Review Article

Access this article online

Quick Response Code:

Website: http://www.vitonline.org/

DOI: 10.4103/VIT.VIT_2_19

Pathophysiological mechanisms of chronic venous disease and their role in C0s clinical class

Andrew Nicolaides[1,2], Eliete Bouskela[3]

Abstract:

The aim of this review is to provide a clear understanding of the pathophysiological mechanisms of chronic venous disease (CVD) at different clinical stages and the possible role of these mechanisms in the development of symptoms in C0s clinical class of the Clinical, Etiologic, Anatomic, and Pathophysiologic classification, which consists of symptomatic patients without any visible or palpable signs of venous disease. The prevalence of C0s class in several epidemiological studies varies between 13% and 23% of the general population.Wall remodeling and valve destruction due to white cell endothelial interaction is the main cause of primary varicose veins, while deep vein thrombosis produces secondary changes leading to the postthrombotic syndrome. The underlying mechanism of the skin changes and ulceration is venous hypertension, which is transmitted to the skin microcirculation. Over the last 10 years, an improved videocapillaroscopic technique, the orthogonal polarization spectral imaging technique demonstrated that quantitative measurements in the skin microcirculation are progressively altered from C_1 to C_6 patients and that values in CVD patients are significantly different from healthy individuals ($P < 0.05$): capillary diameter increases and capillary morphology worsens from C2 to C5; diameter of the dermal papilla and diameter of the capillary bulk increase from C3 to C5; and functional capillary density (FCD) decreases from C4 to C5. In addition, significant changes have been shown between C0a and C0s patients despite the presence of normal conventional duplex scans in the latter: a decrease of FCD and an increase in the diameter of the dermal papilla. Functional abnormalities found to be present in C0s patients by recent studies include increased compliance of the venous wall (hypotonic phlebopathy), dilatation of deep veins in the calf producing an abnormally increased venous volume, reduction in emptying of venous reservoir, reduction in the venoarteriolar response on standing, and blood reflux in small venules despite a normal conventional duplex scan. However, most of the studies are small, and their findings need to be confirmed by larger series. It remains to be seen whether functional changes and microcirculatory changes respond to venoactive medications in parallel to the relief of symptoms.

Keywords:

Chronic venous disease, microcirculation, venous wall remodeling

[1]Department of Surgery, Imperial College, London, UK, [2]Department of Surgery, University of Nicosia Medical School, Engomi, Cyprus, [3]Department of Physiology, State University of Rio De Janeiro, Rio De Janeiro, Brazil

Address for correspondence:
Prof. Andrew Nicolaides, University of Nicosia Medical School, Engomi, Cyprus.
E-mail: anicolaides1@gmail.com

Introduction

Chronic venous disease (CVD) presents with a variable combination of symptoms and signs and is associated with complicated venous hemodynamics that are difficult to understand unless one is familiar with the theory of hemodynamics in collapsible tubes. In addition, it is a progressive condition.

Epidemiological studies have demonstrated that the prevalence of CVD varies between 25% and 85% depending on severity considered and age group[1] with telangiectasias and reticular veins being the most common (80% in men and 85% in women). Varicose

How to cite this article: Nicolaides A, Bouskela E. Pathophysiological mechanisms of chronic venous disease and their role in C0s clinical class. Vasc Invest Ther 2018;1:XX-XX.

veins (VVs) are present in 25%–33% of female and 10%–40% of male adults. The prevalence of edema and skin changes due to CVD such as hyperpigmentation and eczema varies from 3% to 11% of the population. Venous ulcers occur in about 0.3% of the adult population in Western countries.[1]

Symptoms[2] and signs[3] related to CVD are shown in Table 1. Symptoms describe what the patient feels. Signs are elicited by the doctor and are typically used to define the clinical classes of the Clinical, Etiologic, Anatomic, and Pathophysiologic (CEAP) classification. However, what the patient seeks is relief of symptoms, often more than improved appearance. What the doctor aims for is not only to relieve symptoms and improve appearance but also to stop disease progression.

One of the diagnostic problems is that symptoms are not specific for CVD and there is a poor correlation between symptoms and signs. Another problem is that symptoms of CVD are often present without any evidence of an abnormality on clinical examination or routine duplex scanning (C0s CEAP clinical class).

The aim of this review is to provide a clear understanding of the pathophysiological mechanisms of CVD at different clinical stages and the possible role of these mechanisms in the development of symptoms in C0s clinical class.

Primary chronic venous disease

Primary varicose veins are very common in the adult population and are frequently responsible for skin changes as well as 40% of venous leg ulcers despite the presence of normal deep veins.[1] In recent years, the role of leukocyte–endothelium interaction as a key factor in the initiation of primary CVD has become better understood. This process starts with leukocyte adhesion, degranulation, and migration under the endothelium producing chronic inflammation with eventual remodeling of venous wall and valves.[4] The resulting damage produces valve cusp distortion, reflux, and venous hypertension.[5]

Table 1: Symptoms, signs, and CEAP clinical class

Symptoms
Heaviness
Pain
Sensation of swelling
Restless legs
Paresthesias
Night time cramps
Tiredness
Throbbing
Itching
SIGNS and CEAP clinical class
C0: Absence of visible or palpable signs
C0a Normal, asymptomatic subjects
C0s Symptomatic
C1: Telangiectasias, reticular veins
C2: Varicose veins
C3: Oedema
C4: Skin changes
Pigmentation, Eczema
Lipodermatosclerosis, atrophie blanche
C5: Healed venous ulcer

During standing, venous pressure in the veins of the foot and ankle is approximately 90 mmHg depending on the height of the individual. This is the hydrostatic pressure from the level of the heart to the foot. During walking in a normal person with competent venous valves and a healthy calf muscle pump, the pressure decreases to 25 mmHg. In the presence of damaged valves, what determines venous pressure during walking (ambulatory venous pressure) is the volume of blood refluxing (ml/sec) during the period of 1.0–1.5 s when the foot is off the ground, before the onset of the next step and muscular contraction. A steady state is reached after the first ten steps when the amount of blood refluxing during muscle relaxation equals the amount expelled during muscle contraction. A high rate of reflux is associated with rapid filling of the veins before the next muscle contraction ensues and results in rapid elevation of pressure and a high mean venous pressure during walking. A low rate of reflux is associated with a slow filling of the veins before the next muscle contraction ensues and results in a slow elevation of pressure and a low mean venous pressure during walking. The rate of reflux, AVP, and duration of standing or sitting periods determine the mean venous pressure throughout the day and the prevalence of skin changes and ulceration.[5]

Secondary chronic venous disease

The postthrombotic syndrome is responsible for edema, skin changes, and 60% of venous ulcers and also for the development of secondary varicose veins acting as collateral vessels. Persistent obstruction due to failed recanalization and recurrence of deep vein thrombosis or reflux due to damage of the deep venous valves also result in venous hypertension. The combination of both reflux and obstruction of the deep veins is responsible for the most severe symptoms and signs. In cases of severe outflow obstruction, venous pressure during walking may increase to levels above 90 mmHg.

Mechanical dysfunction of the calf muscle pump may enhance the development of leg ulceration emphasizing the importance of the range of ankle motion[6] and patient activity.[7]

Incompetent Perforating Veins

Incompetent perforating veins (IPVs) can be defined as those that penetrate the deep fascia and permit flow

from the deep to the superficial veins. The flow of calf IPVs is often bidirectional, outward during muscular contraction, and inward during relaxation. In normal legs and in the majority of patients with primary uncomplicated varicose veins, the net flow is inward from the superficial to the deep veins (reentry perforators), as first demonstrated in 1891 by Trendelenburg[8] and more recently by Bjordal who used electromagnetic flow meters during exercise.[9] The inward net flow during exercise is the basis of the Perthes test. The net flow in IPVs of the calf is also inward even in patients with femoral vein reflux, provided the popliteal valves are competent. However, in the presence of popliteal valve incompetence (axial reflux), and especially when there is associated deep vein obstruction, the flow is predominantly outward.[9,10]

IPVs are associated with superficial and/or deep-vein reflux but are rarely found in the absence of reflux.[11-13] The prevalence, diameter, volume flow, and velocity of IPVs increase with clinical severity of CVD irrespective of presence or absence of coexisting deep venous incompetence.[9,14-19]

Up to 10% of patients, often women, presenting with CEAP clinical Class 1–3, have nonsaphenous superficial reflux in association with unusually located IPVs.[20]

Changes in the Microcirculation

Venous hypertension is transmitted backward to the microcirculation increasing the hydrostatic pressure in capillaries. This results in transcapillary filtration that exceeds lymphatic drainage and thus contributes to interstitial edema formation. Venous hypertension slows blood flow in capillaries prompting leukocyte adhesion to capillary endothelium and initiating an inflammatory reaction.[21]

In patients with venous hypertension, capillaries become markedly dilated, elongated, and tortuous, especially at skin sites with hyperpigmentation and lipodermatosclerosis (LDS). These changes are associated with a high overall microvascular blood flow in the dermis[22] and a decreased flow in nutritional capillaries with decreased oxygen delivery.[23]

Laser Doppler studies show that in addition to increased blood flow (red cell flux) in the dermis, there is loss of the rhythmic vasomotor activity seen in normal skin and abolition of the venoarteriolar reflex (VAR). The VAR is an axon reflex elicited by any postural change that increases venous pressure by 40 mmHg or more. In normal limbs, the ensuing arteriolar vasoconstriction and reduction in skin blood flow is a protective mechanism in the sitting or standing position.[24]

Over the last 10 years, an improved videocapillaroscopic technique, the orthogonal polarization spectral imaging technique used in the Cytoscan (Lekam Medical Ltd, UK) with sidestream dark field has become available. Combined with the MicroScan Video Microscope (MicroVision Medical, The Netherlands) and lately the Cytocam (Braedius Medical Ltd, The Netherlands), it has allowed alterations of skin capillaries to be studied in patients assigned C_1 to C_5 of the CEAP classification. The Cytoscan has a small handheld probe which can be noninvasively applied to the skin to evaluate microcirculatory parameters such as functional capillary density (FCD), number of capillaries with flowing red blood cells per unit of tissue area, representing tissue perfusion evaluated by capillaries per mm^2, diameter of dermal papilla (DDP, μm) to quantify edema, the largest diameter of the capillary bulk (DCB, μm) to assess its degree of change, capillary limb diameter (CD, μm) to describe diameter changes, and capillary morphology (CM, percentage of abnormal capillaries per field). It has been demonstrated that all these values are progressively altered from C1 to C6 patients and that values in CVD patients are significantly different from healthy subjects ($P < 0.05$): capillary diameter increases and CM worsens from C2 to C5; diameter of the dermal papilla and diameter of the capillary bulk increases from C3 to C5, and FCD decreases from C4 to C5.[25] In a most recent study, significant changes have been shown between (C0a) asymptomatic normal persons and C0s patients despite the presence of normal conventional duplex scans.[26] Using the Cytocam, it was possible to observe changes in CM: a decrease of FCD and an increase in the diameter of the dermal papilla.

Alteration of Lymphatic Vessels

Spontaneous contractility of lymphatic vessels contributes to lymph transport. Internal extensions of lymphatic endothelial cells act as valves and guarantee a one-way lymph flow.[27] In a steady state, extravasation of fluids and proteins from blood vessels is balanced by lymphatic drainage and return into the bloodstream. If microvascular filtration in blood capillaries and venules as occurs in advanced CVD exceeds the capacity for lymphatic drainage for sufficiently long periods, edema develops in afflicted areas by accumulation of tissue fluid.

Vascular Biology and Pathophysiology of the Venous Wall

Varicose veins have different elastic properties from normal veins. The ratio of Collagen I to Collagen III is altered and so do dermal fibroblasts from the same patients, suggesting a systemic disorder with a genetic basis.[28]

Leukocyte activation, adhesion, and migration through the endothelium as a result of altered shear stress[29-31] contribute to the inflammation and subsequent remodeling of the venous wall and valves.[4,32-34]

Reduction in shear stress also stimulates production of tumor growth factor-β1 by activated endothelial cells and smooth muscle cells (SMCs) inducing SMC migration into the intima and subsequent proliferation. Fibroblasts proliferate and synthesize matrix metalloproteinases (MMPs) overcoming the effect of tissue inhibitors of metalloproteinases (TIMPs). The MMP/TIMP imbalance results in degradation of elastin and collagen.[22,30,35] This may contribute to the development of hypertrophic and atrophic venous segments and valve destruction seen in varicose veins. Remodeling of the venous wall and abnormal venous distension prevents valve leaflets from closing properly resulting in reflux.

Compensatory Mechanisms

It should be emphasized that the effects of venous hypertension are modified by three compensatory mechanisms. The first mechanism is the ability of the lymphatic system to compensate for the increased lymph that is produced. In some patients, the lymphatic drainage can increase up to ten times and the leg may look normal despite severe hypertension, while in some others, only two times, when overt skin changes occur for the same severity of venous hypertension.[36] The second mechanism is the fibrinolytic activity in the blood and tissues that can remove excess extracellular protein and particularly fibrin, which varies from person to person. Skin changes are rare in the presence of AVP <35, and skin changes and ulceration are frequent in the presence of AVP >65. Fibrinolytic activity was measured in a series of 37 patients with moderate venous hypertension with AVP in the range of 35–65. Euglobulin lysis time (ELT) was normal (<240 min) in 12 patients of whom only two had LDS but no ulceration, in contrast to the remaining ten patients with low fibrinolytic activity (ELT >240 min) of whom nine had LDS and seven had both LDS and ulceration ($P < 0.001$).[37] The third factor is time which exerts an adverse effect on the microcirculation with progressive deterioration.[38]

Pathophysiology of Symptoms

Pain which is a vague and unpleasant feeling is the result of increase in venous pressure that is transmitted to the microcirculation resulting in activation of sensory multimodal nociceptors of myelinated Aδ and unmyelinated C fibers[39,40] through local inflammatory mediators. Throbbing occurs more often in patients with varicose veins and this observation is indicative of a hemodynamic mechanism. Tightness is common in patients with iliocaval obstruction and is thought to be related to fluid accumulation and increased pressure in the anatomical compartments. Venous claudication is the result of severe venous outflow obstruction when the arterial inflow exceeds the venous outflow. In these patients, the recovery time when they stop walking is long, often more than 15 min.[2] Heaviness and feeling of swelling are often related to edema but can be present in the absence of edema. It is thought that they are produced by microedema at the microcirculation since they are relieved by venoactive drugs without any actual reduction in leg volume.[2] Itching is often associated with skin changes, but it can be an isolated symptom. Inflammation, cytokine, and MMP activation have all been implicated in the pathophysiology.[2] The exact causation of cramps, restless legs, tingling, and burning is not clear.

Pathophysiology of C0s

C0s Class of the CEAP classification consists of symptomatic patients without any visible or palpable signs of venous disease.[3] Recent epidemiological studies have demonstrated that C0s patients are common in the general population. C0s prevalence was 13%–23% in a Polish Study,[41] 15% in the San Diego Vein Study,[42] 19.7% in the worldwide Vein Consult Program,[43] and 14% in the Belgium and Luxemburg Study.[44]

The presence of CVD symptoms in the absence of any visible or palpable signs has been described well before the development of the CEAP classification. Such patients have been considered to have functional phlebopathy[45] or more recently as functional CVD.[46] They were studied in depth with photoplethysmography (PPG), strain gauge plethysmography (SGP), and laser Doppler as well as CW Doppler and Duplex scanning in the Acireale epidemiological study which involved 1031 individuals (age: 30–59).[47] In this study, symptoms of CVD were present in 561 (54%). Of these, 325 (58%), i.e., 31% of the whole population studied did not have any visible or palpable varicose veins (C0s). However, 163 (50%) of these had reflux in some veins (femoral, great saphenous vein (GSV) above and below the knee, popliteal, small saphenous vein, and tibial veins). The remaining 164 (15.9% of all the population) did not have any reflux on routine conventional duplex scanning. They were considered to have hypotonic phlebopathy (HP) on the basis of the findings summarized below.

In patients with HP, the mean (±standard deviation) PPG change in voltage from baseline (ΔR) after 10 plantar flexion movements were lower (200 ± 15 V) compared with normal controls (275 ± 40 V) ($P < 0.005$) indicating reduced emptying of the venous reservoir. The mean

refilling time was also lower (27 ± 5 s) compared with normal controls (35 ± 10 s) ($P < 0.005$) indicating a relatively fuller reservoir or some reflux in small venules not examined by ultrasound. However, it was not as low as in patients with varicose veins (10 ± 6 s).

SGP demonstrated that the mean maximum incremental venous volume during venous occlusion was higher (4.5 ± 0.4 ml%) in patients with HP than normal controls (2.9 ± 0.3 ml%) ($P < 0.005$) indicating increased venous compliance. The decrease in volume (ΔV) in the sitting position after 10 plantar flexion/dorsiflexion movements was 2.7 ± 0.5 ml% compared with 1.5 ± 0.4 ml% in the normal controls ($P < 0.005$) confirming a larger volume in the calf reservoir.

In a subgroup of 20 patients with HP, duplex scanning demonstrated that the increase in vein diameters of the popliteal, tibioperoneal trunk, and gastrocnemial veins on standing were 2–3 times greater than in 10 normal controls.

In a subgroup of 10 patients with HP, laser Doppler showed an increase in standing and resting flux indicating increased cutaneous blood flow. In HP patients, resting flux decreased from 10.4 ± 1.9 in the supine position to 7.28 ± 1.2 in the standing position (only 30% reduction) indicating impaired VAR. In contrast, in 10 normal controls, resting flux decreased from 8.0 ± 0.9 in the supine position to 3.6 ± 0.6 in the standing position indicating normal VAR (>50% reduction) ($P < 0.001$).

In a recent study, 16 C0a normal asymptomatic individuals were compared with 16 C0s patients.[48] Routine duplex scanning in both groups excluded reflux in superficial and deep main trunks. However, using a continuous wave flat probe on visually identified small venules on the lateral thigh and leg, medial leg, and anterior tibial area, it was possible to identify the presence of bidirectional flow in 54 sites in the C0s group and only in 33 sites in the C0a group ($P = 0.05$) during exercise.

Using a different approach, Tsoukanov *et al*. investigated 41 C0s women with duplex scanning in the morning (before 10 am) and in the afternoon (after 6 pm).[49] Fifteen of these patients did not have any reflux at any time. The remaining 26 patients had reflux in the GSV in the evening but not in the morning (situational reflux). Two patients had axial reflux and 24 had segmental reflux. The evening diameter of the GSV was larger in those with reflux in the evening ($P < 0.05$). The difference in the GSV between the evening and morning was also greater in the patients with evening reflux than those without any reflux. After 2 months of Micronized purified flavonoid fraction (MPFF) treatment, 22 patients no longer had reflux in the evening, the GSV diameter decreased and so did the difference in diameter between the morning and evening ($P < 0.0001$). There was a parallel significant decrease in the intensity of symptoms as demonstrated by the VAS score and a significant improvement in quality of life ($P < 0.00001$).

In a subsequent study by Tsoukanov and Tsukanov[50] involving 294 patients, the prevalence of situational reflux in the GSV was investigated. It was detected in 21 (38.2%) of 55 patients classified as C0s, 25 (49.0%) of 51 classified as C1s, and in 32 (17.0%) of 188 classified as C2. After treatment with MPFF 1000 mg for 90 days, in the 46 women with transient reflux in classes C0s and C1s, reflux disappeared in 76.1% and there was a significant decrease in the GSV diameters. The intensity of symptoms decreased from 5.2 to 1.7 ($P < 0.001$) according to the 0–10 visual analog scale. The global index score (CIVIQ-20) decreased from 47.2 ± 7.9 to 28.8 ± 9.1 ($P < 0.001$).

It appears from several studies summarized above that a significant number of patients in C0s clinical class who do not have any reflux or obstruction on routine duplex examination are found to have abnormal venous function such as increased venous compliance and venous volume, reduced emptying of venous reservoir on calf muscle contraction, decreased VAR, evening venous reflux in the main venous trunks, reflux in small venules not normally examined by duplex, and even anatomic changes in the skin dermal papillae. However, the number of patients in most studies is small and the findings need to be confirmed in larger studies. In addition, the future studies should determine the prevalence of these functional abnormalities, their coexistence and which are the most predominant in C0s patients and also the contribution of these abnormalities to individual symptoms, disease progression, and response to venoactive drugs or compression. Knowledge of the above should help one provide a rational plan for investigation and management of C0s patients.[21,51]

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Nicolaides A, Kakkos S, Baekgaard N, Comerota A, de Maeseneer M, Eklof B, *et al.* Management of chronic venous disorders of the lower limbs. Guidelines according to scientific evidence. Part I. Int Angiol 2018;37:181-254.
2. Perrin M, Eklof B, Van Rij A, Labropoulos N, Vasquez M, Nicolaides A, *et al.* Venous symptoms: The SYM vein consensus statement developed under the auspices of the European venous forum. Int Angiol 2016;35:374-98.
3. Eklöf B, Rutherford RB, Bergan JJ, Carpentier PH, Gloviczki P,

Kistner RL, *et al.* Revision of the CEAP classification for chronic venous disorders: Consensus statement. J Vasc Surg 2004;40:1248-52.
4. Bergan JJ, Schmid-Schönbein GW, Smith PD, Nicolaides AN, Boisseau MR, Eklof B, *et al.* Chronic venous disease. N Engl J Med 2006;355:488-98.
5. Nicolaides AN; Cardiovascular Disease Educational and Research Trust, European Society of Vascular Surgery, The International Angiology Scientific Activity Congress Organization, International Union of Angiology, Union Internationale de Phlebologie at the Abbaye des Vaux de Cernay. Investigation of chronic venous insufficiency: A consensus statement (France, March 5-9, 1997). Circulation 2000;102:E126-63.
6. Shiman MI, Pieper B, Templin TN, Birk TJ, Patel AR, Kirsner RS, *et al.* Venous ulcers: A reappraisal analyzing the effects of neuropathy, muscle involvement, and range of motion upon gait and calf muscle function. Wound Repair Regen 2009;17:147-52.
7. Davies JA, Bull RH, Farrelly IJ, Wakelin MJ. A home-based exercise programme improves ankle range of motion in long-term venous ulcer patients. Phlebology 2007;22:86-9.
8. Trendelenburg F. About the collapse of Great Saphenous Varices. Beitr Klin Chir 1891;7:195-210.
9. Bjordal R. Simultaneous pressure and flow recordings in varicose veins of the lower extremity. A haemodynamic study of venous dysfunction. Acta Chir Scand 1970;136:309-17.
10. Al-Mulhim AS, El-Hoseiny H, Al-Mulhim FM, Bayameen O, Sami MM, Abdulaziz K, *et al.* Surgical correction of main stem reflux in the superficial venous system: Does it improve the blood flow of incompetent perforating veins? World J Surg 2003;27:793-6.
11. Darke SG, Penfold C. Venous ulceration and saphenous ligation. Eur J Vasc Surg 1992;6:4-9.
12. Lees TA, Lambert D. Patterns of venous reflux in limbs with skin changes associated with chronic venous insufficiency. Br J Surg 1993;80:725-8.
13. Myers KA, Ziegenbein RW, Zeng GH, Matthews PG. Duplex ultrasonography scanning for chronic venous disease: Patterns of venous reflux. J Vasc Surg 1995;21:605-12.
14. Christopoulos D, Nicolaides AN, Szendro G. Venous reflux: Quantification and correlation with the clinical severity of chronic venous disease. Br J Surg 1988;75:352-6.
15. Delis KT, Ibegbuna V, Nicolaides AN, Lauro A, Hafez H. Prevalence and distribution of incompetent perforating veins in chronic venous insufficiency. J Vasc Surg 1998;28:815-25.
16. Zukowski AJ, Nicolaides AN, Szendro G, Irvine A, Lewis R, Malouf GM, *et al.* Haemodynamic significance of incompetent calf perforating veins. Br J Surg 1991;78:625-9.
17. Stuart WP, Adam DJ, Allan PL, Ruckley CV, Bradbury AW. The relationship between the number, competence, and diameter of medial calf perforating veins and the clinical status in healthy subjects and patients with lower-limb venous disease. J Vasc Surg 2000;32:138-43.
18. Stuart WP, Lee AJ, Allan PL, Ruckley CV, Bradbury AW. Most incompetent calf perforating veins are found in association with superficial venous reflux. J Vasc Surg 2001;34:774-8.
19. Delis KT, Husmann M, Kalodiki E, Wolfe JH, Nicolaides AN. *In situ* hemodynamics of perforating veins in chronic venous insufficiency. J Vasc Surg 2001;33:773-82.
20. Labropoulos N, Tiongson J, Pryor L, Tassiopoulos AK, Kang SS, Mansour MA, *et al.* Nonsaphenous superficial vein reflux. J Vasc Surg 2001;34:872-7.
21. Perrin M, Ramelet AA. Pharmacological treatment of primary chronic venous disease: Rationale, results and unanswered questions. Eur J Vasc Endovasc Surg 2011;41:117-25.
22. Leu AJ, Leu HJ, Franzeck UK, Bollinger A. Microvascular changes in chronic venous insufficiency – A review. Cardiovasc Surg 1995;3:237-45.
23. Fagrell B. Microcirculatory disturbances – The final cause for venous leg ulcers? Vasa 1982;11:101-3.
24. Belcaro G, Grigg M, Rulo A, Nicolaides A. Blood flow in the perimalleolar skin in relation to posture in patients with venous hypertension. Ann Vasc Surg 1989;3:5-7.
25. Virgini-Magalhães CE, Porto CL, Fernandes FF, Dorigo DM, Bottino DA, Bouskela E, *et al.* Use of microcirculatory parameters to evaluate chronic venous insufficiency. J Vasc Surg 2006;43:1037-44.
26. Barros BS. Is there a Link between Symptoms and Microcirculatory Alterations? Presentation at EVF Meeting, Athens, Greece; 28-30 June, 2018.
27. Rovensca E, Rovensky J. Lymphatic vessels: structure and function. Isr Assoc J 2011;13:762-8.
28. Sansilvestri-Morel P, Rupin A, Jaisson S, Fabiani JN, Verbeuren TJ, Vanhoutte PM, *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;106:479-83.
29. Bergan JJ, Schmid-Schönbein GW, Takase S. Therapeutic approach to chronic venous insufficiency and its complications: Place of daflon 500 mg. Angiology 2001;52 Suppl 1:S43-7.
30. Michiels C, Bouaziz N, Remacle J. Role of the endothelium and blood stasis in the appearance of varicose veins. Int Angiol 2002;21:1-8.
31. Weber C. Novel mechanistic concepts for the control of leukocyte transmigration: Specialization of integrins, chemokines, and junctional molecules. J Mol Med (Berl) 2003;81:4-19.
32. Ono T, Bergan JJ, Schmid-Schönbein GW, Takase S. Monocyte infiltration into venous valves. J Vasc Surg 1998;27:158-66.
33. Takase S, Schmid-Schönbein G, Bergan JJ. Leukocyte activation in patients with venous insufficiency. J Vasc Surg 1999;30:148-56.
34. Takase S, Bergan JJ, Schmid-Schönbein G. Expression of adhesion molecules and cytokines on saphenous veins in chronic venous insufficiency. Ann Vasc Surg 2000;14:427-35.
35. Badier-Commander C, Verbeuren T, Lebard C, Michel JB, Jacob MP. Increased TIMP/MMP ratio in varicose veins: A possible explanation for extracellular matrix accumulation. J Pathol 2000;192:105-12.
36. Tanaka H, Zaima N, Sasaki T, Yamamoto N, Samo M, Konno H, *et al.* Loss of lymphatic vessels and regional lipid accumulation is associated with great saphenous vein incompetence. J Vasc Surg 2012;55:1440-8.
37. Whawell SA, Harbourne T, Vasdekis S, Christopoulos D, Clarke H, Nicolaides AN. The significance of fibrinolytic activity in the development of ulceration in patients with chronic venous insufficiency. Br J Surg 1989; 76(6):646-647
38. Papadakis KG, Christopoulos D, Hobbs JT, Nicolaides AN. Descending phlebography in patients with venous ulceration: Hemodynamic implications. Int Angiol 2015;34:263-8.
39. Vital A, Carles D, Serise JM, Boisseau MR. Evidence for unmyelinated C fibres and inflammatory cells in human varicose saphenous vein. Int J Angiol 2010;19:e73-7.
40. Boisseau MR. Leukocyte involvement in the signs and symptoms of chronic venous disease. Perspectives for therapy. Clin Hemorheol Microcirc 2007;37:277-90.
41. Jawien A. The influence of environmental factors in chronic venous insufficiency. Angiology 2003;54 Suppl 1:S19-31.
42. Langer RD, Ho E, Denenberg JO, Fronek A, Allison M, Criqui MH, *et al.* Relationships between symptoms and venous disease: The San Diego population study. Arch Intern Med 2005;165:1420-4.
43. Guex JJ, Rabe E, Escotto SI, Escudero JR, Scuderi A, Yuwono HS, *et al.* The "C0s" patient: Worldwide results from the vein consult program. Phlebolymphology 2012;19:182-92.
44. Vuylsteke ME, Colman R, Thomis S, Guillaume G, Degrande E, Staelens I, *et al.* The influence of age and gender on venous symptomatology. An epidemiological survey in Belgium and Luxembourg. Phlebology 2016;31:325-33.

45. Bassi G. La patologia venosa funzionale. In: Bassi G, editor. Functional venus pathology. Torino: Minerva Medica Ed; 1985.
46. Serra R, Andreucci M, De Caridi G, Massara M, Mastroroberto P, de Franciscis S, *et al.* Functional chronic venous disease: A systematic review. Phlebology 2017;32:588-92.
47. Andreozzi GM, Signorelli S, Di Pino L, Garozzo S, Cacciaguerra G, Leone A, *et al.* Varicose symptoms without varicose veins: The hypotonic phlebopathy, epidemiology and pathophysiology. The acireale project. Minerva Cardioangiol 2000;48:277-85.
48. Lugli M, Maleti O, Iabichella ML, Perrin M. Investigation of non-saphenous veins in C0S patients. Int Angiol 2018;37:169-75.
49. Tsoukanov YT, Tsoukanov AY, Nikolaychuk A. Great saphenous vein transitory reflux in patients with symptoms related to chronic venous disorders, but without visible sins (C0s), and its correction with MPFF treatment. Phlebolymphology 2015;22:18-24.
50. Tsoukanov YT, Tsukanov AY. Diagnosis and treatment of situational great saphenous vein reflux in daily medical practice. Phlebolymphology 2017;24:144-51.
51. Blazek C, Amsler F, Blaettler W, Keo HH, Baumgartner I, Willenberg T, *et al.* Compression hosiery for occupational leg symptoms and leg volume: A randomized crossover trial in a cohort of hairdressers. Phlebology 2013;28:239-47.