophages and EC become activated so that leucocytes become attached to the endothelium of the vein wall and valve leaflets. As a consequence, apoptosis and/or necrosis may be induced as well as activated inflammatory and EC may degrade extracellular matrix components by releasing oxygen free radicals and MMPs[39].

In addition, in patients with CVD, there is a reduction in number of valves in great saphenous veins, as well as an abnormal structure. Alterations in valves include stretching, splitting, tearing, thinning, and adhesion of valve leaflets. Reduced SS together with a sustained elevation in pressure can initiate an inflammatory cascade, with subsequent valve damage and remodeling[19].

Overall, these evidences suggest that inflammatory processes involving leukocyte–endothelial interactions can be triggered in response to abnormal venous flow and may play an important role in causing venous dilation and insufficiency.

5. Conclusion

Research made on the last two decades allowed a great advance in understanding CVD. However, clinical and basic studies all overlie that CVD results from a complex interplay of multiple mechanisms that cannot be thought as individual processes. The current state of art indicates that vein wall dilation precedes valve dysfunction, triggering a mechanical feedback that further increases venous pressure and contributes to chronic and progressive venous disease and VV formation. Moreover, hemodynamic forces, such as venous hypertension and reduced SS, acting on the vein wall, likely initiates an inflammatory cascade, which promotes leukocyte-endothelium interaction, endothelial activation and releasing of pro-inflammatory and pro-thrombotic mediators, contributing to the maintenance of an inflammatory/thrombotic environment. The

development of drugs specifically aimed to stop this endothelial dysfunction/inflammation feedback might be pivotal in the treatment of venous disease. The wide prevalence of CVD and morbidity associated make it imperative to fully understand the mechanisms involved in its pathogenesis. Thus, more investigation is needed in order to clarify the precise role of endothelial dysfunction and inflammation in CVD triggering and progression.

6. Conflict of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

7. Funding

The authors received no special grant from any funding agency in the public, commercial or non-profit sectors.

8. References

1. Pocock ES, Alsaigh T, Mazor R, et al. Cellular and molecular basis of Venous insufficiency. Vascular cell. 2014;6(1):24.

2. Beebe-Dimmer JL, Pfeifer JR, Engle JS, et al. The epidemiology of chronic venous insufficiency and varicose veins. Annals of epidemiology. 2005;15(3):175-84.

3. Nicolaides AN. Investigation of chronic venous insufficiency: A consensus statement (France, March 5-9, 1997). Circulation. 2000;102(20):E126-63.

4. Raffetto JD, Mannello F. Pathophysiology of chronic venous disease. International angiology : a journal of the International Union of Angiology. 2014;33(3):212-21.

5. Launois R, Reboul-Marty J, Henry B. Construction and validation of a quality of life questionnaire in chronic lower limb venous insufficiency (CIVIQ). Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation. 1996;5(6):539-54.

6. Launois R, Le Moine JG, Lozano FS, et al. Construction and international validation of CIVIQ-14 (a short form of CIVIQ-20), a new questionnaire with a stable factorial structure. Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation. 2012;21(6):1051-8.

7. Launois R, Mansilha A, Lozano F. Linguistic validation of the 20 item-chronic venous disease quality-of-life questionnaire (CIVIQ-20). Phlebology / Venous Forum of the Royal Society of Medicine. 2014;29(7):484-7.

8. Clarke GH, Vasdekis SN, Hobbs JT, et al. Venous wall function in the pathogenesis of varicose veins. Surgery. 1992;111(4):402-8.

9. Sansilvestri-Morel P, Rupin A, Jaisson S, et al. Synthesis of collagen is dysregulated in cultured fibroblasts derived from skin of subjects with varicose veins as it is in venous smooth muscle cells. Circulation. 2002;106(4):479-83.

10. Lim CS, Davies AH. Pathogenesis of primary varicose veins. The British journal of surgery. 2009;96(11):1231-42.

11. Raffetto JD, Khalil RA. Mechanisms of varicose vein formation: valve dysfunction and wall dilation. Phlebology / Venous Forum of the Royal Society of Medicine. 2008;23(2):85-98.

12. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. Arteriosclerosis, thrombosis, and vascular biology. 2003;23(2):168-75.

13. Szmitko PE, Wang CH, Weisel RD, et al. New markers of inflammation and endothelial cell activation: Part I. Circulation. 2003;108(16):1917-23.

14. Carrasco OF, Ranero A, Hong E, et al. Endothelial function impairment in chronic venous insufficiency: effect of some cardiovascular protectant agents. Angiology. 2009;60(6):763-71.

15. Constans J, Conri C. Circulating markers of endothelial function in cardiovascular disease. Clinica chimica acta; international journal of clinical chemistry. 2006;368(1-2):33-47.

16. White SJ, Hayes EM, Lehoux S, et al. Characterization of the differential response of endothelial cells exposed to normal and elevated laminar shear stress. Journal of cellular physiology. 2011;226(11):2841-8.

17. Anwar MA, Shalhoub J, Lim CS, et al. The effect of pressure-induced mechanical stretch on vascular wall differential gene expression. Journal of vascular research. 2012;49(6):463-78.

18. Takase S, Bergan JJ, Schmid-Schonbein G. Expression of adhesion molecules and cytokines on saphenous veins in chronic venous insufficiency. Annals of vascular surgery. 2000;14(5):427-35.

19. Takase S, Schmid-Schonbein G, Bergan JJ. Leukocyte activation in patients with venous insufficiency. Journal of vascular surgery. 1999;30(1):148-56.

20. Kirsch D, Dienes HP, Kuchle R, et al. Changes in the extracellular matrix of the vein wall--the cause of primary varicosis? VASA Zeitschrift fur Gefasskrankheiten. 2000;29(3):173-7.

21. Bergan JJ, Schmid-Schonbein GW, Smith PD, et al. Chronic venous disease. The New England journal of medicine. 2006;355(5):488-98.

22. Ono T, Bergan JJ, Schmid-Schonbein GW, et al. Monocyte infiltration into venous valves. Journal of vascular surgery. 1998;27(1):158-66.

23. Pfisterer L, Konig G, Hecker M, et al. Pathogenesis of varicose veins - lessons from biomechanics. VASA Zeitschrift fur Gefasskrankheiten. 2014;43(2):88-99.

24. Feldner A, Otto H, Rewerk S, et al. Experimental hypertension triggers varicosis-like maladaptive venous remodeling through activator protein-1. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2011;25(10):3613-21.

25. Atta HM. Varicose veins: role of mechanotransduction of venous hypertension. International journal of vascular medicine. 2012;2012:538627.

26. Somers P, Knaapen M. The histopathology of varicose vein disease. Angiology. 2006;57(5):546-55.

27. Dignat-George F, Sampol J. Circulating endothelial cells in vascular disorders: new insights into an old concept. European journal of haematology. 2000;65(4):215-20.

28. Cesarone MR, Belcaro G, Pellegrini L, et al. Circulating endothelial cells in venous blood as a marker of endothelial damage in chronic venous insufficiency: improvement with venoruton. Journal of cardiovascular pharmacology and therapeutics. 2006;11(1):93-8.

29. Poredos P, Spirkoska A, Rucigaj T, et al. Do blood constituents in varicose veins differ from the systemic blood constituents? European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2015;50(2):250-6.

30. Blomgren L, Johansson G, Siegbahn A, et al. Coagulation and fibrinolysis in chronic venous insufficiency. VASA Zeitschrift fur Gefasskrankheiten. 2001;30(3):184-7.

31. Criado PR, Alavi A, Kirsner RS. Elevated Levels of Coagulation Factor VIII in Patients With Venous Leg Ulcers. The international journal of lower extremity wounds. 2014;13(2):130-4.

32. Nozaki T, Sugiyama S, Koga H, et al. Significance of a multiple biomarkers strategy including endothelial dysfunction to improve risk stratification for cardiovascular events in patients at high risk for coronary heart disease. Journal of the American College of Cardiology. 2009;54(7):601-8.

33. Colombo PC, Onat D, Harxhi A, et al. Peripheral venous congestion causes inflammation, neurohormonal, and endothelial cell activation. European heart journal. 2014;35(7):448-54.

34. Muller-Buhl U, Leutgeb R, Engeser P, et al. Varicose veins are a risk factor for deep venous thrombosis in general practice patients. VASA Zeitschrift fur Gefasskrankheiten. 2012;41(5):360-5.

35. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation. 2004;109(23 Suppl 1):Iii27-32.

36. Tisato V, Zauli G, Gianesini S, et al. Modulation of circulating cytokine-chemokine profile in patients affected by chronic venous insufficiency undergoing surgical hemodynamic correction. Journal of immunology research. 2014;2014:473765.

37. Smith PD. Neutrophil activation and mediators of inflammation in chronic venous insufficiency. Journal of vascular research. 1999;36 Suppl 1:24-36.

38. Saharay M, Shields DA, Porter JB, et al. Leukocyte activity in the microcirculation of the leg in patients with chronic venous disease. Journal of vascular surgery. 1997;26(2):265-73.

39. Takase S, Pascarella L, Lerond L, et al. Venous hypertension, inflammation and valve remodeling. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2004;28(5):484-93.

9. Figure Legends

Figure 1. Schematic representation of the interplay of mechanisms contributing to CVD development.

Figure 2. Comparison between agents produced by healthy endothelium and damaged endothelium in CVD. Dysfunctional endothelium is characterized by an imbalance in bioavailability of vasodilators, anti-thrombotic and anti-inflamatory to endothelium-derived pro-thrombotic and pro-inflammatory agents.

Figure 3. Schematic diagram of endothelium-leukocyte interaction in CVD pathophysiology.

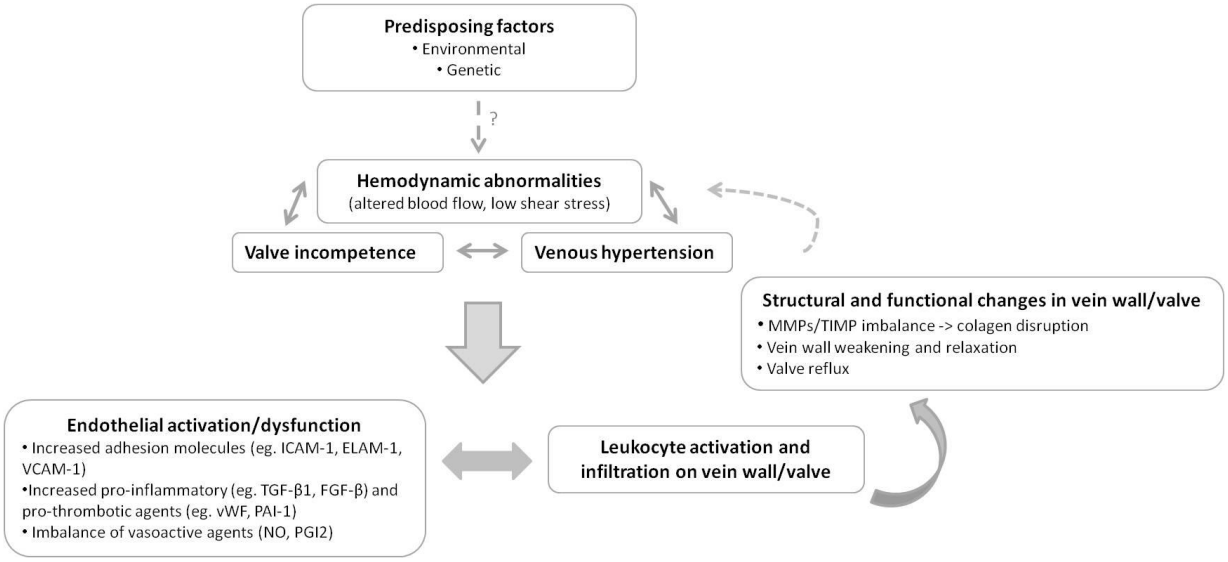

Figure 1. Schematic representation of the interplay of mechanisms contributing to CVD development.

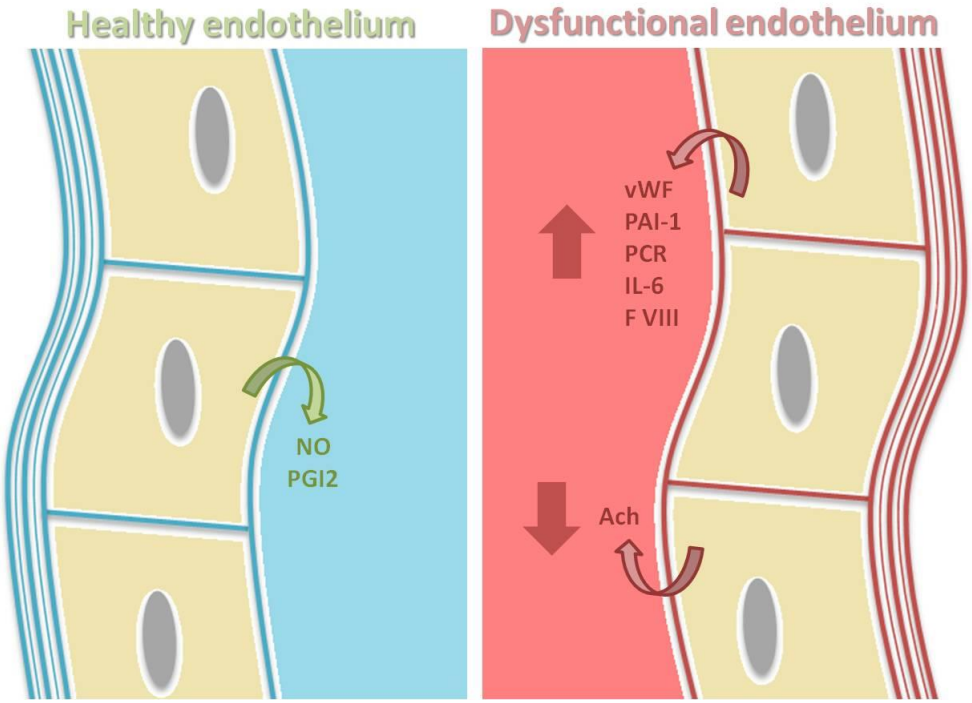

Figure 2. Comparison between agents produced by healthy endothelium and damaged endothelium in CVD. Dysfunctional endothelium is characterized by an imbalance in bioavailability of vasodilators, anti-thrombotic and anti-inflamatory to endothelium-derived pro-thrombotic and pro-inflammatory agents.

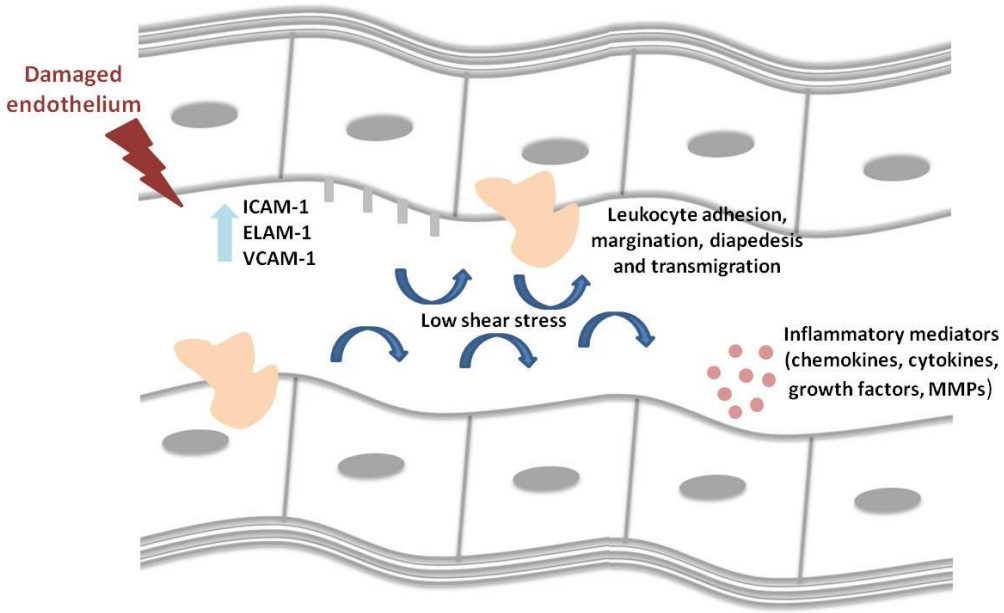

Figure 3. Schematic diagram of endothelium-leukocyte interaction in CVD pathophysiology.