Varicose Veins of the Lower Limbs

Subjects: Allergy

Contributor: Lukasz Gwozdzinski, Anna Pieniazek, Krzysztof Gwozdzinski

One of the early symptoms of chronic venous disease (CVD) is varicose veins (VV) of the lower limbs. There are many etiological environmental factors influencing the development of chronic venous insufficiency (CVI), although genetic factors and family history of the disease play a key role.

Keywords: chronic venous insufficiency ; varicose vein ; pathophysiology ; inflammation ; oxidative stress

1. Introduction

Chronic venous disease (CVD) is associated with a disease of the veins of the lower extremities. The veins develop into varicose veins (VV), which are characterized by the presence of dilated, bulging, twisting veins beneath the surface of the skin. In addition, there are spider veins that may surround the varicose veins. These are smaller red or purple blood vessels near the surface of the skin [1]. It is believed that the formation of varicose veins is caused by valve dysfunction and venous reflux, which consequently leads to venous stasis and hypoxia. Left untreated, VVs can lead to the formation of venous leg ulcers (VLU) [2]. However, in some cases, they can lead to serious health problems such as thromboembolism. A common cause of varicose veins is chronic inflammation, which may have a genetic basis and can cause damage to the valves in the veins of the lower limbs [3]. Blood stasis in varicose veins and hypoxia can lead to cells releasing inflammatory mediators and growth factors through endothelial cells (ECs). Inflammatory mediators attract and activate neutrophils, leading to their infiltration of the venous wall, and initiate damage to extracellular matrix components. In contrast, growth factors cause migration, proliferation, and dedifferentiation of smooth muscle cells, which stimulates the formation of neointima [4]. As a consequence, there is stagnation of blood flow, hypoxia, and further damage to the vein causing the formation of varicose veins. It has been shown that varicose veins are characterized by a greater infiltration of inflammatory cells compared to the normal vein. Macrophages/monocytes and mast cells participate in the damage and remodeling of the vein [5][6]. Numerous studies have shown that growth factors secreted by macrophages, pro-inflammatory cytokines, matrix metalloproteinases (MMPs), and adhesion molecules are involved in the etiology of varicose veins [7]. Of all white blood cells, macrophages/monocytes are most numerous in damaged veins of the lower limbs. Compared to healthy veins, these cells are located near valves and vein walls [7][8]. They were found in adventitia and vasa vasorum but not in the muscular layer [9]. These cells can release proteolytic enzymes and reactive oxygen species (ROS), participating in damage to valves and venous walls [8]. It has been shown that the neutrophils of patients with a chronic venous disease produce more ROS compared to healthy individuals [10][11]. Pro-inflammatory factors, including ROS and matrix metalloproteinases, are released by the vascular endothelium, leading to the accumulation of neutrophils and platelets at the site of inflammation. Inflamed endothelial cells express cell adhesion molecules such as E-selectin, P-selectin, and von Willebrand factor (vWf). Neutrophils and platelets form bonds with these molecules, which initiates an inflammatory cascade and in consequence leads to thrombosis [12].

Factors favoring the development of VV include familial and genetic predisposition associated with CVD, as well as other factors such as behavioral and dietary factors, including obesity and a sedentary lifestyle. Moreover, the etiology and pathophysiology of chronic venous disease include genetic, proteomic, hormonal, and cellular mechanisms that influence the changes induced in the structure and functioning of venous vessels. The expression of several genes related to angiogenesis, vascular hyperplasia, and venous regulation influences the incidence of CVI [13]. In addition, inflammation, changes in mRNA expression, protein levels, and the proteolytic activity of matrix metalloproteinases (MMPs) have been found in VV and VLU [2].

Depending on the severity of the disease, varicose veins can be treated with compression stockings, oral medications, laser therapy, sclerotherapy, or surgery [14]. There are various types of surgical treatment for sealing pathological venous vessels. Some of these methods, such as endovascular laser or radiofrequency ablation use thermal ablation, others mechanical ablation, using a special catheter and foam or chemical ablation using special foam in a similar way to sclerotherapy. Pharmacological treatment, used at all stages of CVD and together with other methods, involves the use of

venotropic drugs of plant origin as well as synthetic drugs. However, if left untreated, varicose veins can lead to serious consequences, such as venous leg ulcer development and thrombosis, including deep vein thrombosis (DVT). Altered venous hemodynamic triggers proteolytic remodeling of the venous wall and inflammatory processes as well as degradation of the protective endothelial glycocalyx, resulting in a wide spectrum of clinical symptoms ranging from varicose veins to venous ulcers, which have been termed chronic venous disorders (CVeD).

2. Chronic Venous Insufficiency

2.1. Factors Influencing the Occurrence and Development of CVeD

Varicose veins are a very common disease, affecting about 1/3 of the adult population. They occur more often in women than men. The factors contributing to the occurrence of varicose veins include the general health and the age of patients (over the age of 50), which is related to the aging of the vein walls (the veins lose elasticity and stiffen) and valves, which no longer function as efficiently as at a younger age. In addition, varicose veins can arise as a result of a sedentary lifestyle, obesity, and smoking. No less important are familial predispositions, and the occurrence of the disease in the family. In the case of women, female hormones can lead to stretching of the walls of the veins. In addition, taking birth control pills can aggravate this process. Menopause is also a factor contributing to the disease. The most common symptoms of chronic venous disease of the lower extremities are pain, heaviness, leg fatigue, cramps, itching, burning sensation, swelling, and restless legs syndrome. In addition, visible changes in the lower extremities are skin changes, induration of subcutaneous fat (lipodermatosclerosis), telangiectasias, reticular veins and varicose veins, edema, pigmentation, and ultimately ulceration [15].

2.2. Changes in the Vein Wall Structure

Chronic venous insufficiency is caused by abnormalities in the wall structure and dysfunction of the venous valves, as well as disorders resulting from previous deep vein thrombosis [16]. Disturbances in the structure of the vein wall are related to the abnormal extensibility of the connective tissue in the vein wall. It has been shown that the veins of patients with varicose veins differ, among other things, in their elastic properties compared to the veins of healthy people [17]. Primary varicose veins are a consequence of venous dilatation and valvular failure without previous deep vein thrombosis. Secondary varicose veins are caused by DVT or superficial thrombophlebitis [18]. In the vessels of the lower limbs, the calf muscle pump, called the peripheral heart, plays an important role influencing proper blood flow and preventing venous stasis.

With properly functioning valves, contraction of the calf muscle compresses the vein and pumps blood upwards [19]. During walking, the calf muscle pump causes a decrease in the capacity of the venous system, which leads to a decrease in the pressure in the veins. In turn, the relaxation of the muscle pump causes the veins to refill with blood. Dysfunction of the valves of the venous system leads to a reverse flow of blood, i.e., venous reflux, and the development of chronic venous insufficiency. This process concerns superficial and deep veins, perforating veins, and venous tributaries [20]. As the insufficiency increases, various changes are observed at the level of the venous walls. Disturbances in the types of collagen in the venous wall affect not only the fibroblasts in the vein walls but also the skin fibroblasts of patients with varicose veins [21]. Moreover, genetic influence on the remodeling of vessel walls has been demonstrated, which is associated with imbalance in tissue MMPs and their inhibitors and a decrease in the expression of desmuslin in smooth muscle cells, as well as thrombomodulin mutations 1208/1209TT [2][21]. It has been shown that the ratio of collagen I to collagen III is disturbed in CVD patients, i.e., there is an increase in the collagen I/collagen III ratio and MMP-2, and the TIMP-2 expression levels are reduced [2][21].

Changes in shear stress leading to activation, leukocyte adhesion, and migration across the endothelium contribute to inflammation and subsequent remodeling of the venous wall and valves [22]. Pathological shear stress with multidirectional orientation can cause the expression of atherogenic and thrombogenic genes, and accelerate endothelial cell proliferation and turnover. In addition, it has pro-inflammatory, pro-coagulant, pro-oxidative, and pro-apoptotic effects, which consequently lead to endothelial dysfunction [23][24].

2.3. The Role of Inflammation in CVD

The main mechanism associated with the pathophysiology of chronic venous insufficiency is an increase in venous pressure, which is a consequence of damaged venous valves, shear stresses, and reflux [25]. These factors cause further damage to the valves, increasing pressure and dilating the vein. Changes in the vein are transferred to microcirculation, disturbing the function of endothelial cells and the vascular microenvironment, which in turn leads to venous microangiopathy, characterized by dilatation and tortuosity of the capillary beds [25]. Changes in vessel hemodynamics

activate various biological processes, such as inflammation, proteolytic enzymes release in the vascular microenvironment, as well as leukocyte adhesion, degranulation, and the release of granules from neutrophils, mastocytes, endothelial cells, and platelets [26].

In patients with varicose veins, the presence of many inflammatory molecules, cytokines, chemokines, vasoactive factors, selectins, and prothrombotic precursors has been demonstrated, including adhesion molecules: ICAM-1, VCAM, chemoattractant protein MCP-1, L-selectin, E-selectin, cytokines: IL-1β, IL-4, IL-6, IL-8, IL-12, IL-13, p40, G-CSF, GM-CSF, IFN-γ, TNF-α, MIP-1a, and matrix metalloproteinases MMP-1, -2, -3, -8, -9, -12, and -13, and macrophage inflammation protein 1b [27]. Platelets reach sites of inflammation and can lead to coagulation as well as an immune response promoting leukocyte–endothelial interaction. Thus, platelets are involved in the pathology of inflammation [28].

2.4. Classification System for Chronic Venous Disorders

The CEAP (clinical–etiology–anatomy–pathophysiology) classification has been adopted throughout the country to enable the assessment of the CVD status and the use of appropriate treatment methods [29]. The pathophysiology of CVD is characterized by different clinical stages, starting with the clinical class C0s, which includes patients without visible or palpable symptoms of a venous disease. Between 13% and 23% of the general population belong to the C0s class. The main cause of CVeD is remodeling of the vein wall and damage to the valves, which is a consequence of blood stasis, hypoxia, and interaction of the endothelium with white blood cells. The formation of varicose veins can cause changes leading to prothrombotic syndrome followed by deep vein thrombosis. In turn, skin changes and ulcerations are caused by venous hypertension, which is transferred to the microcirculation [30]. In cutaneous microcirculation, changes are progressive in groups C1 to C6. It was also found that the diameter of the capillaries increased and their morphology deteriorated in groups from C2 to C5. In turn, an increase in the diameter of the dermal papilla, as well as in the diameter of the volume of capillaries, appears in groups C3 to C5. In contrast, the functional capillary density (FCD) decreases from groups C4 to C5 [30]. The presence of varicose veins may lead to a prothrombotic state and then to deep vein thrombosis.

3. Vascular Endothelium

In the pathogenesis of vascular lesions, an important role is performed by the vascular endothelium, which directly interacts with blood cells. One of the functions of the endothelium is to concentrate various biochemical and biomechanical signals to maintain a barrier function providing selective permeability, vascular tone, proper blood viscosity by regulating laminar flow, and the formation of new vessels. The endothelium covering the inner surface of blood vessels is in constant contact with the morphotic components of blood, but also with its metabolites and immune cells, being responsible for the regulation of homeostasis [31]. In the presence of inflammation, the vascular endothelium is exposed to many factors, for example, ROS, proteases, and others, which may lead to its dysfunction or damage.

3.1. Vascular Endothelium in Inflammation

During inflammation, ROS are overproduced. On the one hand, excessive ROS production inhibits the release of nitric oxide (NO·), which affects vascular stiffness and contractility, leading to endothelial dysfunction [12]. However, the overproduction of NO leads to oxidative stress and cell apoptosis [32]. Many studies have shown a significant contribution of nitric oxide synthases (mainly iNOS and eNOS) and nitric oxide in ROS activity in vascular diseases. In addition, endothelial cells can regulate gene expression. For example, class A genes include E-selectin, thrombomodulin, endothelial protein C receptor, endothelial isoforms of nitric oxide synthase (eNOS), endocan, and vWf. In turn, the vascular endothelial growth factor (VEGF) released by platelets and other cells regulates endothelial cell (EC) proliferation and migration. Endothelium is also involved in the processes of aging, autophagy, and cell death, and releases signaling molecules such as nitric oxide, prostanoids, and others [22]. It participates in inflammation initiated by infection or tissue damage. Inflammatory factors interacting with ECs lead to impaired vascular tone, increased permeability, increased procoagulant dynamics, and impaired vascular formation mechanisms. A dysregulated mechanism related to the inflammatory response is associated with the development of vascular diseases.

3.2. The Role of Adhesion Proteins and Cytokines in Inflammation

Non-activated endothelial cells express intercellular adhesion molecules (ICAM). On the other hand, E-selectin and vascular cell adhesion molecules (VCAM) are found only in stimulated ECs. In inflammatory conditions, cytokines TNF-α, IL-1β, and IFN-γ, acting on endothelial cells, can initiate the synthesis and expression of E-selectin and VCAM, and also lead to an increase in the level of ICAM [33]. In the adhesion of leukocytes to the endothelium under flow conditions, interactions between selectins and their respective ligands perform an important role [34]. In inflammation and infection or vascular damage, the surface of the endothelium becomes susceptible to the adhesion of leukocytes, which is an innate

immune response. This process is regulated and consists of a multi-step cascade that includes successive stages of interaction between leukocytes and the endothelium. Selectins are involved in the initial phase, and after leukocyte activation by chemokines present on the endothelial surface, leukocyte integrins (CD11/CD18) are activated, leading to leukocyte adhesion to the endothelium. In addition, platelets circulating in the bloodstream may participate in the coagulation process as well as in the immune response [28]. The basic integrin ligands that participate in leukocyte adhesion include intercellular adhesion molecules 1–5, vascular cell adhesion molecule-1, and junctional adhesion molecules (JAMs) expressed on endothelial and other cells [35][36]. In addition, the important adhesion receptors involved in leukocyte recruitment include platelet endothelial cell adhesion molecule-1 (PECAM-1) and endothelial cell adhesion molecule (ESAM) [37][38].

Proinflammatory cytokines and adhesion molecules perform important roles in the development of venous thrombosis. In inflammation, neutrophils aggregate and adhere to ECs [39][40]. Initially, P, E, and L selectins participate in the process of adhesion to the endothelium [41]. P-selectin, in particular, performs an important role in diseases associated with damage and thrombosis of blood vessels, selectin initiates the accumulation of leukocytes and adhesion to the endothelium and then in the accumulation of platelets [42]. However, stronger binding to the endothelium occurs through the CD11/CD18 complex and the ICAM-1 and ICAM-2 [43][44]. Adhesion can also be induced by cytokines (IL-8 and IFN-γ), platelet activation factor (PAF), as well as the active complement complex and arachidonic acid metabolites [42][45]. Activated neutrophils produce reactive oxygen species and proteases [46]. On the other hand, TNF-α causes increased activation of neutrophils, their adhesion, degranulation, and the production of ROS, which occurs in the presence of p55 and p7 proteins [47][48].

3.3. Cellular Response in Inflammatory Conditions

There is chronic inflammation in varicose veins as indicated by elevated levels of inflammatory and prothrombotic markers [49][50]. The consequences of chronic inflammation after vascular damage are pathological interactions of the activated endothelium, neutrophils, and platelets, as well as fibrosis and thrombosis [12]. In the case of varicose veins, the developing disease causes damage to the vein, which can lead to the formation of blood clots. Inflammation accompanies the pathophysiology of deep vein thrombosis, pulmonary embolism, and peripheral arterial disease [51]. Patients with varicose veins have a significantly increased risk of DVT [52].

Cells respond to the influence of the environment, which may lead to changes in morphology, mobility, or proliferation, affecting their state of differentiation [53]. The structure and condition of the endothelial cytoskeleton performs an important role in leukocyte recruitment and fibrosis. In endothelial cells stimulated by TNF-α, there is a gradient of cortical stiffness of rolling neutrophils, which causes them to be directed to sites of transmigration [54]. Pro-fibrotic promoting factors, e.g., autotaxin (ATX), responsible for the production of lysophosphatidic acid, lead to the rearrangement of endothelial actin and cell contractility, which causes vascular leakage, leading to the migration of fibroblasts in the underlying tissue [55]. Moreover, changes in the structure of the cytoskeleton contribute to changes in the distribution and function of the glycocalyx [56], which in turn further deepens endothelial dysfunction and leads to vascular damage.

Using two fluorescent probes located at different depths of the lipid monolayer of the membrane, researchers demonstrated lower membrane fluidity of human varicose vein endothelial cells (HVVEC) in comparison to the human umbilical vein endothelial cells (HUVEC) in the subsurface area of the membrane. Greater differences in membrane fluidity were found for the probe located in the deeper region of the monolayer. These results demonstrated a higher stiffness of HVVEC plasma membranes compared to HUVEC cells. Differences in the fluidity of normal and pathological membranes may be the result of lipid–lipid and lipid–protein interactions or may be caused by oxidative stress occurring in the pathological vein [57]. Changes in membrane fluidity are closely related to the passive and active transport of substances across membranes into and out of the cells [58][59]. Previous studies have shown that the fluidity of the plasma membrane of the cells performs a significant role in the mechanism of cell adhesion [60][61]. Membrane fluidity is also an important parameter determining communication between cells [62][63]. The mechanism of cell adhesion is improved by building nanoclusters, ordered lipid rafts heterogeneously distributed in the membrane, which are associated with adhesive complexes [64]. A more fluid environment allows the rapid dispersal of lipid rafts, which leads to a reduction in adhesions [60][61]. The reduced fluidity of HVVEC may result in increased adhesion properties and, therefore, a greater likelihood of venous thrombosis. Interestingly, drugs used in varicose vein therapy such as diosmin and aescin, as well as bromelain, lead to a slight increase in fluidity in the near-surface region of the lipid bilayer of the membrane and in consequence to their lower adhesive properties [57].

References

1. Youn, Y.J.; Lee, J. Chronic venous insufficiency and varicose veins of the lower extremities. Korean J. Intern. Med. 2019, 34, 269–283.
2. Raffetto, J.D.; Khalil, R.A. Mechanisms of Lower Extremity Vein Dysfunction in Chronic Venous Disease and Implications in Management of Varicose Veins. Vessel Plus 2021, 5, 36.
3. Grudzińska, E.; Lekstan, A.; Szliszka, E.; Czuba, Z.P. Cytokines Produced by Lymphocytes in the Incompetent Great Saphenous Vein. Mediat. Inflamm. 2018, 2018, 7161346.
4. Michiels, C.; Bouaziz, N.; Remacle, J. Role of the endothelium and blood stasis in the appearance of varicose veins. Int. Angiol. 2002, 21, 18, PMID: 11941268.
5. Rizzo, C.; La Barbera, L.; Miceli, G.; Tuttolomondo, A.; Guggino, G. The innate face of Giant Cell Arteritis: Insight into cellular and molecular innate immunity pathways to unravel new possible biomarkers of disease. Front. Mol. Med. 2022, 2, 933161.
6. Sayer, G.L.; Smith, P.D.C. Immunocytochemical characterisation of the inflammatory cell infiltrate of varicose veins. Eur. J. Vasc. Endovasc. Surg. 2004, 28, 479–483.
7. Wang, X.; Khalil, R.A. Matrix Metalloproteinases, Vascular Remodeling, and Vascular Disease. Adv. Pharmacol. 2018, 81, 241–330.
8. Ono, T.; Bergan, J.J.; Schmid-Schönbein, G.W.; Takase, S. Monocyte infiltration into venous valves. J. Vasc. Surg. 1998, 27, 158–166.
9. Knaapen, M.W.M.; Somers, P.; Bortier, H.; de Meyer, G.R.Y.; Kockx, M.M. Smooth muscle cell hypertrophy in varicose veins is associated with expression of estrogen receptor-beta. J. Vasc. Res. 2005, 42, 8–12.
10. Grudzińska, E.; Czuba, Z.P. Immunological aspects of chronic venous disease pathogenesis. Cent. Eur. J. Immunol. 2014, 39, 525–531.
11. Whiston, R.J.; Hallett, M.B.; Davies, E.V.; Harding, K.G.; Lane, I.F. Inappropriate neutrophil activation in venous disease. Br. J. Surg. 1994, 81, 695–698.
12. Puhr-Westerheide, D.; Schink, S.J.; Fabritius, M.; Mittmann, L.; Hessenauer, M.E.T.; Pircher, J.; Zuchtriegel, G.; Uhl, B.; Holzer, M.; Massberg, S.; et al. Neutrophils promote venular thrombosis by shaping the rheological environment for platelet aggregation. Sci. Rep. 2019, 9, 15932.
13. Costa, D.; Andreucci, M.; Ielapi, N.; Serraino, G.F.; Mastroroberto, P.; Bracale, U.M.; Serra, R. Molecular Determinants of Chronic Venous Disease: A Comprehensive Review. Int. J. Mol. Sci. 2023, 24, 1928.
14. Raetz, J.; Wilson, M.; Collins, K. Varicose Veins: Diagnosis and Treatment. Am. Fam. Physician 2019, 99, 682–688.
15. Ortega, M.A.; Fraile-Martínez, O.; García-Montero, C.; Álvarez-Mon, M.A.; Chaowen, C.; Ruiz-Grande, F.; Pekarek, L.; Monserrat, J.; Asúnsolo, A.; García-Honduvilla, N.; et al. Understanding Chronic Venous Disease: A Critical Overview of Its Pathophysiology and Medical Management. J. Clin. Med. 2021, 10, 3239.
16. Singh, A.; Zahra, F. StatPearls: Chronic Venous Insufficiency; StatPearls: Treasure Island, FL, USA, 2023. Available online: https://www.ncbi.nlm.nih.gov/books/NBK587341/ (accessed on 15 December 2023).
17. Vekilov, D.P.; Grande-Allen, K.J. Mechanical Properties of Diseased Veins. Methodist Debakey Cardiovasc. J. 2018, 14, 182–187.
18. Nicolaides, A.N.; Allegra, C.; Bergan, J.; Bradbury, A.; Cairols, M.; Carpentier, P.; Comerota, A.; Delis, C.; Eklof, B.; Fassiadis, N.; et al. Management of chronic venous disorders of the lower limbs: Guidelines according to scientific evidence. Int. Angiol. 2008, 27, 1–59.
19. Niccolini, G.; Manuello, A.; Capone, A.; Marongiu, G.; Dell'Osa, A.H.; Fois, A.; Velluzzi, F.; Concu, A. Possible Assessment of Calf Venous Pump Efficiency by Computational Fluid Dynamics Approach. Front. Physiol. 2020, 11, 1003.
20. Labropoulos, N.; Volteas, N.; Leon, M.; Sowade, O.; Rulo, A.; Giannoukas, A.D.; Nicolaides, A.N. The role of venous outflow obstruction in patients with chronic venous dysfunction. Arch. Surg. 1997, 132, 46–51.
21. Boisseau Michel-René. Chronic venous disease and the genetic influence. Phlebolymphology 2014, 21, 100–111.
22. Peng, Z.; Shu, B.; Zhang, Y.; Wang, M. Endothelial Response to Pathophysiological Stress. Arter. Thromb. Vasc. Biol. 2019, 39, e233–e243.
23. Chiu, J.-J.; Chien, S. Effects of disturbed flow on vascular endothelium: Pathophysiological basis and clinical perspectives. Physiol. Rev. 2011, 91, 327–387.

24. Theofilis, P.; Sagris, M.; Oikonomou, E.; Antonopoulos, A.S.; Siasos, G.; Tsioufis, C.; Tousoulis, D. Inflammatory Mechanisms Contributing to Endothelial Dysfunction. Biomedicines 2021, 9, 781.

25. Raffetto, J.D.; Mannello, F. Pathophysiology of chronic venous disease. Int. Angiol. 2014, 33, 212–221.

26. Atta, H.M. Varicose veins: Role of mechanotransduction of venous hypertension. Int. J. Vasc. Med. 2012, 2012, 538627.

27. Tkaczyk, J.; Przywara, S.; Iłżecka, J.; Iłżecki, M. The influence of compression therapy on the level of inflammatory biomarkers in patients with chronic venous disease. Acta Angiol. 2021, 1, 32–36.

28. Langer, H.F.; Chavakis, T. Leukocyte-endothelial interactions in inflammation. J. Cell. Mol. Med. 2009, 13, 1211–1220.

29. Lurie, F.; Passman, M.; Meisner, M.; Dalsing, M.; Masuda, E.; Welch, H.; Bush, R.L.; Blebea, J.; Carpentier, P.H.; Maeseneer, M.d.; et al. The 2020 update of the CEAP classification system and reporting standards. J. Vasc. Surg. Venous Lymphat. Disord. 2020, 8, 342–352.

30. Mansilha, A.; Sousa, J. Pathophysiological Mechanisms of Chronic Venous Disease and Implications for Venoactive Drug Therapy. Int. J. Mol. Sci. 2018, 19, 1669.

31. Neubauer, K.; Zieger, B. Endothelial cells and coagulation. Cell Tissue Res. 2022, 387, 391–398.

32. Howlader, M.H.; Smith, P.D. Increased plasma total nitric oxide among patients with severe chronic venous disease. Int. Angiol. 2002, 21, 180–186, PMID: 12110781.

33. Hua, S. Targeting sites of inflammation: Intercellular adhesion molecule-1 as a target for novel inflammatory therapies. Front. Pharmacol. 2013, 4, 127.

34. Ivetic, A.; Hoskins Green, H.L.; Hart, S.J. L-selectin: A Major Regulator of Leukocyte Adhesion, Migration and Signaling. Front. Immunol. 2019, 10, 1068.

35. Ley, K.; Laudanna, C.; Cybulsky, M.I.; Nourshargh, S. Getting to the site of inflammation: The leukocyte adhesion cascade updated. Nat. Rev. Immunol. 2007, 7, 678–689.

36. Orlova, V.V.; Chavakis, T. Regulation of vascular endothelial permeability by junctional adhesion molecules (JAM). Thromb. Haemost. 2007, 98, 327–332.

37. Muller, W.A. Leukocyte-endothelial-cell interactions in leukocyte transmigration and the inflammatory response. Trends Immunol. 2003, 24, 327–334.

38. Wegmann, F.; Petri, B.; Khandoga, A.G.; Moser, C.; Khandoga, A.; Volkery, S.; Li, H.; Nasdala, I.; Brandau, O.; Fässler, R.; et al. ESAM supports neutrophil extravasation, activation of Rho, and VEGF-induced vascular permeability. J. Exp. Med. 2006, 203, 1671–1677.

39. Dehghani, T.; Panitch, A. Endothelial cells, neutrophils and platelets: Getting to the bottom of an inflammatory triangle. Open Biol. 2020, 10, 200161.

40. Summers, C.; Rankin, S.M.; Condliffe, A.M.; Singh, N.; Peters, A.M.; Chilvers, E.R. Neutrophil kinetics in health and disease. Trends Immunol. 2010, 31, 318–324.

41. Gupta, G.S. Selectins and Associated Adhesion Proteins in Inflammatory disorders. In Animal Lectins; Gupta, G.S., Gupta, A., Gupta, R.K., Eds.; Springer: Vienna, Austria, 2012; pp. 991–1026. ISBN 978-3-7091-1064-5.

42. Mosevoll, K.A.; Johansen, S.; Wendelbo, Ø.; Nepstad, I.; Bruserud, Ø.; Reikvam, H. Cytokines, Adhesion Molecules, and Matrix Metalloproteases as Predisposing, Diagnostic, and Prognostic Factors in Venous Thrombosis. Front. Med. 2018, 5, 147.

43. Golias, C.; Tsoutsi, E.; Matziridis, A.; Makridis, P.; Batistatou, A.; Charalabopoulos, K. Review. Leukocyte and endothelial cell adhesion molecules in inflammation focusing on inflammatory heart disease. In Vivo 2007, 21, 757–769.

44. Mazzone, A.; Ricevuti, G. Leukocyte CD11/CD18 integrins: Biological and clinical relevance. Haematologica 1995, 80, 161–175.

45. Michiels, C.; Arnould, T.; Remacle, J. Endothelial cell responses to hypoxia: Initiation of a cascade of cellular interactions. Biochim. Biophys. Acta 2000, 1497, 1–10.

46. Dahlgren, C.; Karlsson, A.; Bylund, J. Intracellular Neutrophil Oxidants: From Laboratory Curiosity to Clinical Reality. J. Immunol. 2019, 202, 3127–3134.

47. Riedl, M.; Noone, D.G.; Khan, M.A.; Pluthero, F.G.; Kahr, W.H.A.; Palaniyar, N.; Licht, C. Complement Activation Induces Neutrophil Adhesion and Neutrophil-Platelet Aggregate Formation on Vascular Endothelial Cells. Kidney Int. Rep. 2017, 2, 66–75.

48. Zeman, K.; Kantorski, J.; Paleolog, E.M.; Feldmann, M.; Tchórzewski, H. The role of receptors for tumour necrosis factor-alpha in the induction of human polymorphonuclear neutrophil chemiluminescence. Immunol. Lett. 1996, 53, 45–50.
49. Poredoš, P.; Šabovič, M.; Božič Mijovski, M.; Nikolajević, J.; Antignani, P.L.; Paraskevas, K.I.; Mikhailidis, D.P.; Blinc, A. Inflammatory and Prothrombotic Biomarkers, DNA Polymorphisms, MicroRNAs and Personalized Medicine for Patients with Peripheral Arterial Disease. Int. J. Mol. Sci. 2022, 23, 12054.
50. Stark, K.; Massberg, S. Interplay between inflammation and thrombosis in cardiovascular pathology. Nat. Rev. Cardiol. 2021, 18, 666–682.
51. Riva, N.; Donadini, M.P.; Ageno, W. Epidemiology and pathophysiology of venous thromboembolism: Similarities with atherothrombosis and the role of inflammation. Thromb. Haemost. 2015, 113, 1176–1183.
52. Chang, S.-L.; Huang, Y.-L.; Lee, M.-C.; Hu, S.; Hsiao, Y.-C.; Chang, S.-W.; Chang, C.J.; Chen, P.-C. Association of Varicose Veins with Incident Venous Thromboembolism and Peripheral Artery Disease. JAMA 2018, 319, 807–817.
53. Wolfenson, H.; Yang, B.; Sheetz, M.P. Steps in Mechanotransduction Pathways that Control Cell Morphology. Annu. Rev. Physiol. 2019, 81, 585–605.
54. Schaefer, A.; Te Riet, J.; Ritz, K.; Hoogenboezem, M.; Anthony, E.C.; Mul, F.P.J.; de Vries, C.J.; Daemen, M.J.; Figdor, C.G.; van Buul, J.D.; et al. Actin-binding proteins differentially regulate endothelial cell stiffness, ICAM-1 function and neutrophil transmigration. J. Cell Sci. 2014, 127, 4470–4482.
55. Sakai, N.; Bain, G.; Furuichi, K.; Iwata, Y.; Nakamura, M.; Hara, A.; Kitajima, S.; Sagara, A.; Miyake, T.; Toyama, T.; et al. The involvement of autotaxin in renal interstitial fibrosis through regulation of fibroblast functions and induction of vascular leakage. Sci. Rep. 2019, 9, 7414.
56. Li, W.; Wang, W. Structural alteration of the endothelial glycocalyx: Contribution of the actin cytoskeleton. Biomech. Model. Mechanobiol. 2018, 17, 147–158.
57. Gwozdzinski, L.; Bernasinska-Slomczewska, J.; Hikisz, P.; Wiktorowska-Owczarek, A.; Kowalczyk, E.; Pieniazek, A. The Effect of Diosmin, Escin, and Bromelain on Human Endothelial Cells Derived from the Umbilical Vein and the Varicose Vein—A Preliminary Study. Biomedicines 2023, 11, 1702.
58. Guţu, M.; Rusu, V.; Ştefănescu, C. Fluiditatea membranară—Parametru biofizic in relaţie cu procesele de transport membranare. Rev. Med. Chir. Soc. Med. Nat. Iasi 2011, 115, 153–162.
59. Lande, M.B.; Donovan, J.M.; Zeidel, M.L. The relationship between membrane fluidity and permeabilities to water, solutes, ammonia, and protons. J. Gen. Physiol. 1995, 106, 67–84.
60. Juliano, R.L.; Gagalang, E. The effect of membrane-fluidizing agents on the adhesion of CHO cells. J. Cell. Physiol. 1979, 98, 483–489.
61. Schaeffer, B.E.; Curtis, A.S. Effects on cell adhesion and membrane fluidity of changes in plasmalemmal lipids in mouse L929 cells. J. Cell Sci. 1977, 26, 47–55.
62. Salaita, K.; Nair, P.M.; Petit, R.S.; Neve, R.M.; Das, D.; Gray, J.W.; Groves, J.T. Restriction of receptor movement alters cellular response: Physical force sensing by EphA2. Science 2010, 327, 1380–1385.
63. Zhou, Y.; Mao, H.; Joddar, B.; Umeki, N.; Sako, Y.; Wada, K.-I.; Nishioka, C.; Takahashi, E.; Wang, Y.; Ito, Y. The significance of membrane fluidity of feeder cell-derived substrates for maintenance of iPS cell stemness. Sci. Rep. 2015, 5, 11386.
64. Ratajczak, M.K.; Chi, E.Y.; Frey, S.L.; Cao, K.D.; Luther, L.M.; Lee, K.Y.C.; Majewski, J.; Kjaer, K. Ordered nanoclusters in lipid-cholesterol membranes. Phys. Rev. Lett. 2009, 103, 28103.
