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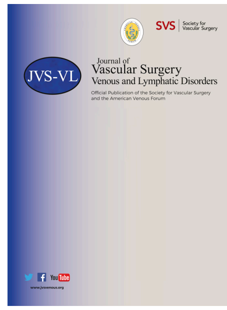
Journal Pre-proof



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PII: S2213-333X(20)30635-1

DOI: https://doi.org/10.1016/j.jvsv.2020.11.010

Reference: JVSV 1130

To appear in: *Journal of Vascular Surgery: Venous and Lymphatic Disorders*

Received Date: 7 August 2020

Accepted Date: 11 November 2020

Please cite this article as: S. Mestre, J. Triboulet, C. Demattei, F. Veye, M. Nou, A. Pérez-Martin, M. Dauzat, I. Quéré, Noninvasive measurement of venous wall deformation induced by changes in transmural pressure shows altered viscoelasticity in patients with chronic venous disease, *Journal of Vascular Surgery: Venous and Lymphatic Disorders* (2020), doi: https://doi.org/10.1016/j.jvsv.2020.11.010.

Noninvasive measurement of venous wall deformation induced by changes in transmural pressure shows altered viscoelasticity in patients with chronic venous disease

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Key-Words:

Chronic venous disease, lower limb veins, viscoelasticity, diameter, ultrasonography

Sources of Funding:

Technical developments required by the present study are covered by patent WO 2017005642 A1 for equipment (ultrasound probe instrumentation for force measurement) and by IDDN.FR.001.090044.000.S.P.2013.000.21000 and IDDN.FROO1.150022.000.S.C.2017.000.31230 for software (automatic image and signal analysis and extraction of viscoelasticity variables), detained by the institutions (Montpellier University and Nimes University Hospital consortium) the authors are affiliated to.

Clinical Trial Registration—URL: https://clinicaltrials.gov/ct2/show/NCT01558024

This study was approved by the Ethics Committee CP Sud-Méditerranée (RCB-2014-A00737-40) and all participants provided a written informed consent.

Article Highlights

Type of Research: Single-center case-control clinical research.

Key Findings: In 57 patients with chronic venous disease and 54 controls, the small saphenous vein showed diverse postural diameter changes but marked and consistent viscoelasticity changes as evidenced by its cross-sectional area variation induced by compression with the ultrasound probe. Viscoelasticity features discriminated patients from controls.

Take home Message: The non-invasive assessment of viscoelasticity is a promising technique for the evaluation of vein biomechanics and pathophysiology.

Table of Contents Summary

Leg vein ultrasonography during compression with the probe in 57 patients with chronic venous disease and 54 controls showed highly diverse postural changes in vein cross-sectional area, but marked and consistent viscoelasticity changes, differentiating patients from controls. Non-invasive viscoelasticity measurement is a promising technique for the evaluation of vein pathophysiology.

Abstract

Objective: The noninvasive measurement of venous wall deformation induced by changes in transmural pressure may allow assessing viscoelasticity and differentiating normal from diseased veins.

Methods: In 57 patients with limbs in C_{1S}, C_3, or C_5 CEAP category of chronic venous disease (CVD) and 54 matched healthy controls, we measured with ultrasonography the changes in cross-sectional area of the small saphenous vein and of a deep calf vein in the supine and in the standing position, and under compression with the ultrasound probe.

Results: The small saphenous, but not the deep calf vein cross-sectional area was smaller in controls than in limbs with category C_3 or C_5 disease while not different from C_{1s}. When changing from the supine to the standing position, a greater force was required to collapse leg veins, of which the cross-sectional area increased in most subjects but decreased in 31.5% of subjects for the small saphenous and 40.5% for the deep calf vein. The small saphenous vein area *versus* compression force function followed a *hysteresis* loop, demonstrating viscoelastic features. Its area, which represents the viscosity component, was greater ($p<0.001$) in pooled C_3 and C_5 limbs (median 2.40 [lower–upper quartile 1.65–3.88] $N.mm^2$) than in controls (1.24 [0.64–2.14] $N.mm^2$) and C_{1s} limbs (1.15 [0.71-2.97] $N.mm^2$). It increased ($p<0.0001$) in the standing position in all groups.

Conclusion: Postural changes in cross-sectional area of leg veins are highly diverse among patients with chronic venous diseases as well as among healthy subjects, and appear unsuitable for pathophysiological characterization, whereas small saphenous vein viscoelasticity increases consistently in the standing position and viscosity is greater in limbs with C_3 and C_5 CEAP categories than in controls.

Introduction

Chronic rise in venous blood pressure increases venous wall stress, altering the endothelium vasomotor function.[1] The smooth muscle contractile response of the venous wall to angiotensine-2, norepinephrine, and endothelin-1 is impaired in primary chronic venous insufficiency,[2,3] together with Ca^{2+} mobilization,[4] while post-receptor contraction mechanisms are preserved.[5] Such changes in smooth muscle tone may alter the biomechanical properties of the vessel wall.[6] Chronic venous wall stress and inflammation, notably with TGF-b1 activation, result in an imbalance between matrix metalloproteases and their tissue inhibitors and lead to wall remodeling.[7] Loss of elastin and type III collagen has been observed in varicose veins, together with disorganization of the extracellular matrix, disturbed expression of matrix remodeling enzymes, and loss of smooth muscle cells.[8-11] These structural changes also alter the vein biomechanical characteristics.[12,13] Noninvasive assessment of vein biomechanics could therefore contribute to early detection of the venous wall distress.

The volume–pressure function reflects the vein biomechanics. In the low venous blood pressure range, as in the supine position, a minimal transmural pressure rise produces a large volume increase by changing the venous cross-section from bimodal to elliptical to circular. At higher blood pressure, as in the standing position, the slope of the venous volume–pressure function flattens, eventually reaching a plateau where a further rise in blood pressure no longer translate in a significant volume increase.[14] Only in this high pressure range is the venous wall elasticity solicited, and diameter changes correlate with pressure (at least in superficial veins with incompetent valves).[15] Therefore, venous biomechanics cannot be inferred from static measurements of vein diameter. Postural changes, *e.g.* the difference in leg vein diameter between the standing and the supine position, would provide more relevant information. In limbs with saphenous vein reflux, they were found smaller in CVD patients with C_4-C_6 than in patients with C_0-C_1 or C_2-C_3 CEAP category.[16]

Blood vessel walls are viscoelastic, combining features of elastic solids and viscous fluids.[17] Elasticity, illustrated by the slope of the volume–pressure function, is the ability of the vessel wall to resist a distending force and return to its original shape and size when this force recedes. Conversely, viscosity absorbs energy, slowing dilation when blood pressure rises suddenly, and slowing deformation under external compression. Viscoelasticity produces a horizontal shift between the ascending (at increasing transmural pressure) and descending (at decreasing transmural pressure) parts of the volume–pressure function, drawing a *hysteresis* loop, the area of which represents energy losses due to viscosity.[18] Viscosity damps down the pulse waveform in arteries, but little is known of the venous viscosity and its role in the pathophysiology of CVD,[19-21] although viscoelasticity may be as essential for veins as it is for arteries.[11]

Venous distensibility increases in patients with CVD,[22,23] even in unaffected veins.[13,24] However, the smaller postural diameter changes that has been reported in C_4-C_6 than in C_0-C_1 or C_2-C_3 patients, and in enlarged than in unaffected veins,[16] suggest reduced venous distensibility. If CVD results from a systemic disorder altering venous tone, structure, and biomechanics, the proper interpretation of these data would require assessing the vein biomechanics in the high-pressure range, and comparing CVD patients to healthy subjects, which was done only by a few studies[13,22] while others compared veins with and without reflux[16,25,26] or limbs with different CEAP categories.[27]

Our aim was to assess non-invasively the biomechanics of normal and diseased lower limb veins. Measuring, with B-mode ultrasonography (US),[28] the changes in cross-sectional area of leg veins when applying an increasing force on the US probe to compress and collapse the vein, we obtained typical *hysteresis* loops, thus offering a noninvasive technique for the evaluation of viscoelasticity of veins in their natural environment, involving the physical characteristics of the venous wall and surrounding tissues, the luminal blood viscosity, and the resistance to blood displacement. Using this technique, we investigated viscoelasticity features of the small saphenous vein (*saphena parva*, SSV) and measured the postural changes in cross-sectional area of the SSV and of a deep calf vein (DCV, the soleal vein or a gastrocnemial vein, as available),[29] in CVD patients for whom compression was the main therapeutic option, and in normal controls. These veins were chosen because they were lesion-free, could be examined at the same calf level, and their US examination was not hampered by bone structures while leaving the GSV available for blood pressure measurement.

Material and Methods

Population sample

We recruited CVD patients whose lower limbs presented with C_{1s}, C_3, or C_5 CEAP category, diagnosed on the basis of thorough clinical and ultrasonographic examinations by two independent physicians. Any other etiology of signs and symptoms (heart, kidney, liver or skin disease, lymph stasis, other sources of leg pain…) was investigated and excluded before concluding to CVD. We included in the C_{1S} group patients with bilateral and symmetrical signs (telangiectasies or reticular veins) and symptoms (aching legs, pain, tightness, skin irritation, leg heaviness, muscle cramps) attributed to CVD. We included in the C_3 group patients with bilateral leg edema as the prominent sign of CVD, and in the C_5 group patients with healed venous ulcer (investigation was performed on the lower limb with healed ulcer). Controls were healthy subjects volunteering for biomedical research recruited by the Montpellier Center for Clinical

Investigation and matched with patients for age and body mass index (BMI), in three subgroups depending on their regular activity (<2h, 2–6h, and >6h of weekly physical exercise) thus covering the whole spectrum of the normal population. Pregnant or breastfeeding women, subjects or patients under 18 years of age, and subjects or patients unable or unwilling to sign the informed consent form, were not included. Patients who had had either sclerotherapy, phlebectomy, or any lower limb venous interventional treatment were not included during the 6 following weeks and were not investigated on the treated limb. The SSV and DCV were free of detectable lesion in the lower limb chosen for the study. The anticipated sample size was 54 patients and 54 controls (**Appendix**). We measured intravenous (IVP) and intramuscular (IMP) pressures in 18 of the CVD patients and in 18 of the controls with the same CEAP or activity repartition.

This study was approved by the Ethics Committee CP-Sud-Méditerranée (RCB-2014-A00737-40) and all participants signed an informed consent.

Methods

US examinations were performed with a Logiq-e system (GE-Ultrasound, Chicago, IL) of which the 12L-RS linear probe was instrumented with a XFTC300 sensor and ARD154 amplifier (Measurement Specialties, Hampton, VI) measuring the force (PF) applied on the ultrasound probe by the operator. The US video signal was captured by a Picolo frame-grabber (Euresys, Liege, Belgium) and stored on a personal computer.

Intramuscular pressure was measured with a 1.2 mm external diameter IMP-Cath catheter (Alcis, Besançon, France), inserted, under local anesthesia by 6 to 8 mL of 5 mg/mL lidocaine, into the *triceps surae* muscle at 4 cm approximate depth, slightly above the maximum girth of the calf. Intravenous blood pressure was measured with a 22G Cathlon catheter (Smiths-Medical, St-Paul, MN) inserted into the great saphenous vein at mid-calf height (**Appendix**). Both catheters were filled with heparinized isotonic saline and connected to DPT-6000 pressure sensors (Codan-Medical, Lensahn, Deutschland) of which analog signals were sent, together with PF, to a MP150 signal acquisition and processing system, then analyzed offline with Acqknowledge V4.2 (Biopac-Systems, Goleta, CA). Calibration at atmospheric pressure and against a mercury column was performed before each session.

On the subject lying supine on his or her side (lateral *decubitus*) with a small wedge under the heel to avoid contact of calf muscles with the examination table, the observer recorded B-mode US images of the SSV, then of the DCV, at mid-calf height. The observer increased PF progressively until the vein collapsed, then released it, allowing the vein to reopen and expand. Finally, the subject moved to the standing position and remained motionless (*orthostasis*) for more than one minute, bearing the body weight on the other leg, before the compression test was reiterated.

Measurements and calculations

Measurements were independently performed on recorded signals and images by observers blinded from the subject's status.

Using ***Fiji*** software (https://fiji.sc/), the observer measured the SSV and DCV cross-sectional area, of which postural change (PAC) was calculated in percentage as 100x(AS-AL)/AS, with AL and AS = cross sectional area respectively in the supine and the standing position. SSV and DCV depth (US probe-to-vein distance) was measured at null PF and at vein collapse.

Recorded US sequences were also analyzed with a custom-made LabView-2016 (National-Instruments, Austin, TX) software that detected the vein walls and tracked their displacements.[28] The vein lumen was approximated to an ellipse of which the cross-sectional area was calculated on each frame (**Appendix, Supplemental Video 1**). The SSV cross-sectional area *versus* PF function was drawn, and appeared as a *hysteresis* loop from which were automatically extracted[30] variables related to blood pressure (probe force at which the vein collapsed, then reopened), to viscosity (area of the loop and its compression and decompression parts), and to elasticity (first and second slopes of the compression part) (**Fig 1**).

Mean intravenous (IVPm) and intramuscular (IMPm) pressures were obtained by averaging instantaneous values over about 10s. Were also recorded the subjects' age, weight, height, leg length, and calf circumference, and the presence of reflux or obstruction in veins other than the investigated SSV and DCV.

Statistical analysis

Categorical data were compared by Fisher exact test, with Freeman-Halton extension when appropriate. Quantitative variables are reported as median [lower–upper quartile]. Differences between two groups (independent data) and changes within one group (paired data) were evaluated with Wilcoxon-Mann-Whitney test and Wilcoxon signed-rank test, respectively. Comparisons between controls, C_{1s}, and pooled C_3 and C_5 patients ($C_{3\&5}$) were performed with Kruskal-Wallis test followed by Dunn's multiple comparison. Values of $p<0.05$ were considered significant. Relationships between continuous variables were investigated by Spearman ***r*** coefficient and with random effects models, and described by linear regression. Receiver operating characteristic (ROC) curves were drawn and the area under the curve (AUC) was calculated for each variable. The performance of combined variables for discriminating CEAP groups was estimated from the AUC calculated by introducing independent variables with $p<0.2$ at univariate logistic regression analysis in multivariate logistic regression models. Intra-observer reproducibility is reported in **Appendix**. Statistical analyses were performed using Prism V.5 (GraphPad, San Diego, CA) and *R* V3.5.1 (*R*-Foundation for Statistical Computing, Vienna, Austria).

Results

Characteristics of the population sample

For matching purposes, we recruited three additional C_{1S} patients, so that the population sample comprised 57 CVD patients (41 females) with 21 C_{1s}, 18 C_3, and 18 C_5 (**Fig 2**), and 54 controls (36 females).

Neither age nor BMI differed between CVD patients and controls, but weight and height were greater in C_5 patients than in controls and C_{1S} patients. Calf circumference was greater in C_3 patients than in controls, whereas ankle circumference was greater in C_3 and C_5 patients than in controls (**Supplemental Table I**).

Vein cross-sectional area and depth

SSV and DCV depth was slightly smaller in the standing than in the supine position without difference between groups at null PF or at collapse (**Appendix**).

The SSV and DCV cross-sectional area (**Supplemental Table II, Appendix**) was greater in $C_{3\&5}$ patients than in controls ($p<0.01$ for all). There was no significant difference in DCV cross-sectional area between groups. Among controls, there was no difference in SSV or DCV cross-sectional area between physical activity subgroups.

The SSV and DCV cross-sectional areas were neither related between them nor with IVPm or IMPm. In the whole population sample, cross-sectional area correlated, in the supine position, with age for SSV and DCV, and with body weight for DCV. In the whole population sample and in $C_{3\&5}$ patients, SSV cross-sectional area correlated positively with body weight and BMI in both positions. (**Supplemental Table III**).

SSV and DCV cross-sectional areas were greater in the standing than in the supine position (respectively p<0.0001 and p=0.015), but SSV and DCV PACs were negative, respectively, in 31.5% and 40.5% of the 111 subjects (**Fig 3, Appendix**), without difference between groups and without correlation between SSV and DCV values.

Intravenous and intramuscular pressure

Intravenous and intramuscular pressures could be obtained in 31 and 35 subjects, respectively. Baseline IVPm was not different between groups in the supine position but greater (p<0.01) in $C_{3\&5}$ patients (60.1[55.8–71.8] mmHg) than in controls (46.7[-6.6–57.9]) in the standing position. Changing from supine to standing increased IVPm (**Appendix**).

In the whole population sample, IMPm was lower in the standing than in the supine position (p<0.0001). It was higher in CVD patients than in controls at baseline in the standing (p=0.013) but not in the supine position (**Appendix**).

Viscoelasticity variables

Hysteresis loops were obtained for 108 subjects. All *Hysteresis* loop variables were greater in the standing than in the supine position for all groups (p<0.0001 for all), and differed between controls, C_{1S}, and $C_{3\&5}$ patients (**Fig 4, Table I, Appendix**).

In the supine, but not in the standing position, viscosity-related *hysteresis* variables in the whole population sample and in CVD patients, and pressure-related variables in CVD patients, increased with age (**Appendix**).

ROC curves showed that most *hysteresis* variables differentiated controls from CVD patients. Using different combinations of *hysteresis* variables, multivariate logistic regression analysis yielded an AUC reaching 0.80 to 0.83 for differentiating controls from C_3 and C_5 patients, 0.78 for differentiating controls from C_{1S} patients, and 0.75 for differentiating C_{1S} from C_3 and C_5 patients (**Table II, Appendix**).

Discussion

Our main results were: 1) Postural changes in SSV and DCV cross-sectional area showed large inter-individual differences in all groups. 2) All the variables derived from the *hysteresis* loops drawn by the SSV cross-sectional area *vs.* PF function were greater in the standing than in the supine position, and 3) their combination discriminated controls from C_{1S} patients and from C_3 and C_5 patients.

The greater SSV cross-sectional area we found in CVD patients than in controls is in agreement with previous studies about GSV diameter[15,25,26,31] and CEAP categories.[27,32,33] We found no difference in DCV cross-sectional area. Deep calf veins are thought to be supported by surrounding tissues and muscles,[34] but intramuscular pressure decreased in the standing position, in our study as in another.[35]

Our most striking result is the extent of interindividual differences in PAC, independently of the healthy or CVD *status*, since the vein area increased in some subjects, staid unchanged or even decreased in others in the standing position. As we took care to avoid residual muscle contraction, the absence, in some subjects, of vein area increase in spite of greater hydrostatic blood pressure[15] could be due to multiple, possibly opposite factors. Although a linear correlation has been reported between intravenous pressure and diameter of saphenous veins with reflux,[15] the relationship may be more complex in unaffected veins. Increased venous tone could explain the negative PAC we observed in a noticeable proportion of control subjects, but probably not for CVD patients in whom the venous wall contractile response to angiotensine-2, norepinephrine, and endothelin-1 is impaired.[2,3] Van der Velden *et al*. found a negative postural diameter change in 10% of their subjects, but dismissed it as measurement error.[16] We limited errors by measuring the cross-sectional area rather than only the larger diameter, and ensuring that the subject's weight rested on the other leg. Therefore, we must consider that the interindividual differences we observed are not meaningless. Nevertheless, pending further studies clarifying this issue, postural changes in diameter or cross-sectional area would not be sufficient to characterize CVD.

The *hysteresis* loops we obtained displayed a horizontal swap relative to conventional *hysteresis* loops since increasing PF actually reduced transmural pressure.[36] Observing calf veins with US through a modified pneumatic cuff, Partsch *et al* [37] found that the cuff pressure required to occlude leg veins was greater in the standing than in the sitting position. We also found that a greater probe force was needed to collapse the SSV and DCV in the standing position, reflecting greater hydrostatic blood pressure. In the supine position, the probe force at which the SSV collapsed was greater in C_5 than in C_{1S} patients or in controls. In the standing position, the force at which SSV reopened was greater in C_3 and C_5 patients than in controls, suggesting higher venous transmural pressure and/or greater wall stiffness.

When evaluated in vivo, either by venous occlusion plethysmography or by our technique, venous viscoelasticity features are affected by the venous wall but also by surrounding tissues, blood viscosity, and resistance to blood displacement. Venous compliance or distensibility are commonly calculated from changes in limb circumference or vein diameter produced by incremental venous occlusion-cuff pressure,[38] Valsalva maneuver,[13] or posture.[39] Venous compliance is large at low transmural pressure where a minimal increase in blood pressure generates a large increase in volume through wall deformation. It is smaller at high transmural pressure (as in the standing position), where the vein cross-section becomes circular and diameter changes induced by further rises in blood pressure reflect volume change and depend on wall elasticity.[14,40,41] This may explain why we obtained steeper *hysteresis* loop slopes, corresponding to greater distensibility (i.e. lower elastic *modulus*), in the supine than in the standing position in all groups. Regardless of posture, these slopes were steeper in CVD patients, also suggesting greater vein distensibility. This is consistent with previous reports of greater proximal lower limb vein distensibility in patients with varicose veins than in healthy controls,[13] and of endothelium and smooth muscle abnormalities in CVD patients,[42] even in non-varicose veins,[43] suggesting systemic alteration of venous wall resistance to stress.[19] Such abnormalities should affect viscoelasticity.[44] It is plausible that, beside or before remodeling, changes in smooth muscle cells contractility[2-4,45] alter the venous wall viscoelasticity.[12] This could have contributed to our findings in unaffected veins of CVD patients.

Venous wall *hysteresis*, relating to viscoelasticity, has been demonstrated by invasive volume–pressure measurements[24] and plethysmography.[30,46] However, viscoelasticity is frequency-dependent,[36] and venous-occlusion plethysmography relies on long periods of venous filling. Our technique innovates in that it allows the direct, non-invasive evaluation of a specific vein rather than of a limb segment, in a more physiological frequency range.

The *hysteresis* loop variables we measured discriminated controls from CVD patients. Interestingly, they also discriminated C_{1S} from controls and from $C_{3\&5}$ patients. As telangiectasias or spider-veins are the only objective signs in C_{1S} patients, such quantitative data should help characterizing this distinct entity, which may have some features in common with C_{0s} patients described by Andreozzi *et al.* as suffering from 'hypotonic phlebopathy'.[47] Our results suggest that viscosity is higher in unaffected veins of CVD patients in whom only reduced distensibility had been demonstrated so far.[13,24,38]

Limitations:

CVD also involves skin and soft tissues.[19,48] Therefore, the viscoelasticity variables we measured also depended on the biomechanics of blood and surrounding tissues. Differences in blood viscosity and/or upstream and downstream resistance to blood displacement during focal compression may have played a role, but the present study did not allow their separate evaluation. Skin stiffness, subcutaneous fat thickness, and interstitial fluid may also have contributed, although we found no statistical difference between groups in vein depth and depth changes under compression. Moreover, we performed the compression test at mid-calf level, some distance away from the upper limit of tissue alteration associated with lipodermatosclerosis in patients with advanced CVD.

We restricted invasive measurements to the number of subjects and patients allowing proper characterization of the population samples since ample literature is already available regarding intravenous and intramuscular pressure in CVD, but this limited the statistical power and precluded further correlations. We measured intravenous pressure in the great saphenous vein and performed the ultrasonographic examination on the small saphenous vein (a superficial vein) and on the soleal or gastrocnemial veins (muscular veins).[29] Nevertheless, all measurements were performed at the same calf level. Comparing axial and muscular calf veins, which exhibit different anatomical features, would be necessary in future studies for a more comprehensive assessment. We recruited patients with C_{1s}, C_3, and C_5 CEAP categories because compression is the main therapeutic option for them, whereas C_2 and C_4 categories may be more representative of CVD. We included CVD patients with various etiologies, topographies, and severity of venous lesions, precluding subgroup analyses for lack of statistical power. Foot or knee deformation and body weight distribution may affect saphenous vein caliber and should be specifically studied. Evaluating leg tissues and measuring blood viscosity would be useful for thorough pathophysiological assessment.

Conclusion:

Although the cross-sectional area of the small saphenous, but not the deep calf vein, was greater in CVD patients than in controls, postural changes in cross-sectional area were highly diverse and did not allow differentiating patients from controls. These postural changes may result from multiple, potentially opposite factors that must be specifically investigated before they can be used for characterization of chronic venous disease. Tracking the cross-sectional area of leg veins under compression by the US probe yielded typical *hysteresis* loops, reflecting viscoelasticity. We found higher viscosity in unaffected small saphenous veins of CVD patients than in healthy controls, supporting the hypothesis of global changes to the venous wall. Postural changes of venous viscoelasticity variables appeared much more marked and consistent than cross-sectional area changes.

Acknowledgments:

We thank Professor Patrick Carpentier (Grenoble, France) for his advices and revision of the manuscript.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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Figure Legends:

Fig 1. A typical hysteresis loop of the short saphenous vein. Legend: Cross-sectional area (in mm^2) plotted as a function of the force (in N) exerted by the operator on the ultrasound probe. CPF: vein–closing probe force; OPF: vein–opening probe force; CAH and DAH: area of the compression and decompression parts, respectively, of the loop; S1H and S2H: first and second slopes, respectively, of the compression part of the loop.

Fig 2. CEAP characteristics of the examined lower limb of patients with C_{1s}, C_3, and C_5 class of chronic venous disease. Legend: *per* CEAP classification, C_{1s}: *telangectasia* or reticular veins and symptoms; C_3: edema; C_5: healed venous ulcer; Ep: primary; Es (PTS): secondary (post-thrombotic syndrome); En: no venous cause identified but presence of several potential causes and risk factors (obesity, ankylosis, limb deformity, history of trauma…); As: disease involving superficial veins; Ad: disease involving deep veins; An: no venous location identified; Po: venous obstruction; Pr: venous reflux; Pn: no venous pathophysiology identifiable.

Fig 3: Histogram of relative postural changes in vein cross-sectional area. Legend: Histogram of relative (%) changes in cross-sectional area of the small saphenous vein and of the deep calf vein between the supine and the standing position in the whole population sample (n=111).

Fig 4: Schematic drawing of the *hysteresis* loops of controls and patients. Legend: Hysteresis loops redrawn from the median values of the small saphenous vein cross-sectional area during the compression test for normal controls and for limbs with C_{1S}, C_3, and C_5 CEAP category of chronic venous disease, in the supine and in the standing position.

Supplemental Video 1: Example of B-mode sequence with automatic detection of the small saphenous vein lumen during the compression test.

Table I. *Hysteresis* loop variables of the small saphenous vein in patients and controls.

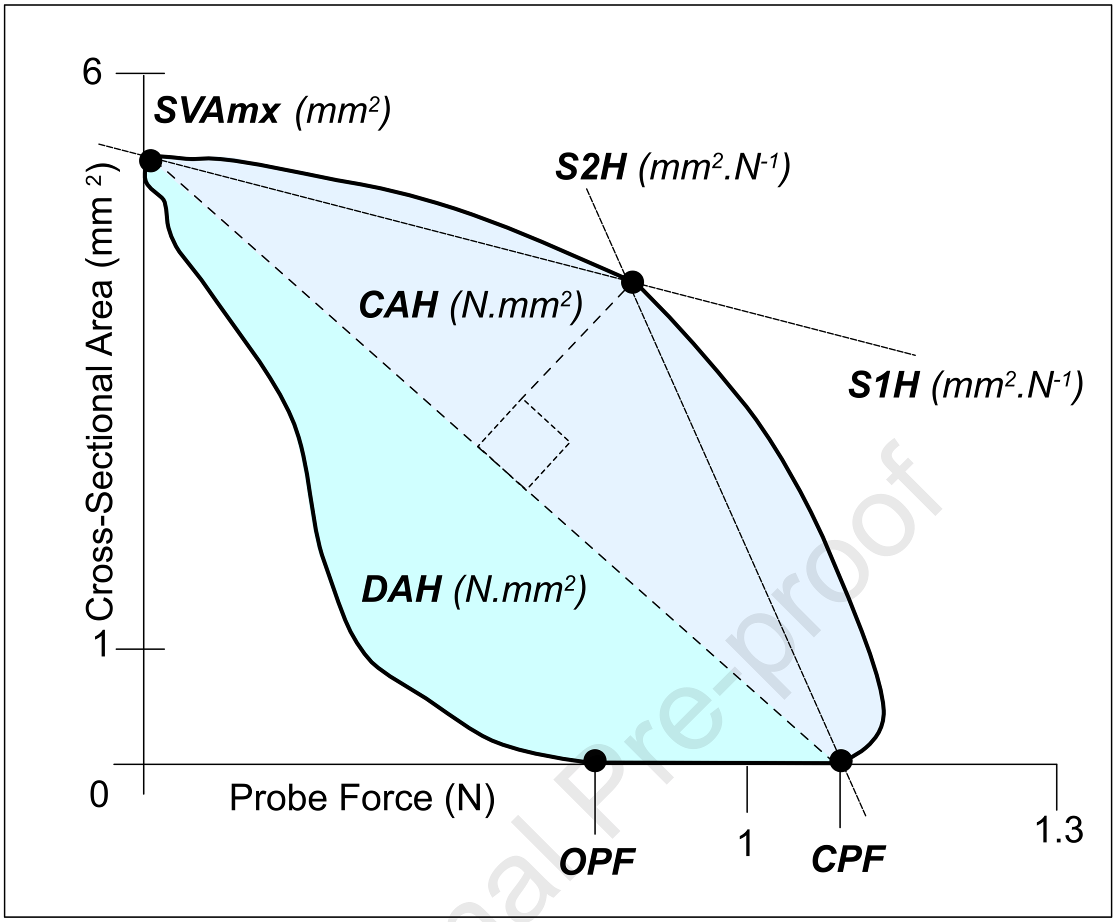
	Controls	C_{1s}	C_3 & C_5	Controls vs C_{1s}	Controls vs $C_{3\&5}$	C_{1s} vs $C_{3\&5}$
			SVAmx (mm^2)			
Supine	2.94 [1.76-5.18]	3.95 [2.33-4.97]	4.87 [3.57-7.06]		p=.005	
	AUC=0.58	AUC=0.70	AUC=0.64			
Standing	3.75 [2.12-5.41]	4.70 [2.56-6.16]	7.07 [2.96-9.90]		p=.002	
	AUC=0.59	AUC=0.60	AUC=0.65			
Supine vs Standing	p=.005	p=.047	p=.002			
			CPF (N)			
Supine	1.03[0.75—1.35]	0.87[0.60—1.23]	1.22[0.89—1.64]			
	AUC=0.59	AUC=0.60	AUC=0.65			
Standing	2.71[2.20—3.13]	2.51[2.04—2.89]	3.15[2.54—4.03]		p=.047	p=.039
	AUC=0.55	AUC=0.66	AUC=0.69			
Supine vs Standing	p<.001	p<.001	p<.001			
			OPF (N)			
Supine	0.36[0.21—0.56]	0.35[0.14—0.58]	0.52[0.19—0.76]			
	AUC=0.53	AUC=0.62	AUC=0.63			
Standing	0.98[0.63—1.56]	1.42[1.19—1.77]	1.76[1.12—2.07]	p=.027	p<.001	
	AUC=0.70	AUC=0.77	AUC=0.59			
Supine vs Standing	p<.001	p<.001	p<.001			
			DPF (N)			
Supine	0.64[.38—.94]	0.50[.32—.90]	0.65[.42—1.02]			
	AUC=0.54	AUC=0.53	AUC=0.57			
Standing	1.65[1.25—2.09]	0.86[.59—1.32]	1.27[.75—2.06]	p=.001		
	AUC=0.77	AUC=0.60	AUC=0.63			
Supine vs Standing	p<.001	p<.001	p<.001			
			TAH ($N.mm^2$)			
Supine	1.24[0.66—2.11]	1.15[0.79—2.89]	2.40[1.65—3.84]		p=.001	
	AUC=0.54	AUC=0.72	AUC=0.68			
Standing	4.16[2.73—8.43]	4.25[2.71—5.21]	8.95[3.87—15.96]		p=.011	p=.019
	AUC=0.53	AUC=0.68	AUC=0.73			
Supine vs Standing	p<.001	p<.001	p<.001			
			CAH ($N.mm^2$)			
Supine	0.38[0.13—0.70]	0.31[0.09—1.02]	0.65[0.32—1.68]			
	AUC=0.51	AUC=0.62	AUC=0.63			
Standing	1.36[1.02—3.52]	1.70[0.97—2.19]	3.70[1.16—7.13]		p=.019	p=.048
	AUC=0.52	AUC=0.67	AUC=0.69			
Supine vs Standing	p<.001	p<.001	p<.001			
			DAH ($N.mm^2$)			
Supine	0.79[0.42—1.46]	0.75[0.58—1.84]	1.86[1.07—2.54]		P<.001	
	AUC=0.58	AUC=0.75	AUC=0.69			
Standing	2.72[1.49—5.05]	2.28[1.37—3.85]	4.24[2.02—9.32]		p=.049	p=.041
	AUC=0.55	AUC=0.65	AUC=0.70			
Supine vs Standing	p<.001	p<.001	p<.001			
			S1H ($mm^2.N^{-1}$)			
Supine	-1.06[-1.86—-0.47]	-1.98[-3.42—-0.53]	-2.04[-3.28—-1.10]		p=.012	
	AUC=0.66	AUC=0.68	AUC=0.52			
Standing	-0.37[-0.68—-0.24]	-0.55[-1.37—-0.28]	-0.52[-0.91—-0.23]			
	AUC=0.62	AUC=0.55	AUC=0.54			
Supine vs Standing	p<.001	p<.001	p<.001			
			S2H ($mm^2.N^{-1}$)			
Supine	-5.49[-8.37—-3.41]	-6.52[-10.31—-3.15]	-9.21[-15.45—-3.54]			
	AUC=0.57	AUC=0.64	AUC=0.59			
Standing	-2.71[-4.07—-1.86]	-3.46[-7.68—-1.83]	-4.29[-6.68—-2.96]		p=.001	
	AUC=0.62	AUC=0.69	AUC=0.55			
Supine vs Standing	p<.001	p<.026	p<.001			

Legend: C_{1S}: limbs with C_{1s} CEAP class of chronic venous disease; $C_{3\&5}$: limbs with either C_3 or C_5 class of chronic venous disease; **p** = p-value (when significant) of Dunn's multiple comparison post-Kruskal-Wallis test for group comparison, and of paired t-test for supine versus standing position. AUC: area under the receiver operating characteristic curve. SVAmx: maximum cross-sectional area of the small saphenous vein; CPF: vein-closing probe force; OPF: vein-opening probe force; TAH: total area of the *hysteresis* loop; CAH and DAH: area of the compression and decompression phase, respectively, of the *hysteresis* loop; S1H and S2H: slope of the first and second part, respectively, of the compression phase of the *hysteresis* loop.

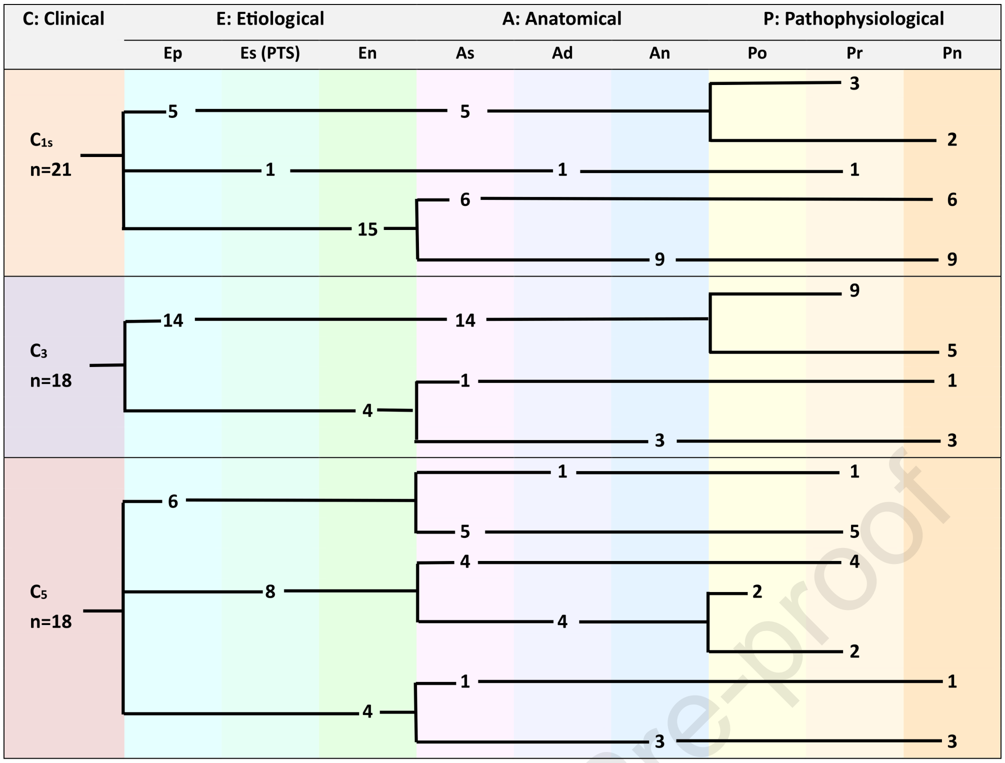
Table II. Discriminative value of *hysteresis* variables.

		Controls vs. CVD	Controls vs. C_{1s}	Controls vs. C_3 & C_5	C_{1s} vs. C_3 & C_5
SVAmx	Supine	p=.015		p=.006	p=.007
	Standing	**p=.007**		**p=.003**	**p=.054**
CPF	Supine				**p=.198**
	Standing	**p=.059**		**p=.007**	**p=.039**
OPF	Supine			**p=.038**	**p=.090**
	Standing	**P<.001**	**p=.013**	**P<.001**	
TAH	Supine	p=.022		p=.008	p=.049
	Standing	p=.035		p=.005	**p=.022**
CAH	Supine			p=.085	p=.113
	Standing	**p=.025**		**p=.004**	p=.027
DAH	Supine	**p=.004**		**p=.001**	**p=.045**
	Standing	p=.074		p=.022	p=.046
S1H	Supine	**p=.007**	**p=.032**	**p=.015**	
	Standing	**p=.078**	**p=.032**		
S2H	Supine	**p=.110**		**p=.085**	
	Standing	**p=.005**	**p=.056**	**p=.004**	
Number of variables introduced in the model		**9**	**4**	**9**	**6**
Multivariate AUC with selected variables		**0.796**	**0.777**	**0.826**	**0.744**
IC95% AUC (Delong method)		0.710—0.882	0.662—0.892	0.739—0.9141	0.614—0.873
IC95% AUC (boot-strap 10000)		0.707—0.878	0.657—0.884	0.731—0.908	0.609—0.866

Legend: p-values of univariate logistic regression analysis, and multivariate logistic regression analysis of eligible *Hysteresis* variables. Variables were eligible if yielding a p value <0.2. Among strongly correlated variables, only the one with the smaller p-value was included in the multivariate model. Other variables (shaded background) were not included. CVD: chronic venous disease (all categories); C_{1S}, C_3, C_5: CEAP categories of CVD; SVAmx: maximum cross-sectional area of the small saphenous vein; CPF: vein-closing probe force; OPF: vein-opening probe force; TAH: total area of the *hysteresis* loop; CAH and DAH: area of the compression and decompression phase, respectively, of the *hysteresis* loop; S1H and S2H: slope of the first and second part, respectively, of the compression phase of the *hysteresis* loop; AUC: area under the receiver operating characteristic curve.



6
SVAmx (mm²)
S2H (mm².N⁻¹)
CAH (N.mm²)
S1H (mm².N⁻¹)
Cross-Sectional Area (mm ²)
DAH (N.mm²)
1
0
Probe Force (N)
OPF
1
CPF
1.3


C: Clinical
E: Etiological
A: Anatomical
P: Pathophysiological
Ep
Es (PTS)
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n=21
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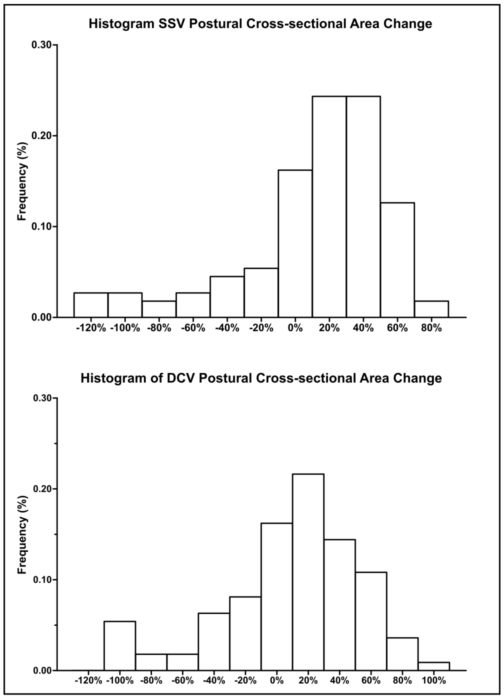
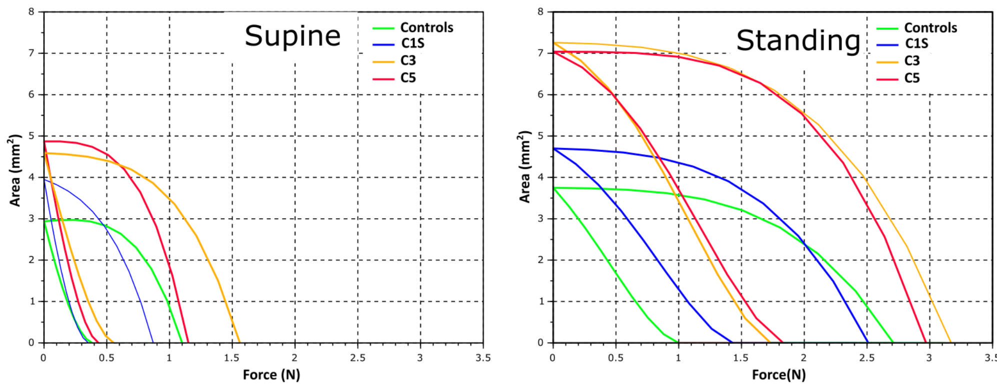

Histogram SSV Postural Cross-sectional Area Change
Frequency (%)
0.30
0.20
0.10
0.00
-120%
-100%
-80%
-60%
-40%
-20%
0%
20%
40%
60%
80%
Histogram of DCV Postural Cross-sectional Area Change
Frequency (%)
0.30
0.20
0.10
0.00
-120%
-100%
-80%
-60%
-40%
-20%
0%
20%
40%
60%
80%
100%


Supine
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Force (N)
Standing
Controls
C1S
C3
C5
Area (mm²)
Force(N)

Noninvasive measurement of venous wall deformation induced by changes in transmural pressure shows altered viscoelasticity in patients with chronic venous disease

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Additional Material

Material and Methods: additional information

Determination of population sample size

Based on studies involving 8 to 35 subjects and reporting significant differences in venous distensibility[1,2] or *hysteresis*[3] between CVD patients and controls, and between young and elderly subjects,[4] we estimated that we needed to include 54 CVD patients (18 for each CEAP subgroup), and 54 controls (18 in each physical activity subgroup). We measured intravenous and intramuscular pressures in 18 of the CVD patients and 18 of the controls with the same CEAP or activity repartition.

Detailed methods

US examinations were performed with a Logiq-e system and its 12L-RS linear probe (GE Ultrasound, Chicago, IL, USA). Settings were harmonics mode, 75 dB dynamic range, and one focal zone. We adjusted emitting frequency, depth, gain, time-gain compensation, and focus to obtain the best image of the vein. Frame rate was ≥26 images per second. The ultrasound probe was mounted on a berth gliding on a rail and instrumented with a XFTC300 sensor (Measurement Specialties, Hampton, VI, USA), with range 2-2000 N, linearity ≤±0.5% of full scale, and *hysteresis* ≤±0.5% of full scale, for the measurement of probe force (PF, in N), *i.e.* the force applied on the ultrasound probe by the operator. The sensor was connected to an ARD154 signal amplifier with -120 to 10 000 Ohm bridge impedance, 20 kHz maximum bandwidth, and accuracy 0.01% of full scale (Measurement Specialties, Hampton, VI, USA). The amplifier was connected through an UIM100C universal interface module to a MP150 data acquisition and processing system (Biopac Systems, Goleta, CA, USA) with 16 Bits A/D resolution and ±0.003 accuracy, at 100 Hz sample rate. The PAL Y/C S-video signal from the US system was captured by a Picolo frame-grabber (Euresys, Liege, Belgium) with 720×576 pixels resolution at 25 images per second, and stored as consecutives images on a personal computer.

Intramuscular pressure was measured with a 1.2 mm external diameter, 275 mm long IMP-Cath catheter (Alcis, Besançon, France), inserted, under local anesthesia by 6 to 8 mL of 5 mg/mL lidocaine, into the *triceps surae* muscle at 4 cm approximate depth, slightly above the maximum girth of the calf. Intravenous blood pressure was measured with a 22G, 1” long Cathlon catheter (Smiths-Medical, St-Paul, MN, USA) inserted into the great saphenous vein at mid-calf height. Both catheters were filled with heparinized isotonic saline and connected to DPT-6000 pressure sensors (Codan-Medical, Lensahn, Deutschland) of which analog signals were sent to a Biopac-MP150 data acquisition system, then measured and analyzed offline with Acqknowledge V4.2 (Biopac Systems, Goleta, CA, USA). Calibration at atmospheric pressure and against a mercury column was performed before each session.

The experiment took place in a quiet, neutral temperature-controlled room. The subject was lying supine on his or her side (lateral *decubitus*) with a small wedge under the heel to avoid any contact of calf muscles with the examination table. The observer recorded B-mode US images of the small saphenous vein (SSV) at mid-calf height, then of a deep calf vein (DCV, the *soleus* vein or a *gastrocnemius* vein, as available) at the same calf level, avoiding buckling or dilated veins or venous segments. These veins were chosen because they could be examined at the same calf level, and their US examination was not hampered by bone structures, while leaving the great saphenous vein available for blood pressure measurement. The observer increased PF progressively until the vein collapsed, then released it, allowing the vein to reopen and expand, at a rate of 0.25—1 cycle per second. The subject was then asked to stand motionless (*orthostasis*), with no effort or muscular contraction of the examined leg, bearing the weight of the body on the other leg, and the vein-compression test was repeated.

Detailed measurements and calculations

Measurements were independently performed on recorded signals and images by observers blinded from the subject's status.

We used the 'fit ellipse' function of ***Fiji*** image processing software (https://fiji.sc/) to measure the SSV and DCV cross-sectional area on recorded US images. The postural cross-sectional area change (PAC) was calculated in percentage as 100x(AS-AL)/AS, with AL and AS = vein cross sectional area, respectively in the supine and the standing position. We measured, on the same image sequences, the SSV and DCV depth (US probe-to-vein distance).

Recorded US images were also analyzed off-line with a custom-made software that detected the vein walls and approximated the lumen to an ellipse.[5] Within the rectangular area of interest (ROI) drawn by the observer to enclose the observed vein on the first image of the recorded sequence, the software automatically adjusted the grey scale threshold for image binarization, then proceed to morphology adjustment for edge smoothing.[5] This allowed the detection of the venous wall along the horizontal (X) and vertical (Y) axes, and the computation of the X and Y lengths for ellipse approximation. The calculated ellipse was then overlaid on the initial B-mode image for visual control. The ROI center was calculated for each approximated ellipse, allowing to track automatically the movements of the vein all along the sequence.

A LabView-2016 (National Instruments Corp., Austin, TX, USA) routine drew the SSV cross-sectional area *versus* PF function, which followed a *hysteresis* loop, from which the following variables were automatically extracted:[6,6]

1) Pressure-related variables: the maximum (with null PF) cross-sectional area (SVAmx), the PF at which the vein collapsed (CPF) during the compression phase, the PF at which the vein reopened (OPF) during the decompression phase, and the difference between CPF and OPF (DPF).
2) Viscosity-related variables: the total area (TAH) of the *hysteresis* loop, and the area of the compression (CAH) and decompression (DAH) phases of the loop.
3) Elasticity-related variables: the first (S1H) and second (S2H) slopes of the compression phase of the loop. We also measured, on recorded images, the vein depth from the skin at zero PF and at collapse.

Mean intravenous (IVPm) and intramuscular (IMPm) pressures were obtained by averaging instantaneous values over about 10s. Were also recorded the subjects' age, weight, height, leg length, and calf circumference, and the presence of reflux or obstruction in veins other than the investigated SSV and DCV.

Additional Results

Reproducibility

Reproducibility was evaluated on two independent readings of the same recorded image or signal by Lin concordance correlation coefficient (ρc)

Intra-observer reading reproducibility of cross-sectional area measurements yielded ρc=0.988 and 0.985 for the SSV, and 0.878 and 0.955 for the DCV, respectively in the supine and in the standing position.

The intra-observer reading reproducibility ρc ranged from 0.95 to 0.9996 for mean intravenous blood pressure (IVPm) and 0.956 to 0.9999 for intramuscular pressure (IMPm) along the procedure.

Inter-observer reading reproducibility ρc was =0.981 for CPF, 0.845 for OPF, 0.978 for TAH, 0.939 for CAH, 0.897 for DAH, 0.706 for S1H, and 0.897 for S2H.

Hysteresis Variables

For the whole population sample, TAH (p=0.0006), CAH (p=0.016), and DAH (p=0.0003) increased with age in the supine position. In controls, only DAH changed with age (p=0.034). In CVD patients, CPF (p=0.019), OPF (p=0.044), TAH (p=0.006), CAH (p=0.032), and DAH (p=0.003) increased with age. There was no significant relation between hysteresis variables and age in the standing position.

Analysis of ROC curves showed that most *hysteresis* variables differentiated controls from CVD patients. Multivariate logistic regression analysis yielded an AUC reaching 0.83 for the differentiation of controls from C_3 and C_5 limbs when OPF, DAH, S1H, and S2H in the supine position, and CPF, OPF, CAH, and S2H in the standing position were included. The AUC reached 0.78 for the differentiation of controls from C_{1S} limbs when S1H in the supine position, and OPF, S1H, and S2H in the standing position were included. It reached 0.80 for the differentiation of controls from C_3 and C_5 patients when DAH, S1H, and S2H in the supine position, and CPF, OPF, CAH, S1H, S2H and SVA in the standing position were included. It reached 0.75 for differentiating C_{1S} from C_3 and C_5 limbs when CPF, OPF, and DAH in the supine position, and CPF, TAH, and SVA in the standing position were included.

Supplemental Discussion

Intravenous blood pressure

We found the expected relation between vein cross-sectional area and body weight[7], BMI, and age.[8] The great saphenous vein blood pressure, although not different between groups at baseline, correlated with weight in agreement with previous reports.[8] Intravenous pressure increased, whereas intramuscular pressure decreased slightly, in the standing position,[9] but intramuscular pressure remained higher in CVD patients than in controls.

Vein cross-sectional area

The greater SSV, but not DCV, cross-sectional area we found in CVD patients than in controls is in agreement with previous studies about GSV diameter[10-12] and CEAP classes.[13-15] However, these studies included no controls, while we included normal controls and measured unaffected superficial but also deep calf veins. Saphenous veins are thought to be more prone to dilation because they are not supported by surrounding tissues and muscles, contrary to deep veins.[16] However, the contribution of surrounding tissues to the limitation of transmural pressure of deep calf veins at rest appears limited since intramuscular pressure decreases in the standing position, as shown by our study and another.[9]

We found the expected relation between vein cross-sectional area and body weight[7], BMI, and age.[8] The great saphenous vein blood pressure, although not different between groups at baseline, correlated with weight in agreement with previous reports.[8] Intravenous pressure increased, whereas intramuscular pressure decreased slightly, in the standing position,[9] but intramuscular pressure remained higher in CVD patients than in controls.

Postural changes in cross-sectional area

Our most striking result is the extent of interindividual differences in PAC, independently of the healthy or CVD *status*, since the vein area increased in some subjects, staid unchanged or even decreased in others in the standing position. As we took care to avoid residual muscle contraction, the absence, in some subjects, of vein area increase in spite of greater hydrostatic blood pressure could be due to multiple, possibly opposite factors such as greater venous wall stiffness and/or stronger venous tone and/or higher interstitial pressure and/or lower distensibility of skin and/or of surrounding soft tissues. For instance, edema and lipodermatosclerosis[17] may form an inelastic sleeve around the calf, limiting vein expansion. This may be reflected by the greater calf circumference we found in C_3 but not in C_5 limbs than in controls, and the greater ankle circumference we found in C_3 and C_5 patients, who also had greater intramuscular pressure in the standing position. Different mechanisms (e.g. reflex orthostatic increase of venous tone in healthy subjects, lower skin distensibility and greater interstitial pressure in C_5 limbs) could lead to the same results by limiting vein expansion. Increased venous tone could explain the negative PAC we observed in a noticeable proportion of subjects. Each of these mechanisms should be specifically investigated.

Van der Velden *et al*. found a negative postural diameter change in 10% of their subjects, but dismissed it as measurement error.[18] We limited errors by measuring the cross-sectional area rather than only the larger diameter, and ensuring that the subject's weight rested on the other leg. We included healthy controls and examined unaffected veins of CVD patients whereas they compared limbs with to limbs without venous reflux in the same CVD patients. Although a linear correlation has been reported between intravenous pressure and diameter of saphenous veins with reflux,[19] the relationship may be more complex in unaffected veins. Therefore, we must consider that the interindividual differences we observed are not meaningless. Different factors may be involved in different veins, as suggested, in our study, by the absence of correlation between saphenous and deep vein PAC. Pending further studies clarifying this issue, postural changes in diameter or cross-sectional area would not be sufficient to characterize CVD.

Vessel wall viscoelasticity

Although viscosity is a characteristic of fluids and a major feature of blood, the walls of arteries and veins do present viscoelastic characteristics, combining features of elastic solids and viscous fluids.[20] The elastic component represents the amount of energy stored during loading, while the viscous component is responsible for energy dissipation. The ratio of the viscous to elastic component increases with strain and strain rate.[21,22] The viscosity component of the vessel wall is mainly attributed to smooth muscle cells[23] but a contribution of collagen (in the extracellular matrix and in the SMC membrane) to the nonlinearity of the stress-strain curve has also been shown. The role of viscosity in the damping of the arterial pulse wave and in the ventricular afterload has been largely demonstrated[24-28] and illustrated in cardiovascular diseases, including arterial hypertension.[23] Viscoelasticity of venous walls has been much less studied but is nevertheless acknowledged as essential.[29] Most studies have been performed *in vitro*, in animal[30] or human specimens, especially for the evaluation of saphenous veins used as homografts since their viscoelastic properties are essential for proper function when implanted in the arterial system.[31-34] *In vivo*, venous occlusion plethysmographic studies also demonstrated hysteresis,[6,35] which implies viscosity.

Ex vivo biomechanical and immuno-histochemical studies have clearly demonstrated the presence of structural changes in the venous wall of patients with chronic venous disease, with subsequent alteration of vein biomechanics.[36,37] Loss of elastin and type III collagen has been found in varicose veins, together with disorganization of the extracellular matrix, disturbed expression of matrix remodeling enzymes, and loss of smooth muscle cells.[37-40] These changes result in increased distensibility,[2,41] which means decreased elastic *modulus*. This also applies to plethysmography, which allowed to record *hysteresis* loops and showed greater leg veins distensibility in patients with varicose veins than in controls.[3] On the other hand, although data remain scarce, a decrease in calf muscle tissue viscoelasticity with age have been demonstrated,[42] and biopsy specimens showed structural and biochemical changes in the gastrocnemius muscles of patients with chronic venous disease.[43] Therefore, our results, obtained noninvasively *in vivo*, are consistent with previous *in vitro* and *ex vivo* findings demonstrating altered venous viscoelasticity in patients with chronic venous disease (CVD). However, most of these studies were performed on varicose veins whereas we studied unaffected veins of CVD patients, and we could not specifically identify the role of the venous wall in the viscoelasticity differences we observed.

Viscoelasticity is typically strain-rate dependent, although little differences have been observed in hysteresis curves of bovine jugular and lumbar veins between 1, 5, and 10 Hz (10, 50, and 100%.s^{-1}).[30] Nevertheless, viscoelasticity components related to blood, vessel wall, skeletal muscles, surrounding tissues, and skin may display different rate-dependence. In view of the limited footprint of the ultrasound probe, and the relatively high rate of the compression test (0.25—1 Hz, compared to the 0.05 to 0.015 Hz range of venous occlusion plethysmography), we hypothesize that blood displacement was not significantly involved, but this remains to be demonstrated.

Appendix: Additional Tables

Appendix Table I. Small saphenous and deep calf vein depth.

	Small saphenous vein depth (mm)		
	Supine	**Standing**	Supine vs. Standing
Baseline	7.3 [5.2—9.7]	6.8 [4.7—8.8]	p<.0001
At collapse	7.5 [5.4—9.1]	6.8 [5.2—9.0]	p<.0001
Baseline vs. collapse	p=.93	p=.06	
	Deep calf vein depth (mm)		
	Supine	**Standing**	Supine vs. Standing
Baseline	18.9 [15.7—22.9]	18.6 [14.1—23.4]	p=.018
At collapse	17.4 [14.6—21.7]	16.7[13.6—20.8]	p=.0005
Baseline vs. collapse	p=.05	p=.05	

Legend: small saphenous and deep calf vein depth from the skin in the Supine and in the standing position, in the whole population sample (n=111). Values are provided as median [lower-upper quartile]. p: p-value of Wilcoxon signed rank test for comparison between the Supine and the standing position and between depth at baseline and at vein collapse.

Appendix Table II. Depth of the small saphenous vein according to CEAP class.

	Controls n=54	C_{1s} limbs n=21	C_3 limbs n=18	C_5 limbs n=18
		Small Saphenous Vein		
Supine				
Baseline	7.6 [6.0—10.1]	7.1 [4.4—8.5]	7.0 [4.3—11.2]	6.9 [5.2—9.9]
At collapse	7.3 [5.0—8.7]	7.6 [6.0—9.9]	6.8 [4.4—9.9]	8.5 [5.4—10.3]
Standing				
Baseline	6.8 [5.6—8.8]	6.3 [4.4—8.0]	7.0 [4.3—11.2]	6.7 [3.9—9.3]
At collapse	6.6 [5.0—8.3]	7.4 [5.6—9.8]	6.8 [4.4—9.9]	7.4 [5.4-10.3]
		Deep Calf Vein		
Supine down				
Baseline	18.9 [15.2–23.6]	18.7 [16.0–26.6]	19.2 [14.8–21.9]	18.9 [15.2–23.2]
At collapse	17.0 [14.5–20.8]	18.5 [16.2–24.8]	17.2 [14.0–24.3]	17.7 [13.4–22.0]
Standing				
Baseline	18.9 [13.9–23.72]	18.3 [15.9–26.5]	18.3 [12.3–21.6]	17.7 [13.8–23.0]
At collapse	16.6 [13.2–19.9]	17.8 [13.9–22.8]	17.0 [12.5–24.1]	16.8 [14.2–20.4]

Legend: Depth, in mm, of the small saphenous and of the deep calf vein at baseline (with void compression force) and at vein collapse, in normal controls, and in limbs with C_{1s}, C_3, and C_5 CEAP category of chronic venous disease. Values are reported as median [lower—upper quartile].

Appendix Table III. Relative postural changes in cross-sectional area of the small saphenous vein and of the deep calf vein.

	Controls (n=54)	C_{1s} (n=21)	C_3 (n=18)	C_5 (n=18)
SSV	22.26 [-9.40—41.66]	19.34 [-5.42—46.21]	26.97 [1.46—36.30]	20.18 [-17.52—36.86]
DCV	17.34 [-13.31—41.34]	-12.18 [-117.20—18.19]	19.73 [-72.84—44.43]	18.09 [-11.84—36.04]

Legend: Relative (%) postural changes in cross-sectional area of the small saphenous vein (**SSV**) and of the deep calf vein (**DCV**) in normal controls and in limbs with C_{1s}, C_3, and C_5 CEAP category of chronic venous disease. Values are provided as median [lower-upper quartile].

Appendix Table IV. Mean intravenous blood pressure in the great saphenous vein.

	Supine	Standing	Supine vs Standing
Controls (n=15)	10.6 [4.9–15.3]	46.7 [-6.6–57.9]	**p=0.030**
CVD (n=16)	14.3 [8.3–22.0]	58.0 [51.0–65.0]	**p=0.0001**
Controls vs CVD	p=0.093	**p=0.011**	

Legend: Mean intravenous blood pressure (in mm Hg) in the great saphenous vein of normal controls and of limbs with chronic venous disease (CVD) in the supine and in the standing position. Values are provided as median [lower–upper quartile]. p: p-value of Wilcoxon signed rank rest for comparison between the supine and the standing position, and of Mann-Whitney test for comparison between normal controls and limbs with chronic venous disease.

Appendix Table V. Mean calf intramuscular pressure.

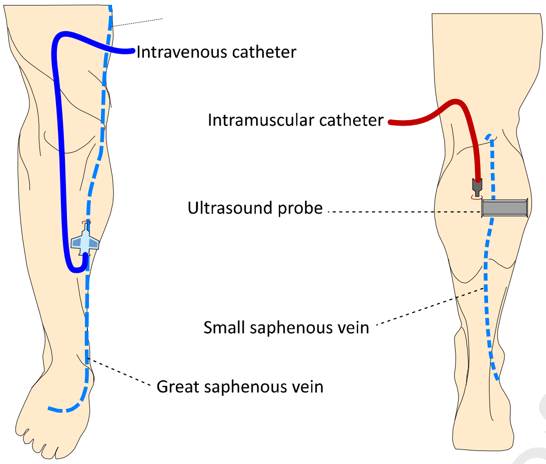
Controls	CVD Patients	p
	Supine	
1.5 [-2.7 — 4.13]	2.7 [-0.1 — 7.7]	p=0.523
	Standing	
-16.8 [-20.1 — -8.4]	-7.3 [-11.0 — -2.4]	p=0.007

Legend: Mean calf intramuscular pressure (in mm Hg) at rest in the supine and in the standing position in normal controls (n=17) and in limbs (n=17) with chronic venous disease (CVD). Results are provided as median [lower – upper quartile]. p: p-value of Wilcoxon-Mann-Whitney test for comparison between controls and CVD patients.

Appendix: Additional Figures

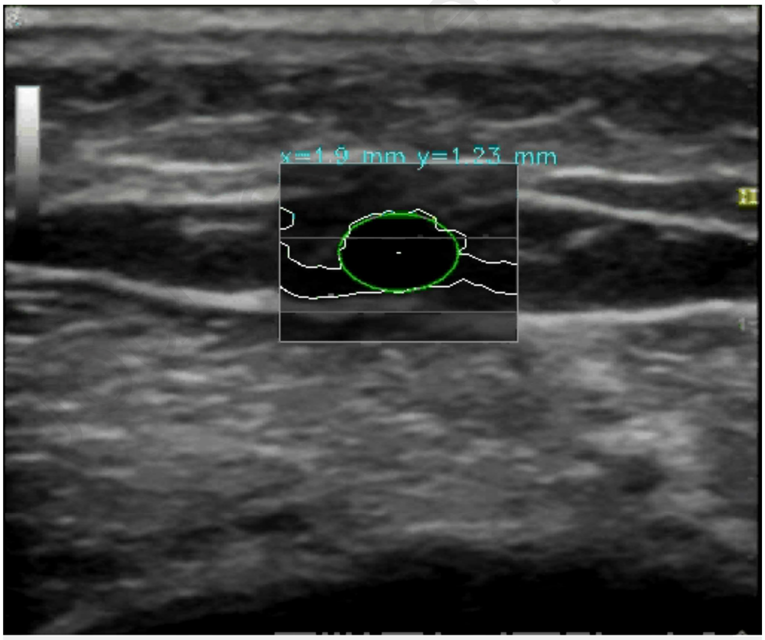
Appendix Figure 1

Location of intravenous and intramuscular catheters and of the ultrasound probe.



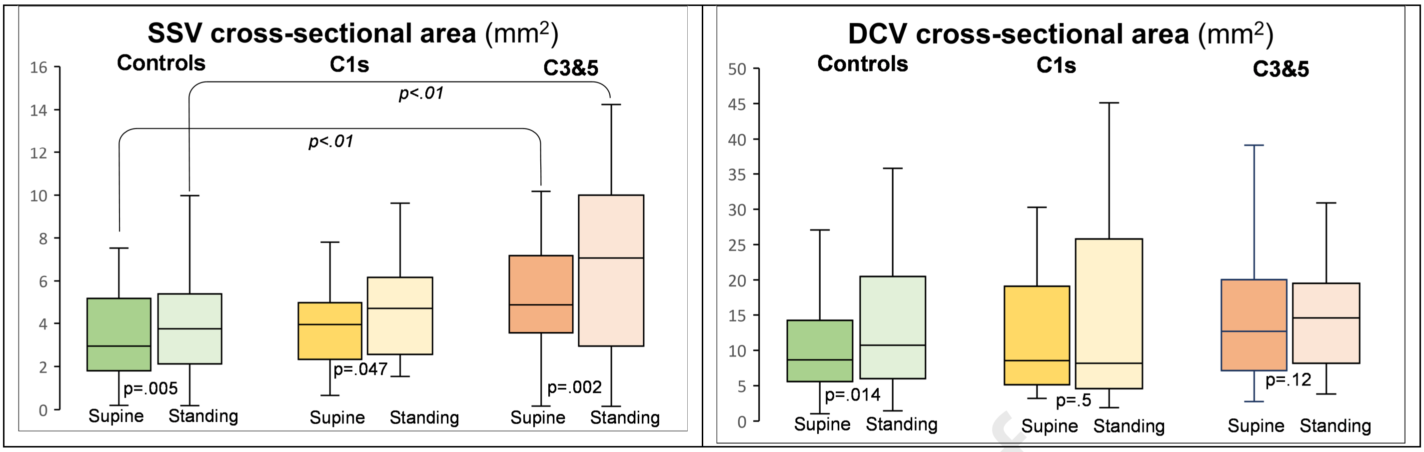
Appendix Figure 2

B-mode ultrasonographic image of the short saphenous vein with automatic wall detection and ellipse approximation.



Appendix Figure 3

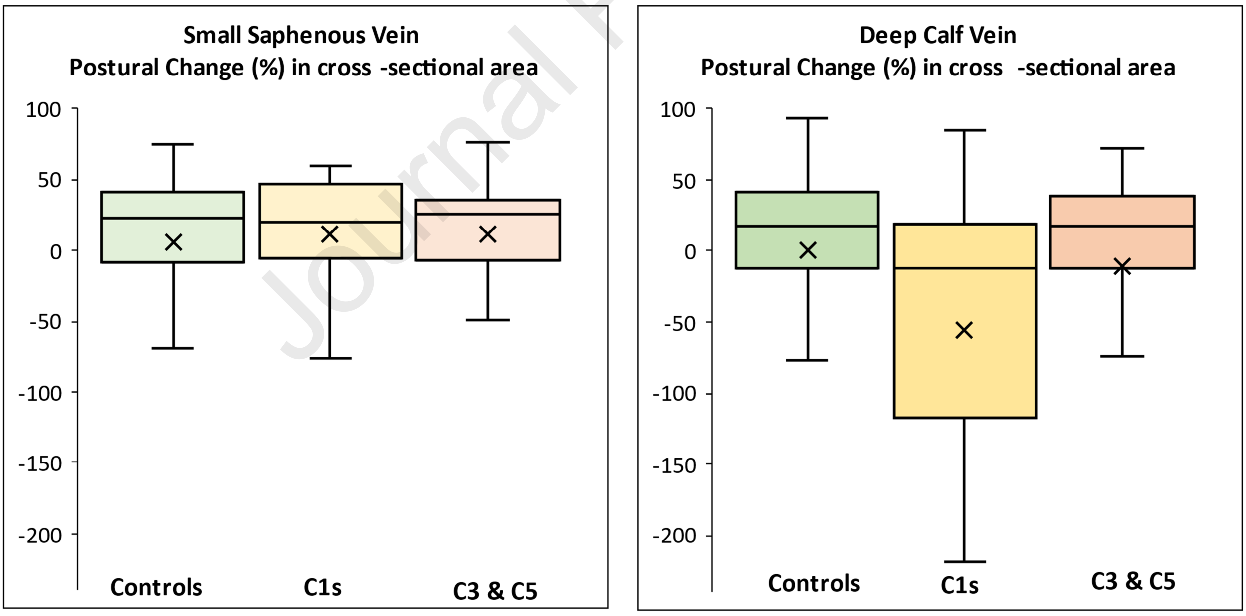
Cross-sectional area of the small saphenous vein and of the deep calf vein.



Legend: Box-and-Whiskers plots of the cross-sectional area (in mm2) of the small saphenous vein (SSV) and of the deep calf vein (DCV) in the supine and in the standing position, in normal controls, in limbs with C_{1s}, and in limbs with C_3 or C_5 CEAP category of chronic venous disease. p: p-value of comparison between the supine and the standing position. Comparison between groups are shown, when significant, as horizontal brackets with p-value.

Appendix Figure 4

Relative postural change in cross-sectional area of the small saphenous and of the deep calf vein.



Legend: Box-and-Whiskers plots of relative (%) postural changes of vein cross-sectional area in normal controls, in limbs with C_{1s}, and in limbs with C_3 or C_5 category of chronic venous disease. The horizontal line dividing the boxes represents the median value, and "X" represents the mean.

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Supplemental Table I. Biometrics of the population sample.

	Controls (n=54)	$\mathbf{C_{1S}}$ (n=21)	$\mathbf{C_3}$ (n=18)	$\mathbf{C_5}$ (n=18)
Age (years)	63.5 [53.0—70.0]	61.0 [44.0—72.0]	61.0 [52.3—67.0]	66.0 [60.0—76.5]
Weight (kg)	63.0 [60.0—74.5]	63.0 [58.5—80.0]	79.0 [64.0—88.5]	82.0 [68.5—111.5]
Height (cm)	164.5 [160.0—169.8]	162.0 [157.0—170.0]	166.5 [161.0—170.0]	169.0 [164.0—180.5]
BMI ($kg.m^{-2}$)	24.8 [21.5—27.3]	25.6 [21.5—28.5]	29.0 [23.0—33.1]	27.3 [22.6—36.4]
Leg length (cm)	42.0 [39.0—43.5]	40.0 [39.0—42.0]	41.0 [39.6—42.0]	43.0 [41.5—44.0]
Calf Circumference (cm)	34.8 [32.9—37.0]	35.8 [34.0—37.0]	38.5 [36.3—42.7]	37.0[32.5—40.5]
Ankle Circumference (cm)	21.0 [20.0—22.0]	21.8 [20.8—23.4]	23.8 [22.2—25.4]	23.1 [22.0—25.9]

Legend: age, body weight, height, body mass index (BMI), leg length, calf circumference, and ankle circumference of the examined lower limbs in normal controls and in patients with limbs in C_{1S}, C_3, and C_5 CEAP category of chronic venous disease. Values are reported as median [lower—upper quartile].

Supplemental Table II. Small saphenous vein and deep calf vein cross-sectional area.

	Controls (n=54)	**C_{1s} (n=21)**	**C_3 & C_5 (n=36)**
	Small Saphenous Vein cross-sectional area (mm^2)		
Supine	2.9 [1.8—5.2]	4.0 [2.3—5.0]	4.9 [3.6—7.1]
Standing	3.8 [2.1—5.4]	4.7 [2.6—6.2]	7.07 [3.0—9.9]
Wilcoxon signed-rank test	**p=.005**	**p=.047**	**p=.002**
	Deep Calf Vein cross-sectional area (mm^2)		
Supine	8.7 [5.6—14.3]	8.6 [5.1—19.1]	12.7 [7.2—20.0]
Standing	10.7 [6.0—20.8]	8.2 [4.6—25.8]	14.6 [8.2—19.4]
Wilcoxon signed-rank test	**p=.014**	p=.500	p=.120

Legend: Cross-sectional area (in mm^2) of the small saphenous vein and of the deep calf vein in normal controls, in patients in C_{1S}, and in patients with limbs in C_3 or C_5 CEAP category of chronic venous disease in the supine and in the standing position. Values are provided as median [lower-upper quartile]. p: p-value of the comparison between the supine and the standing position by Wilcoxon signed-rank test.

Supplemental Table III. Correlation of cross-sectional area of leg veins with age, body mass, and height.

	Age	Body mass	Height	BMI
		All subjects and patients (n=111)		
		Supine		
SSV	0.277 (**p=.003**)	0.382 (**p<.001**)	0.101 (p=.300)	0.326 (**p<.001**)
DCV	0.305 (**p=.001**)	0.191 (**p=.047**)	0.140 (p=.150)	0.170 (p=.080)
		Standing		
SSV	0.181 (p=.060)	0.412 (**p<.001**)	0.148 (p=.130)	0.357 (**p<.001**)
DCV	0.083 (p=.390)	0.080 (p=.410)	0.070 (p=.470)	0.057 (p=.560)
		Normal controls (n=54)		
		Supine		
SSV	0.336 (**p=.010**)	0.224 (p=.110)	-0.118 (p=.410)	0.232 (p=.100)
DCV	0.251 (p=.070)	0.134 (p=.340)	0.020 (p=.890)	0.126 (p=.380)
		Standing		
SSV	0.248 (p=.070)	0.255 (p=.070)	-0.124 (p=.390)	0.322 (**p=.020**)
DCV	0.117 (p=.400)	0.130 (p=.350)	-0.001 (p=.990)	0.141 (p=.330)
		C_{1s} patients (n=21)		
		Supine		
SSV	0.292 (p=.200)	0.308 (p=.190)	0.161 (p=.500)	0.181 (p=.450)
DCV	0.305 (p=.180)	0.128 (p=.590)	0.310 (p=.180)	-0.063 (p=.790)
		Standing		
SSV	0.513 (**p=.017**)	0.236 (p=.320)	0.365 (p=.110)	0.012 (p=.960)
DCV	-0.214 (p=.350)	-0.291 (p=.210)	-0.071 (p=.770)	-0.275 (p=.240)
		C_3 & C_5 patients (n=36)		
		Supine		
SSV	0.106 (p=.540)	0.514 (**p=.001**)	0.213 (p=.210)	0.407 (**p=.010**)
DCV	0.305 (p=.070)	0.055 (p=.750)	-0.021 (p=.900)	0.139 (p=.420)
		Standing		
SSV	-0.054 (p=.750)	0.398 (**p=.020**)	0.248 (p=.150)	0.298 (p=.080)
DCV	0.235 (p=.170)	0.002 (p=.990)	0.227 (p=.180)	-0.017 (p=.920)

Legend: Spearman ***r*** and significance (p) of the cross-sectional area in the supine and in the standing position, of the small saphenous vein (**SSV**) and of the deep calf vein (**DCV**) correlation with age, weight, height, and body mass index (BMI), in the whole population sample, in normal controls, in patients with limbs in C_{1s}, and in patients with limbs in C_3 or C_5 CEAP category of chronic venous disease.