# 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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## ABSTRACT

**Objective:** The noninvasive measurement of venous wall deformation induced by changes in transmural pressure could allow for the assessment of viscoelasticity and differentiating normal from diseased veins.

**Methods:** In 57 patients with limbs in the C1s (telangiectasia or reticular veins and symptoms), C3 (edema), or C5 (healed venous ulcer) CEAP (clinical, etiologic, anatomic, pathophysiologic) category of chronic venous disease and 54 matched healthy controls, we measured the changes in the cross-sectional area of the small saphenous vein and a deep calf vein in the supine and standing positions and under compression with an ultrasound probe using ultrasonography.

**Results:** The small saphenous vein, but not the deep calf vein, cross-sectional area was smaller in the limbs of the controls than in the limbs with C3 or C5 disease but was not different from that in C1s limbs. When changing from the supine to the standing position, a greater force was required to collapse the leg veins. Their cross-sectional area increased in most subjects but decreased in 31.5% of them as for the small saphenous veins and 40.5% for the deep calf vein. The small saphenous vein area vs compression force function followed a hysteresis loop, demonstrating viscoelastic features. Its area, which represents the viscosity component, was greater ($P < .001$) in the pooled C3 and C5 limbs (median, 2.40 $N \cdot mm^2$; lower quartile [Q1] to upper quartile [Q3], 1.65-3.88 $N \cdot mm^2$) than in the controls (median, 1.24 $N \cdot mm^2$; Q1-Q3, 0.64-2.14 $N \cdot mm^2$) and C1s limbs (median, 1.15 $N \cdot mm^2$; Q1-Q3, 0.71-2.97 $N \cdot mm^2$). The area increased ($P < .0001$) in the standing position in all groups.

**Conclusions:** Postural changes in the cross-sectional area of the leg veins were highly diverse among patients with chronic venous disease and among healthy subjects and appear unsuitable for pathophysiologic characterization. In contrast, small saphenous vein viscoelasticity increased consistently in the standing position and the viscosity was greater in limbs with C3 and C5 CEAP disease than in controls. (J Vasc Surg: Venous and Lym Dis 2020;■:1-11.)

**Keywords:** Chronic venous disease; Diameter; Lower limb veins; Ultrasonography; Viscoelasticity

Chronic increases in venous blood pressure increase venous wall stress, altering the endothelium vasomotor function.[1] The smooth muscle contractile response of the venous wall to angiotensin-2, norepinephrine, and endothelin-1 is impaired in patients with primary chronic venous insufficiency,[2,3] together with $Ca^{2+}$ mobilization,[4] although the postreceptor contraction mechanisms are preserved.[5] Such changes in smooth muscle tone can alter the biomechanical properties of the vessel wall.[6] Chronic venous wall stress and inflammation, notably with transforming growth factor-β1 activation, result in an imbalance between matrix metalloproteases and their tissue inhibitors and lead to wall remodeling.[7] The loss of elastin and type III collagen has been observed

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in varicose veins, together with disorganization of the extracellular matrix, disturbed expression of matrix remodeling enzymes, and the 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 the early detection of venous wall distress.

Volume–pressure function reflects the vein biomechanics. In the low venous blood pressure range, such as in the supine position, a minimal transmural pressure increase will produce a large volume increase by changing the venous cross-section from bimodal to elliptical to circular. At higher blood pressure, such as in the standing position, the slope of the venous volume–pressure function flattens, eventually reaching a plateau at which an additional increase in blood pressure will no longer translate into a significant volume increase.[14] Only in this high pressure range will the venous wall elasticity be solicited and the diameter changes will correlate with pressure (at least in superficial veins with incompetent valves).[15] Therefore, venous biomechanics cannot be inferred from static measurements of the vein diameter. Postural changes (eg, the difference in the leg vein diameter between the standing and supine positions) can provide more relevant information. In limbs with saphenous vein reflux, the changes were smaller in patients with chronic venous disease (CVD) with a CEAP (clinical, etiologic, anatomic, pathophysiologic) category of C4 to C6 than in patients with C0 to C1 or C2 to C3.[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. In contrast, viscosity absorbs energy, slowing dilation when the blood pressure increases suddenly, and slowing deformation under external compression. Viscoelasticity produces a horizontal shift between the ascending (increasing transmural pressure) and descending (decreasing transmural pressure) parts of the volume–pressure function, resulting in a hysteresis loop, the area of which represents the energy losses due to viscosity.[18] Viscosity damps down the pulse waveform in arteries. However, little is known of the venous viscosity and its role in the pathophysiology of CVD,[19-21] although viscoelasticity might 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, smaller postural diameter changes have been reported in patients with C4 to C6 than in those with C0 to C1 or C2 to C3, and in enlarged veins than in unaffected veins,[16] which suggests reduced venous distensibility in CVD. If CVD results from a systemic disorder altering venous tone, structure, and biomechanics, the proper interpretation of these data would require assessment of the vein biomechanics in the high-pressure range and comparisons of CVD patients to healthy subjects. Such assessments and comparison have only been performed in a few studies,[13,22] and others have compared veins with and without reflux[16,25,26] or limbs with different CEAP categories.[27]

Our aim was to noninvasively assess the biomechanics of normal and diseased lower limb veins. Measuring, using B-mode ultrasonography (US),[28] the changes in the cross-sectional area of leg veins during application of an increasing force of the US probe on the skin to compress and collapse the vein, we obtained typical hysteresis loops. This method offered a noninvasive technique for the evaluation of the viscoelasticity of the veins in their natural environment, including the physical characteristics of the venous wall and surrounding tissues, luminal blood viscosity, and resistance to blood displacement. Using this technique, we investigated the viscoelasticity features of the small saphenous vein (SSV; saphena parva) and measured the postural changes in the cross-sectional area of the SSV and a deep calf vein (DCV; soleal vein or gastrocnemius vein, as available),[9] in CVD patients for whom compression was the main therapeutic option and normal controls. These veins were chosen because they were lesion free and could be examined at the same calf level, and the US examination was not hampered by bone structures, leaving the great saphenous vein (GSV) available for blood pressure measurement.

## ARTICLE HIGHLIGHTS

- **Type of Research:** A single-center, case-control, clinical research study
- **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 the cross-sectional area variation induced by compression with the ultrasound probe. The viscoelasticity features discriminated patients from controls.
- **Take Home Message:** The noninvasive assessment of viscoelasticity is a promising technique for the evaluation of vein biomechanics and pathophysiology.

## METHODS

**Population sample.** We recruited CVD patients who had presented with lower limbs with CEAP category (telangiectasia or reticular veins and symptoms), C3 (edema), or C5 (healed venous ulcer), diagnosed on the basis of thorough clinical and US examinations by two independent physicians. Any other etiology appropriate to the signs and symptoms (ie, heart, kidney, liver or skin disease, lymph stasis, other sources of leg pain) was investigated and excluded before diagnosing CVD.

We included patients with bilateral and symmetrical signs (ie, telangiectasia, reticular veins) and symptoms (ie, aching legs, pain, tightness, skin irritation, leg heaviness, muscle cramps) attributed to CVD in the C1S group. We included patients with bilateral leg edema as the prominent sign of CVD in the C3 group and patients with healed venous ulcer (investigation performed on the lower limb with the healed ulcer) in the C5 group. The controls were healthy subjects volunteering for biomedical research recruited by the Montpellier Center for Clinical Investigation and matched with the patients for age and body mass index (BMI) in three subgroups depending on their regular activity (<2 hours, 2 to 6 hours, and >6 hours of weekly physical exercise), covering the whole spectrum of the normal population. Pregnant or breastfeeding women, subjects or patients aged <18 years, and those unable or unwilling to provide written informed consent were excluded. Patients who had undergone sclerotherapy, phlebectomy, or any lower limb venous interventional treatment within 6 weeks previously were also excluded and, if included after this delay, the investigations were performed on the nontreated limb. The SSV and DCV were free of detectable lesions in the lower limb chosen for the study. The anticipated sample size was 54 patients and 54 controls (Appendix, online only). We measured the intravenous pressure (IVP) and intramuscular pressure (IMP) in 18 of the CVD patients and 18 of the controls with the same CEAP or activity repartition.

The ethics committee CP-Sud-Méditerranée approved the present study (approval no. RCB-2014-A00737-40), and all the participants provided written informed consent.

**Examination protocol.** The US examinations were performed using the Logiq-e system (GE Ultrasound, Chicago, Ill), with a 12L-RS linear probe equipped with a XFTC300 sensor connected to an ARD154 amplifier (Measurement Specialties, Hampton, Va) measuring the probe force (PF) applied on the skin by the ultrasound probe. The US video signal was captured using a Picolo frame-grabber (Euresys, Liege, Belgium) and stored on a personal computer.

The IMP was measured using a 1.2-mm external diameter IMP-Cath catheter (Alcis, Besançon, France) that had been inserted under local anesthesia (6-8 mL of 5 mg/mL lidocaine) into the triceps surae muscle at a depth of ~4 cm, slightly above the maximum girth of the calf. The intravenous blood pressure was measured using a 22-gauge Cathlon catheter (Smiths-Medical, St Paul, Minn) inserted into the GSV at the mid-calf (Appendix, online only). Both catheters were filled with heparinized isotonic saline and connected to DPT-6000 pressure sensors (Codan-Medical, Lensahn, Germany). The analog signals were sent, together with the PF, to an MP150 signal acquisition and processing system and analyzed offline using Acqknowledge, version 4.2 (Biopac-Systems, Goleta, Calif). Calibration at atmospheric pressure and against a mercury column was performed before each session.

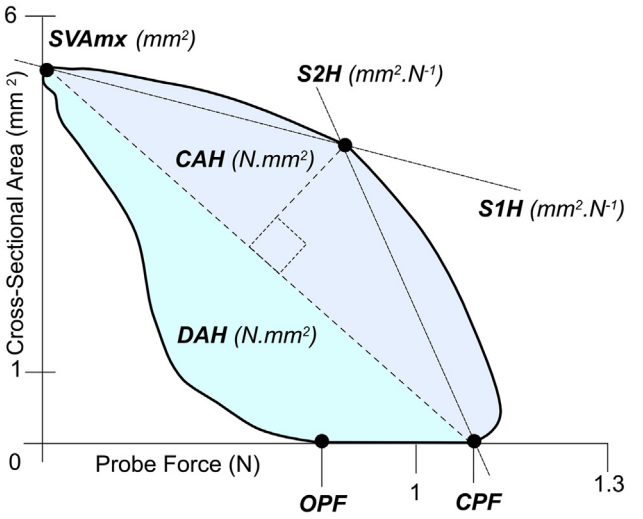


**Fig 1.** A typical hysteresis loop of the short saphenous vein. The cross-sectional area ($mm^2$) plotted as a function of the force (N) exerted by the operator on the ultrasound probe. *CAH,* Area of compression phase of the loop; *CPF,* vein–closing probe force; *DAH,* area of decompression phase of the loop; *OPF,* vein–opening probe force; *S1H,* first slope of compression phase of the loop; *S2H,* second slope of compression phase of the loop.

With the subjects lying on their side (lateral decubitus), with a small wedge under the heel to avoid contact of the calf muscles with the examination table, the observer recorded B-mode US images of the SSV and then the DCV at mid-calf height. The observer increased the PF progressively until the vein collapsed and then released the PF to allow the vein to reopen and expand. Finally, the subject moved to the standing position and remained motionless (orthostasis) for >1 minute, then stood on the leg not being examined, while the compression test was repeated.

**Measurements and calculations.** The measurements were independently performed, using the recorded signals and images, by observers who were unaware of the subject's status. Using Fiji software (available at: https://fiji.sc/), the observer measured the cross-sectional area of the SSV and DCV. The postural area change (PAC) was calculated as follows: $\% = 100 \times (AS - AL)/AS$, with AL indicating the cross-sectional area in the supine position and AS, the cross-sectional area in the standing position. The SSV and DCV depth (US probe-to-vein distance) was measured at 0 PF and at vein collapse.

The recorded US sequences were also analyzed using custom-made LabView-2016 software (National Instruments, Austin, Tex) to detect the vein walls and track the displacement.[28] The vein lumen was

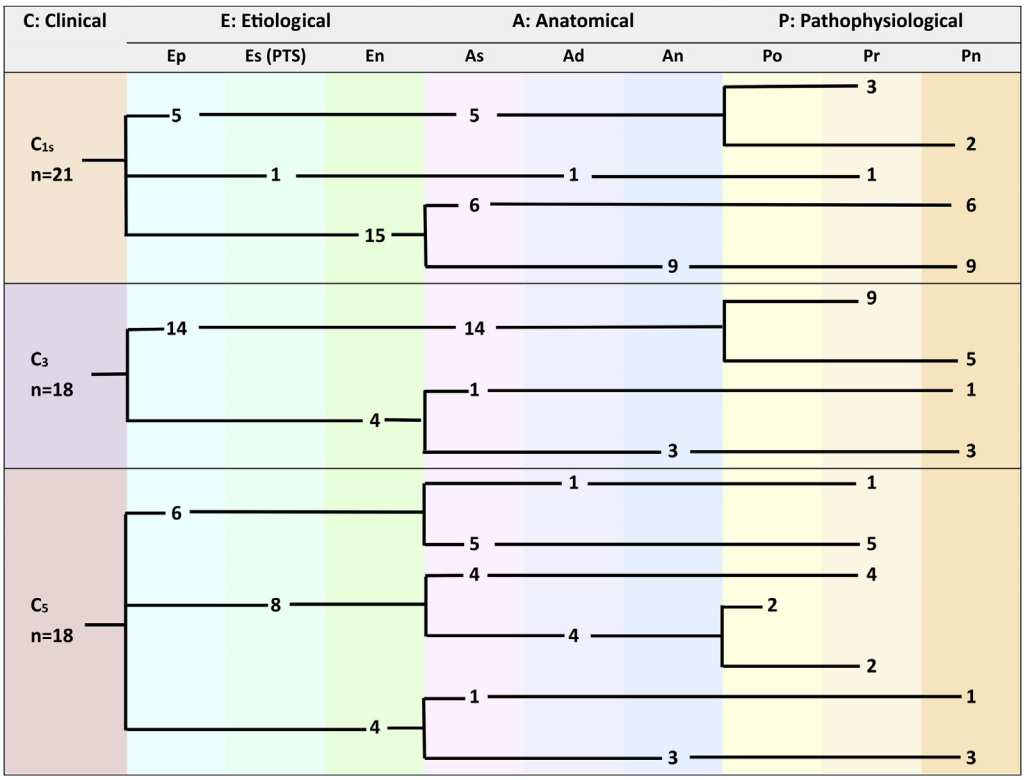


**Fig 2.** CEAP (clinical, etiologic, anatomic, pathophysiologic) characteristics of examined lower limb of patients with C1s (telangiectasia or reticular veins and symptoms), C3 (edema), and C5 (healed venous ulcer) chronic venous disease (CVD). *Ad,* Disease involving deep veins; *An,* no venous location identified; *As,* disease involving superficial veins; *En,* no venous cause identified but several potential causes and risk factors (eg, obesity, ankylosis, limb deformity, history of trauma) present; *Ep,* primary; *Es (PTS),* secondary (postthrombotic syndrome); *Pn,* no venous pathophysiology identifiable; *Po,* venous obstruction; *Pr,* venous reflux.

approximated to an ellipse and the cross-sectional area was calculated on each frame (Appendix, online onlyand Supplementary Video 1, online only). The SSV cross-sectional area vs PF function was drawn and appeared as a hysteresis loop from which the variables related to blood pressure (PF at which the vein had collapsed and then reopened), viscosity (total area of the loop and area of its compression and decompression parts), and elasticity (first and second slopes of the compression phase) were automatically extracted[30] (Fig 1).

The mean IVP (IVPm) and mean IMP (IMPm) were obtained by averaging the instantaneous values recorded for ~10 seconds. We 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.** The categorical data were compared using the Fisher exact test, with the Freeman-Halton extension used as appropriate. The quantitative variables are reported as the median and lower (Q1) to upper quartile (Q3). Differences between two groups (independent data) and changes within one group (paired data) were evaluated using the Wilcoxon-Mann-Whitney test and Wilcoxon signed rank test, respectively. Comparisons between the controls, C1s, and pooled C3 and C5 patients (C3&5) were performed using the Kruskal-Wallis test, followed by Dunn's multiple comparison. Values of $P < .05$ were considered statistically significant. Relationships between continuous variables were investigated using the Spearman $r$ coefficient and random effects models and described by linear regression. Receiver operating characteristic curves were drawn, and the area under the curve (AUC) was calculated for each variable. Evaluation of the combined variables to discriminate among the CEAP groups was estimated using the AUC by introducing independent variables with $P < .2$ on univariate logistic regression analysis into multivariate logistic regression models. Intraobserver reproducibility is reported in the Appendix (online only). Statistical analyses were performed using Prism, version 5 (GraphPad, San Diego, Calif) and R, version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

**Characteristics of population sample.** For matching purposes, we recruited three additional C1s patients; thus, the population sample included 57 CVD patients

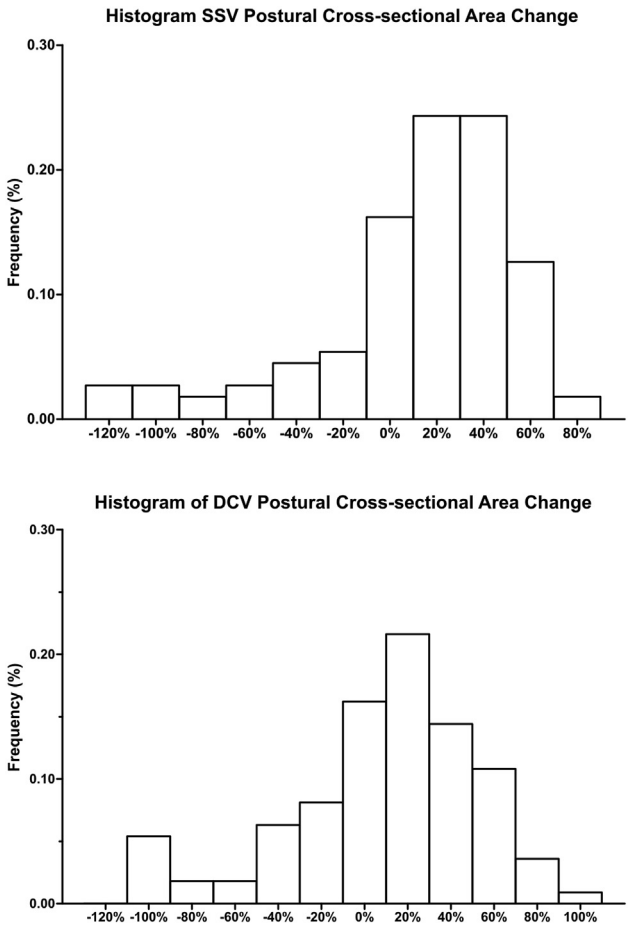


**Fig 3.** Histogram of relative (%) changes in cross-sectional area of small saphenous vein (*SSV*) and deep calf vein (*DCV*) between supine and standing positions in whole population sample (n = 111).

(41 women), 21 with C1s, 18 with C3, and 18 with C5 (Fig 2), and 54 controls (36 women). Neither age nor BMI differed between the CVD and control groups. However, the weight and height were greater in the C5 group compared with the control and C1s groups. The calf circumference was greater in those with C3 than in the controls, and the ankle circumference was greater in those with C3 and C5 than in the controls (Supplementary Table I, online only).

**Vein cross-sectional area and depth.** The SSV and DCV depth was slightly smaller in the standing than in the supine position without differences among groups at 0 PF or vein collapse (Appendix, online only). The SSV and DCV cross-sectional areas (Supplementary Table II and Appendix, online only) were greater in the C3&5 patients than in the controls ($P < .01$ for all). No significant difference was found in the DCV cross-sectional area among the groups. In the controls, no difference was found in the SSV or DCV cross-sectional areas among the physical activity subgroups.

The SSV and DCV cross-sectional areas were not related to each other nor to the IVPm or IMPm. In the whole population sample, the cross-sectional area correlated in the supine position with age for the SSV and DCV and with body weight for the DCV. In the whole population sample and the C3&5 patients, the SSV cross-sectional area correlated positively with the body weight and BMI in both positions (Supplementary Table III, online only).

The SSV and DCV cross-sectional areas were greater in the standing than in the supine position ($P < .0001$ and $P = .015$, respectively). However, the SSV and DCV PAC was negative in 31.5% and 40.5% of the 111 subjects, respectively (Fig 3 and Appendix, online only), without differences among groups and without a correlation with the SSV or DCV values.

**IVP and IMP.** IVP and IMP could be obtained for 31 and 35 subjects, respectively. The baseline IVPm was not different among the groups in the supine position but was greater in the C3&5 patients (median, 60.1 mm Hg; Q1-Q3, 55.8-71.8 mm Hg) than in the controls (median, 46.7 mm Hg; Q1-Q3, −6.6 to 57.9 mm Hg) in the standing position ($P < .01$). Changing from the supine to the standing position increased the IVPm (Appendix, online only). In the whole population sample, the IMPm was lower in the standing than in the supine position ($P < .0001$). It was higher in the CVD patients than in the controls at baseline in the standing position ($P = .013$) but not in the supine position (Appendix, online only).

**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 ($P < .0001$ for all) and differed among the controls and C1s and C3&5 patients (Fig 4 and Table I; Appendix, online only). In the supine position, but not in the standing position, the viscosity-related hysteresis variables in the whole population sample and in the CVD patients and the pressure-related variables in the CVD patients increased with increasing age (Appendix, online only).

The receiver operating characteristic curves showed that most hysteresis variables could differentiate between the controls and CVD patients. Using different combinations of hysteresis variables, multivariate logistic regression analysis yielded an AUC of 0.80 to 0.83 to differentiate controls from C3 and C5 patients, 0.78 to differentiate controls from C1s patients, and 0.75 to differentiate C1s from C3 and C5 patients (Table II; Appendix, online only).

## DISCUSSION

Our main results were as follows. First, the PACs in the SSV and DCV cross-sectional areas showed large

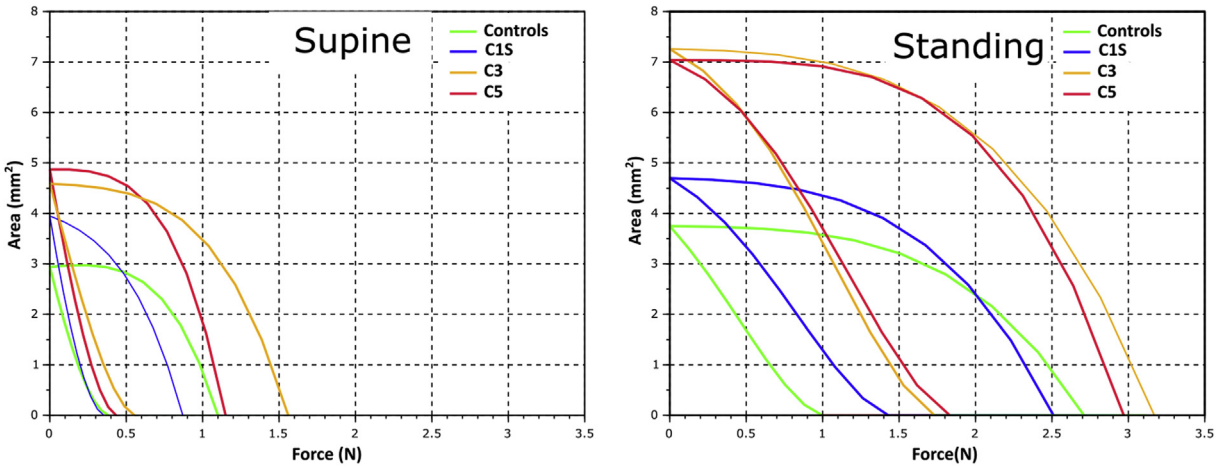


**Fig 4.** Schematic drawing of the hysteresis loops of controls and patients. Hysteresis loops were redrawn from the median values of the small saphenous vein (SSV) cross-sectional area during the compression test for normal controls and limbs with C1s (telangiectasia or reticular veins and symptoms), C3 (edema), and C5 (healed venous ulcer) CEAP (clinical, etiologic, anatomic, pathophysiologic) category of chronic venous disease (CVD), in the supine and in the standing position.

interindividual differences in all groups. Second, all the variables derived from the hysteresis loops drawn from the SSV cross-sectional area vs PF function were greater with the subjects in the standing position than in the supine position. Finally, their combination could discriminate between the controls and C1s patients and between the controls and C3 and C5 patients.

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

Our most striking result was the extent of the interindividual differences in the PACs, independently of healthy or CVD status, because the vein area had increased in some subjects, had remained unchanged, and had even decreased in other subjects in the standing position. Because we had taken care to avoid residual muscle contractions, the absence, in some subjects, of a vein area increase despite greater hydrostatic blood pressure,[15] could have resulted from multiple, and possibly opposing, factors. Although a linear correlation has been reported between the IVP and the diameter of saphenous veins with reflux,[15] the relationship could be more complex in unaffected veins. An increased venous tone could explain the negative PAC we observed in a noticeable proportion of the control subjects but probably not for the CVD patients because the venous wall contractile response to angiotensin-2, norepinephrine, and endothelin-1 is impaired in the latter.[2,3] van der Velden et al[16] reported a negative postural diameter change in 10% of their subjects but dismissed it as measurement error. We limited errors by measuring the cross-sectional area rather than only the larger diameter and ensuring that the subject's weight had remained on the other leg for ≥1 minute before the examination. Therefore, we must consider that the interindividual differences we observed were not meaningless. Nevertheless, pending further studies clarifying this issue, the postural changes in the 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 because increasing the PF reduced the transmural pressure.[36] Observing the calf veins using US and a modified pneumatic cuff, Partsch and Partsch[37] found that the cuff pressure required to occlude the 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 the greater hydrostatic blood pressure. In the supine position, the probe force at which the SSV collapsed was greater in the C5 than in the C1s patients or the controls. In the standing position, the force at which SSV reopened was greater in the C3 and C5 patients than in the controls, suggesting greater venous transmural pressure and/or greater wall stiffness.

When evaluated in vivo, using either venous occlusion plethysmography or our technique, the venous viscoelasticity features were affected by the venous wall but also by the surrounding tissues, blood viscosity, and resistance to blood displacement. Venous compliance or distensibility are commonly calculated from changes

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

| Variable | Controls | Patients | | *P* value[a] | | |
|---|---|---|---|---|---|---|
| | | C1s | C3&C5 | Controls vs C1s | Controls vs C3&C5 | C1s vs C3&C5 |
| SVAmax, $mm^2$ | | | | | | |
| Supine | 2.94 (1.76-5.18); 0.58 | 3.95 (2.33-4.97); 0.70 | 4.87 (3.57-7.06); 0.64 | | .005 | |
| Standing | 3.75 (2.12-5.41); 0.59 | 4.70 (2.56-6.16); 0.60 | 7.07 (2.96-9.90); 0.65 | | .002 | |
| *P* value (supine vs standing) | .005 | .047 | .002 | | | |
| CPF, N | | | | | | |
| Supine | 1.03 (0.75-1.35); 0.59 | 0.87 (0.60-1.23); 0.60 | 1.22 (0.89-1.64); 0.65 | | | |
| Standing | 2.71 (2.20-3.13); 0.55 | 2.51 (2.04-2.89); 0.66 | 3.15 (2.54-4.03); 0.69 | | .047 | .039 |
| *P* value (supine vs standing) | <.001 | <.001 | <.001 | | | |
| OPF, N | | | | | | |
| Supine | 0.36 (0.21-0.56); 0.53 | 0.35 (0.14-0.58); 0.62 | 0.52 (0.19-0.76); 0.63 | | | |
| Standing | 0.98 (0.63-1.56); 0.70 | 1.42 (1.19-1.77); 0.77 | 1.76 (1.12-2.07); 0.59 | .027 | <.001 | |
| *P* value (supine vs standing) | <.001 | <.001 | <.001 | | | |
| DPF, N | | | | | | |
| Supine | 0.64 (0.38-0.94); 0.54 | 0.50 (0.32-0.90); 0.53 | 0.65 (0.42-1.02); 0.57 | | | |
| Standing | 1.65 (1.25-2.09); 0.77 | 0.86 (0.59-1.32); 0.60 | 1.27 (0.75-2.06); 0.63 | .001 | | |
| *P* value (supine vs standing) | <.001 | <.001 | <.001 | | | |
| TAH, $N \cdot mm^2$ | | | | | | |
| Supine | 1.24 (0.66-2.11); 0.54 | 1.15 (0.79-2.89); 0.72 | 2.40 (1.65-3.84); 0.68 | | .001 | |
| Standing | 4.16 (2.73-8.43); 0.53 | 4.25 (2.71-5.21); 0.68 | 8.95 (3.87-15.96); 0.73 | | .011 | .019 |
| *P* value (supine vs standing) | <.001 | <.001 | <.001 | | | |
| CAH, $N \cdot mm^2$ | | | | | | |
| Supine | 0.38 (0.13-0.70); 0.51 | 0.31 (0.09-1.02); 0.62 | 0.65 (0.32-1.68); 0.63 | | | |
| Standing | 1.36 (1.02-3.52); 0.52 | 1.70 (0.97-2.19); 0.67 | 3.70 (1.16-7.13); 0.69 | | .019 | .048 |
| *P* value (supine vs standing) | <.001 | <.001 | <.001 | | | |
| DAH, $N \cdot mm^2$ | | | | | | |
| Supine | 0.79 (0.42-1.46); 0.58 | 0.75 (0.58-1.84); 0.75 | 1.86 (1.07-2.54); 0.69 | | <.001 | |
| Standing | 2.72 (1.49-5.05); 0.55 | 2.28 (1.37-3.85); 0.65 | 4.24 (2.02-9.32); 0.70 | | .049 | .041 |
| *P* value (supine vs standing) | <.001 | <.001 | <.001 | | | |
| S1H, $mm^2/N$ | | | | | | |
| Supine | −1.06 (−1.86 to −0.47); 0.66 | −1.98 (−3.42 to −0.53); 0.68 | −2.04 (−3.28 to −1.10); 0.52 | | .012 | |
| Standing | −0.37 (−0.68 to −0.24); 0.62 | −0.55 (−1.37 to −0.28); 0.55 | −0.52 (−0.91 to −0.23); 0.54 | | | |
| *P* value (supine vs standing) | <.001 | <.001 | <.001 | | | |
| S2H, $mm^2/N$ | | | | | | |
| Supine | −5.49 (−8.37 to −3.41); 0.57 | −6.52 (−10.31 to −3.15); 0.64 | −9.21 (−15.45 to −3.54); 0.59 | | | |

*(Continued on next page)*

**Table I.** Continued.

| Variable | Controls | Patients | | *P* value[a] | | |
|---|---|---|---|---|---|---|
| | | C1s | C3&C5 | Controls vs C1s | Controls vs C3&C5 | C1s vs C3&C5 |
| Standing | −2.71 (−4.07 to −1.86); 0.62 | −3.46 (−7.68 to −1.83); 0.69 | −4.29 (−6.68 to −2.96); 0.55 | | .001 | |
| *P* value (supine vs standing) | <.001 | <.026 | <.001 | | | |

*C1s,* Limbs with telangiectasia or reticular veins and symptoms; *C3,* limbs with edema; *C5,* limbs with healed venous ulcer; *C3&5,* pooled data for limbs with C3 or C5; *CAH,* area of compression phase of hysteresis loop; *CPF,* vein-closing probe force; *DAH,* area of decompression phase of hysteresis loop; *OPF,* vein-opening probe force; *S1H,* slope of first portion of compression phase of hysteresis loop; *S2H,* slope of second portion of compression phase of hysteresis loop; *SVAmax,* maximum cross-sectional area of small saphenous vein; *TAH,* total area of hysteresis loop.
Data presented as median (lower quartile to upper quartile); area under the receiver operating characteristic curve.
[a] *P* value provided when significant; Dunn's multiple comparison post–Kruskal-Wallis test used for group comparisons and paired *t* test for supine vs standing positions.

in the limb circumference or vein diameter produced by incremental venous occlusion cuff pressure,[38] Valsalva maneuver,[13] or posture.[39] Venous compliance is large at a low transmural pressure; thus, a minimal increase in blood pressure will generate a large increase in volume through wall deformation. It will be smaller at a high transmural pressure (eg, a standing position), because the vein cross-section area becomes circular and the diameter changes induced by further increases in blood pressure reflect the volume change and are dependent on wall elasticity.[14,40,41] This might explain why we obtained steeper hysteresis loop slopes, corresponding to greater distensibility (ie, lower elastic modulus), in the supine than in the standing position in all groups. Regardless of posture, these slopes were steeper in the CVD patients, also suggesting greater vein distensibility. This finding is consistent with findings from previous reports of greater proximal lower limb vein distensibility in patients with varicose veins than in healthy controls[13] and endothelium and smooth muscle abnormalities in CVD patients,[42] even in nonvaricose veins,[43] suggesting systemic alterations in venous wall resistance to stress.[19] Such abnormalities should affect viscoelasticity.[44] It is plausible that, in addition to 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 the unaffected veins of CVD patients.

Venous wall hysteresis, relating to viscoelasticity, has been demonstrated from 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 is innovative in that it allows for the direct, noninvasive evaluation of a specific vein rather than of a limb segment in a more physiologic frequency range.

The hysteresis loop variables we measured discriminated between controls and CVD patients. These variables also discriminated between patients with C1s and controls and patients with C1s and those with C3&5. Because telangiectasias or spider veins are the only objective signs in C1s patients, such quantitative data should help in characterizing this distinct entity, which might have some features in common with C0s, described by Andreozzi et al[47] as having "hypotonic phlebopathy." Our results suggest that viscosity will be greater in the unaffected veins of CVD patients in whom only reduced distensibility had been demonstrated previously.[13,24,38]

**Study limitations.** CVD also involves the 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 might have played a role. However, the present study did not allow for their separate evaluation. Skin stiffness, subcutaneous fat thickness, and interstitial fluid could also have contributed, although we found no statistically significant differences between the groups for vein depth or depth changes under compression. Moreover, we performed the compression test at the mid-calf level, some distance from the upper limit of the tissue alterations associated with lipodermatosclerosis in patients with advanced CVD.

We restricted the number of controls and patients who underwent invasive measurements since they were needed only to allow for proper characterization of the population samples, as ample data are already available for IVP and IMP in patients with CVD. However, this limited the statistical power and precluded further correlations. We measured IVP in the GSV and performed the US examination on the SSV (a superficial vein) and on the soleal or gastrocnemius veins (muscular veins).[29] Nevertheless, all measurements were performed at the same calf level. A comparison of the axial and muscular calf veins, which have different anatomic features, would be necessary in future studies for a more comprehensive assessment. We recruited patients with C1s, C3, and C5 CEAP because compression is the main therapeutic option. In contrast, the C2 and C4 categories might be more representative

**Table II.** Discriminative value of hysteresis variables

| Variable | *P* value[a] | | | |
|---|---|---|---|---|
| | Controls vs CVD | Controls vs C1s | Controls vs C3&C5 | C1s vs C3&C5 |
| SVAmax | | | | |
| Supine | .015 | | .006 | .007 |
| Standing | **.007** | | **.003** | **.054** |
| CPF | | | | |
| Supine | | | | **.198** |
| Standing | **.059** | | **.007** | **.039** |
| OPF | | | | |
| Supine | | | **.038** | **.090** |
| Standing | **<.001** | **.013** | **<.001** | |
| TAH | | | | |
| Supine | .022 | | .008 | .049 |
| Standing | .035 | | .005 | **.022** |
| CAH | | | | |
| Supine | | | .085 | .113 |
| Standing | **.025** | | **.004** | .027 |
| DAH | | | | |
| Supine | **.004** | | **.001** | **.045** |
| Standing | .074 | | .022 | .046 |
| S1H | | | | |
| Supine | **.007** | **.032** | **.015** | |
| Standing | **.078** | **.032** | | |
| S2H | | | | |
| Supine | **.110** | | **.085** | |
| Standing | **.005** | **.056** | **.004** | |
| Variables introduced in model, No. | 9 | 4 | 9 | 6 |
| Multivariate AUC with selected variables | 0.796 | 0.777 | 0.826 | 0.744 |
| 95% CI AUC | | | | |
| Delong method | 0.710-0.882 | 0.662-0.892 | 0.739-0.9141 | 0.614-0.873 |
| Boot-strap 10,000 | 0.707-0.878 | 0.657-0.884 | 0.731-0.908 | 0.609-0.866 |

*AUC,* Area under receiver operating characteristic curve; *CI,* confidence interval; *CVD,* chronic venous disease (all categories); *C1s,* limbs with telangiectasia or reticular veins and symptoms; *C3,* limbs with edema; *C5,* limbs with healed venous ulcer; *C3&5,* pooled data for limbs with C3 or C5; *CAH,* area of compression phase of hysteresis loop; *CPF,* vein-closing probe force; *DAH,* area of decompression phase of hysteresis loop; *OPF,* vein-opening probe force; *S1H,* slope of first portion of compression phase of hysteresis loop; *S2H,* slope of second portion of compression phase of hysteresis loop; *SVAmax,* maximum cross-sectional area of small saphenous vein; *TAH,* total area of hysteresis loop.
Boldface *P* values represent statistical significance.
[a] *P* values presented from univariate logistic regression analysis and then multivariate logistic regression analysis of eligible hysteresis variables (variables were eligible if they yielded a *P* value $< 0.2$ on univariate analysis); for strongly correlated variables, only the variable with the smaller *P* value was included in the multivariate model. Other variables (those with nonstatistically significant *P* values [no boldface]) were not included.

of CVD. We included CVD patients with various etiologies, topographies, and severity of venous lesions, precluding subgroup analyses owing to lack of statistical power. Foot or knee deformation and body weight distribution can also affect the saphenous vein caliber and should be specifically studied. Evaluating the leg tissues and measuring the blood viscosity would be useful for a thorough pathophysiologic assessment.

## CONCLUSIONS

Although the cross-sectional area of the SSV, but not the DCV, was greater in the CVD patients than in controls, the PACs in the cross-sectional area were highly diverse and did not allow for a differentiation between patients and controls. These postural changes could result from multiple, potentially opposite, factors that must be specifically investigated before they can be used to characterize CVD. Tracking the cross-sectional area of the leg veins under compression by the US probe yielded typical hysteresis loops, reflecting viscoelasticity. We found greater viscosity in the unaffected SSVs of CVD patients than in healthy controls, supporting the hypothesis of global changes to the venous wall. The postural changes in the venous viscoelasticity variables appeared much more marked and consistent compared with the cross-sectional area changes.

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## AUTHOR CONTRIBUTIONS

Conception and design: SM, JT, CD, APM, MD, IQ
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**Supplementary Table I (online only).** Biometrics of population sample

| Variable | Controls (n = 54) | C1s (n = 21) | C3 (n = 18) | C5 (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) |

*BMI,* Body mass index; *C1s,* telangiectasia or reticular veins and symptoms; *C3,* edema; *C5,* healed venous ulcer.
Data presented as median (lower-upper quartile).

**Supplementary Table II (online only).** Small saphenous vein and deep calf vein cross-sectional areas

| | Cross-sectional area, mm$^2$ | | |
|---|---|---|---|
| Variable | Controls (n = 54) | C1s (n = 21) | C3&C5 (n = 36) |
| SSV | | | |
| 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) |
| *P* value[a] | **.005** | **.047** | **.002** |
| DCV | | | |
| 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) |
| *P* value[a] | **.014** | .500 | .120 |

*C1s,* Telangiectasia or reticular veins and symptoms; *C3,* edema; *C5,* healed venous ulcer; *DCV,* deep calf vein; *SSV,* small saphenous vein.
Data presented as median (lower-upper quartile). Boldface *P* values represent statistical significance.
[a]Comparison between supine and standing positions using Wilcoxon signed-rank test.

**Supplementary Table III (online only).** Correlation of cross-sectional area of leg veins with demographic factors

| | Spearman correlation coefficient (*P* value) | | | |
|---|---|---|---|---|
| **Group** | **Age** | **Weight** | **Height** | **BMI** |
| All participants (N = 111) | | | | |
| Supine | | | | |
| SSV | 0.277 (**.003**) | 0.382 (**<.001**) | 0.101 (.300) | 0.326 (**<.001**) |
| DCV | 0.305 (**.001**) | 0.191 (**.047**) | 0.140 (.150) | 0.170 (.080) |
| Standing | | | | |
| SSV | 0.181 (.060) | 0.412 (**< .001**) | 0.148 (.130) | 0.357 (**<.001**) |
| DCV | 0.083 (.390) | 0.080 (.410) | 0.070 (.470) | 0.057 (.560) |
| Normal controls (n = 54) | | | | |
| Supine | | | | |
| SSV | 0.336 (**.010**) | 0.224 (.110) | −0.118 (.410) | 0.232 (.100) |
| DCV | 0.251 (.070) | 0.134 (.340) | 0.020 (.890) | 0.126 (.380) |
| Standing | | | | |
| SSV | 0.248 (.070) | 0.255 (.070) | −0.124 (.390) | 0.322 (**.020**) |
| DCV | 0.117 (.400) | 0.130 (.350) | −0.001 (.990) | 0.141 (.330) |
| C1s (n = 21) | | | | |
| Supine | | | | |
| SSV | 0.292 (.200) | 0.308 (.190) | 0.161 (.500) | 0.181 (.450) |
| DCV | 0.305 (.180) | 0.128 (.590) | 0.310 (.180) | −0.063 (.790) |
| Standing | | | | |
| SSV | 0.513 (**.017**) | 0.236 (.320) | 0.365 (.110) | 0.012 (.960) |
| DCV | −0.214 (.350) | −0.291 (.210) | −0.071 (.770) | −0.275 (.240) |
| C3&C5 (n = 36) | | | | |
| Supine | | | | |
| SSV | 0.106 (.540) | 0.514 (**.001**) | 0.213 (.210) | 0.407 (**.010**) |
| DCV | 0.305 (.070) | 0.055 (.750) | −0.021 (.900) | 0.139 (.420) |
| Standing | | | | |
| SSV | −0.054 (.750) | 0.398 (**.020**) | 0.248 (.150) | 0.298 (.080) |
| DCV | 0.235 (.170) | 0.002 (.990) | 0.227 (.180) | −0.017 (.920) |

*BMI,* Body mass index; *C1s,* telangiectasia or reticular veins and symptoms; *C3,* edema; *C5,* healed venous ulcer; *DCV,* deep calf vein; *SSV,* small saphenous vein.
Boldface *P* values represent statistical significance.