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### ► To cite this version:

Sandrine Mestre, Jean Triboulet, Christophe Demattei, Florent Veye, Monira Nou, et al.. 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*, 2021, 9 (4), pp.987-997. 10.1016/j.jvsv.2020.11.010 . hal-03228579

**HAL Id: hal-03228579**

**<https://hal.umontpellier.fr/hal-03228579>**

Submitted on 27 May 2021

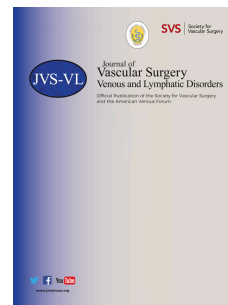
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# Journal Pre-proof



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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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>.

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

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#### 14 **Key-Words:**

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

16

#### 17 **Sources of Funding:**

18 Technical developments required by the present study are covered by patent

19 WO 2017005642 A1 for equipment (ultrasound probe instrumentation for force measurement)

20 and by IDDN.FR.001.090044.000.S.P.2013.000.21000 and

21 IDDN.FROO1.150022.000.S.C.2017.000.31230 for software (automatic image and signal

22 analysis and extraction of viscoelasticity variables), detained by the institutions (Montpellier

23 University and Nimes University Hospital consortium) the authors are affiliated to.

1

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

3

4 This study was approved by the Ethics Committee CP Sud-Méditerranée (RCB-2014-A00737-

5 40) and all participants provided a written informed consent.

Journal Pre-proof

## 1 **Article Highlights**

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

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

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

9

## 10 **Table of Contents Summary**

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

16

## 1 **Abstract**

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

5 **Methods:** In 57 patients with limbs in C<sub>1s</sub>, C<sub>3</sub>, or C<sub>5</sub> CEAP category of chronic venous disease  
6 (CVD) and 54 matched healthy controls, we measured with ultrasonography the changes in  
7 cross-sectional area of the small saphenous vein and of a deep calf vein in the supine and in the  
8 standing position, and under compression with the ultrasound probe.

9 **Results:** The small saphenous, but not the deep calf vein cross-sectional area was smaller in  
10 controls than in limbs with category C<sub>3</sub> or C<sub>5</sub> disease while not different from C<sub>1s</sub>. When  
11 changing from the supine to the standing position, a greater force was required to collapse leg  
12 veins, of which the cross-sectional area increased in most subjects but decreased in 31.5% of  
13 subjects for the small saphenous and 40.5% for the deep calf vein. The small saphenous vein area  
14 *versus* compression force function followed a *hysteresis* loop, demonstrating viscoelastic  
15 features. Its area, which represents the viscosity component, was greater ( $p < 0.001$ ) in pooled C<sub>3</sub>  
16 and C<sub>5</sub> limbs (median 2.40 [lower–upper quartile 1.65–3.88] N.mm<sup>2</sup>) than in controls (1.24  
17 [0.64–2.14] N.mm<sup>2</sup>) and C<sub>1s</sub> limbs (1.15 [0.71–2.97] N.mm<sup>2</sup>). It increased ( $p < 0.0001$ ) in the  
18 standing position in all groups.

19 **Conclusion:** Postural changes in cross-sectional area of leg veins are highly diverse among  
20 patients with chronic venous diseases as well as among healthy subjects, and appear unsuitable  
21 for pathophysiological characterization, whereas small saphenous vein viscoelasticity increases  
22 consistently in the standing position and viscosity is greater in limbs with C<sub>3</sub> and C<sub>5</sub> CEAP  
23 categories than in controls.

## 1 Introduction

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

14 The volume–pressure function reflects the vein biomechanics. In the low venous blood  
15 pressure range, as in the supine position, a minimal transmural pressure rise produces a large  
16 volume increase by changing the venous cross-section from bimodal to elliptical to circular. At  
17 higher blood pressure, as in the standing position, the slope of the venous volume–pressure  
18 function flattens, eventually reaching a plateau where a further rise in blood pressure no longer  
19 translates in a significant volume increase.<sup>14</sup> Only in this high pressure range is the venous wall  
20 elasticity solicited, and diameter changes correlate with pressure (at least in superficial veins  
21 with incompetent valves).<sup>15</sup> Therefore, venous biomechanics cannot be inferred from static  
22 measurements of vein diameter. Postural changes, *e.g.* the difference in leg vein diameter  
23 between the standing and the supine position, would provide more relevant information. In limbs



1 with saphenous vein reflux, they were found smaller in CVD patients with C<sub>4</sub>-C<sub>6</sub> than in patients  
2 with C<sub>0</sub>-C<sub>1</sub> or C<sub>2</sub>-C<sub>3</sub> CEAP category.<sup>16</sup>

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

14 Venous distensibility increases in patients with CVD,<sup>22,23</sup> even in unaffected veins.<sup>13,24</sup>  
15 However, the smaller postural diameter changes that has been reported in C<sub>4</sub>-C<sub>6</sub> than in C<sub>0</sub>-C<sub>1</sub> or  
16 C<sub>2</sub>-C<sub>3</sub> patients, and in enlarged than in unaffected veins,<sup>16</sup> suggest reduced venous distensibility.  
17 If CVD results from a systemic disorder altering venous tone, structure, and biomechanics, the  
18 proper interpretation of these data would require assessing the vein biomechanics in the high-  
19 pressure range, and comparing CVD patients to healthy subjects, which was done only by a few  
20 studies<sup>13,22</sup> while others compared veins with and without reflux<sup>16,25,26</sup> or limbs with different  
21 CEAP categories.<sup>27</sup>

22 Our aim was to assess non-invasively the biomechanics of normal and diseased lower  
23 limb veins. Measuring, with B-mode ultrasonography (US),<sup>28</sup> the changes in cross-sectional area

1 of leg veins when applying an increasing force on the US probe to compress and collapse the  
2 vein, we obtained typical *hysteresis* loops, thus offering a noninvasive technique for the  
3 evaluation of viscoelasticity of veins in their natural environment, involving the physical  
4 characteristics of the venous wall and surrounding tissues, the luminal blood viscosity, and the  
5 resistance to blood displacement. Using this technique, we investigated viscoelasticity features of  
6 the small saphenous vein (*saphena parva*, SSV) and measured the postural changes in cross-  
7 sectional area of the SSV and of a deep calf vein (DCV, the soleal vein or a gastrocnemial vein,  
8 as available),<sup>29</sup> in CVD patients for whom compression was the main therapeutic option, and in  
9 normal controls. These veins were chosen because they were lesion-free, could be examined at  
10 the same calf level, and their US examination was not hampered by bone structures while leaving  
11 the GSV available for blood pressure measurement.

## 12 **Material and Methods**

### 13 **Population sample**

14 We recruited CVD patients whose lower limbs presented with C<sub>1S</sub>, C<sub>3</sub>, or C<sub>5</sub> CEAP  
15 category, diagnosed on the basis of thorough clinical and ultrasonographic examinations by two  
16 independent physicians. Any other etiology of signs and symptoms (heart, kidney, liver or skin  
17 disease, lymph stasis, other sources of leg pain...) was investigated and excluded before  
18 concluding to CVD. We included in the C<sub>1S</sub> group patients with bilateral and symmetrical signs  
19 (telangiectasies or reticular veins) and symptoms (aching legs, pain, tightness, skin irritation, leg  
20 heaviness, muscle cramps) attributed to CVD. We included in the C<sub>3</sub> group patients with bilateral  
21 leg edema as the prominent sign of CVD, and in the C<sub>5</sub> group patients with healed venous ulcer  
22 (investigation was performed on the lower limb with healed ulcer). Controls were healthy  
23 subjects volunteering for biomedical research recruited by the Montpellier Center for Clinical

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

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

#### 14 **Methods**

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

20 Intramuscular pressure was measured with a 1.2 mm external diameter IMP-Cath catheter  
21 (Alcis, Besançon, France), inserted, under local anesthesia by 6 to 8 mL of 5 mg/mL lidocaine,  
22 into the *triceps surae* muscle at 4 cm approximate depth, slightly above the maximum girth of  
23 the calf. Intravenous blood pressure was measured with a 22G Cathlon catheter (Smiths-Medical,

1 St-Paul, MN) inserted into the great saphenous vein at mid-calf height (**Appendix**). Both  
2 catheters were filled with heparinized isotonic saline and connected to DPT-6000 pressure  
3 sensors (Codan-Medical, Lensahn, Deutschland) of which analog signals were sent, together  
4 with PF, to a MP150 signal acquisition and processing system, then analyzed offline with  
5 Acqknowledge V4.2 (Biopac-Systems, Goleta, CA). Calibration at atmospheric pressure and  
6 against a mercury column was performed before each session.

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

#### 14 **Measurements and calculations**

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

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

22 Recorded US sequences were also analyzed with a custom-made LabView-2016  
23 (National-Instruments, Austin, TX) software that detected the vein walls and tracked their

1 displacements.<sup>28</sup> The vein lumen was approximated to an ellipse of which the cross-sectional  
2 area was calculated on each frame (**Appendix, Supplemental Video 1**). The SSV cross-sectional  
3 area *versus* PF function was drawn, and appeared as a *hysteresis* loop from which were  
4 automatically extracted<sup>30</sup> variables related to blood pressure (probe force at which the vein  
5 collapsed, then reopened), to viscosity (area of the loop and its compression and decompression  
6 parts), and to elasticity (first and second slopes of the compression part) (**Fig 1**).

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

### 11 **Statistical analysis**

12 Categorical data were compared by Fisher exact test, with Freeman-Halton extension  
13 when appropriate. Quantitative variables are reported as median [lower–upper quartile].  
14 Differences between two groups (independent data) and changes within one group (paired data)  
15 were evaluated with Wilcoxon-Mann-Whitney test and Wilcoxon signed-rank test, respectively.  
16 Comparisons between controls, C<sub>1s</sub>, and pooled C<sub>3</sub> and C<sub>5</sub> patients (C<sub>3&5</sub>) were performed with  
17 Kruskal-Wallis test followed by Dunn's multiple comparison. Values of  $p < 0.05$  were considered  
18 significant. Relationships between continuous variables were investigated by Spearman  $r$   
19 coefficient and with random effects models, and described by linear regression. Receiver  
20 operating characteristic (ROC) curves were drawn and the area under the curve (AUC) was  
21 calculated for each variable. The performance of combined variables for discriminating CEAP  
22 groups was estimated from the AUC calculated by introducing independent variables with  $p < 0.2$   
23 at univariate logistic regression analysis in multivariate logistic regression models. Intra-observer

1 reproducibility is reported in **Appendix**. Statistical analyses were performed using Prism V.5  
2 (GraphPad, San Diego, CA) and R V3.5.1 (R-Foundation for Statistical Computing, Vienna,  
3 Austria).

## 4 **Results**

### 5 **Characteristics of the population sample**

6 For matching purposes, we recruited three additional C<sub>1S</sub> patients, so that the population  
7 sample comprised 57 CVD patients (41 females) with 21 C<sub>1S</sub>, 18 C<sub>3</sub>, and 18 C<sub>5</sub> (**Fig 2**), and 54  
8 controls (36 females).

9 Neither age nor BMI differed between CVD patients and controls, but weight and height  
10 were greater in C<sub>5</sub> patients than in controls and C<sub>1S</sub> patients. Calf circumference was greater in  
11 C<sub>3</sub> patients than in controls, whereas ankle circumference was greater in C<sub>3</sub> and C<sub>5</sub> patients than  
12 in controls (**Supplemental Table I**).

### 13 **Vein cross-sectional area and depth**

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

16 The SSV and DCV cross-sectional area (**Supplemental Table II, Appendix**) was greater  
17 in C<sub>3&5</sub> patients than in controls ( $p < 0.01$  for all). There was no significant difference in DCV  
18 cross-sectional area between groups. Among controls, there was no difference in SSV or DCV  
19 cross-sectional area between physical activity subgroups.

20 The SSV and DCV cross-sectional areas were neither related between them nor with  
21 IVPm or IMPm. In the whole population sample, cross-sectional area correlated, in the supine  
22 position, with age for SSV and DCV, and with body weight for DCV. In the whole population

1 sample and in C<sub>3&5</sub> patients, SSV cross-sectional area correlated positively with body weight and  
2 BMI in both positions. (**Supplemental Table III**).

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

### 7 **Intravenous and intramuscular pressure**

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

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

### 15 **Viscoelasticity variables**

16 *Hysteresis* loops were obtained for 108 subjects. All *Hysteresis* loop variables were  
17 greater in the standing than in the supine position for all groups ( $p < 0.0001$  for all), and differed  
18 between controls, C<sub>1S</sub>, and C<sub>3&5</sub> patients (**Fig 4, Table I, Appendix**).

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

22 ROC curves showed that most *hysteresis* variables differentiated controls from CVD  
23 patients. Using different combinations of *hysteresis* variables, multivariate logistic regression

1 analysis yielded an AUC reaching 0.80 to 0.83 for differentiating controls from C<sub>3</sub> and C<sub>5</sub>  
2 patients, 0.78 for differentiating controls from C<sub>1S</sub> patients, and 0.75 for differentiating C<sub>1S</sub> from  
3 C<sub>3</sub> and C<sub>5</sub> patients (**Table II, Appendix**).

#### 4 **Discussion**

5 Our main results were: 1) Postural changes in SSV and DCV cross-sectional area showed  
6 large inter-individual differences in all groups. 2) All the variables derived from the *hysteresis*  
7 loops drawn by the SSV cross-sectional area vs. PF function were greater in the standing than in  
8 the supine position, and 3) their combination discriminated controls from C<sub>1S</sub> patients and from  
9 C<sub>3</sub> and C<sub>5</sub> patients.

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

15 Our most striking result is the extent of interindividual differences in PAC, independently  
16 of the healthy or CVD *status*, since the vein area increased in some subjects, staid unchanged or  
17 even decreased in others in the standing position. As we took care to avoid residual muscle  
18 contraction, the absence, in some subjects, of vein area increase in spite of greater hydrostatic  
19 blood pressure<sup>15</sup> could be due to multiple, possibly opposite factors. Although a linear  
20 correlation has been reported between intravenous pressure and diameter of saphenous veins  
21 with reflux,<sup>15</sup> the relationship may be more complex in unaffected veins. Increased venous tone  
22 could explain the negative PAC we observed in a noticeable proportion of control subjects, but  
23 probably not for CVD patients in whom the venous wall contractile response to angiotensine-2,



1 norepinephrine, and endothelin-1 is impaired.<sup>2,3</sup> Van der Velden *et al.* found a negative postural  
2 diameter change in 10% of their subjects, but dismissed it as measurement error.<sup>16</sup> We limited  
3 errors by measuring the cross-sectional area rather than only the larger diameter, and ensuring  
4 that the subject's weight rested on the other leg. Therefore, we must consider that the  
5 interindividual differences we observed are not meaningless. Nevertheless, pending further  
6 studies clarifying this issue, postural changes in diameter or cross-sectional area would not be  
7 sufficient to characterize CVD.

8         The *hysteresis* loops we obtained displayed a horizontal swap relative to conventional  
9 *hysteresis* loops since increasing PF actually reduced transmural pressure.<sup>36</sup> Observing calf veins  
10 with US through a modified pneumatic cuff, Partsch *et al.*<sup>37</sup> found that the cuff pressure required  
11 to occlude leg veins was greater in the standing than in the sitting position. We also found that a  
12 greater probe force was needed to collapse the SSV and DCV in the standing position, reflecting  
13 greater hydrostatic blood pressure. In the supine position, the probe force at which the SSV  
14 collapsed was greater in C<sub>5</sub> than in C<sub>15</sub> patients or in controls. In the standing position, the force  
15 at which SSV reopened was greater in C<sub>3</sub> and C<sub>5</sub> patients than in controls, suggesting higher  
16 venous transmural pressure and/or greater wall stiffness.

17         When evaluated *in vivo*, either by venous occlusion plethysmography or by our  
18 technique, venous viscoelasticity features are affected by the venous wall but also by  
19 surrounding tissues, blood viscosity, and resistance to blood displacement. Venous compliance  
20 or distensibility are commonly calculated from changes in limb circumference or vein diameter  
21 produced by incremental venous occlusion-cuff pressure,<sup>38</sup> Valsalva maneuver,<sup>13</sup> or posture.<sup>39</sup>  
22 Venous compliance is large at low transmural pressure where a minimal increase in blood  
23 pressure generates a large increase in volume through wall deformation. It is smaller at high

1 transmural pressure (as in the standing position), where the vein cross-section becomes circular  
2 and diameter changes induced by further rises in blood pressure reflect volume change and  
3 depend on wall elasticity.<sup>14,40,41</sup> This may explain why we obtained steeper *hysteresis* loop  
4 slopes, corresponding to greater distensibility (i.e. lower elastic *modulus*), in the supine than in  
5 the standing position in all groups. Regardless of posture, these slopes were steeper in CVD  
6 patients, also suggesting greater vein distensibility. This is consistent with previous reports of  
7 greater proximal lower limb vein distensibility in patients with varicose veins than in healthy  
8 controls,<sup>13</sup> and of endothelium and smooth muscle abnormalities in CVD patients,<sup>42</sup> even in non-  
9 varicose veins,<sup>43</sup> suggesting systemic alteration of venous wall resistance to stress.<sup>19</sup> Such  
10 abnormalities should affect viscoelasticity.<sup>44</sup> It is plausible that, beside or before remodeling,  
11 changes in smooth muscle cells contractility<sup>2-4,45</sup> alter the venous wall viscoelasticity.<sup>12</sup> This  
12 could have contributed to our findings in unaffected veins of CVD patients.

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

18 The *hysteresis* loop variables we measured discriminated controls from CVD patients.  
19 Interestingly, they also discriminated C<sub>1S</sub> from controls and from C<sub>3&5</sub> patients. As  
20 telangiectasias or spider-veins are the only objective signs in C<sub>1S</sub> patients, such quantitative data  
21 should help characterizing this distinct entity, which may have some features in common with  
22 C<sub>0s</sub> patients described by Andreozzi *et al.* as suffering from ‘hypotonic phlebopathy’.<sup>47</sup> Our

1 results suggest that viscosity is higher in unaffected veins of CVD patients in whom only  
2 reduced distensibility had been demonstrated so far.<sup>13,24,38</sup>

3

#### 4 **Limitations:**

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

14 We restricted invasive measurements to the number of subjects and patients allowing  
15 proper characterization of the population samples since ample literature is already available  
16 regarding intravenous and intramuscular pressure in CVD, but this limited the statistical power  
17 and precluded further correlations. We measured intravenous pressure in the great saphenous  
18 vein and performed the ultrasonographic examination on the small saphenous vein (a superficial  
19 vein) and on the soleal or gastrocnemial veins (muscular veins).<sup>29</sup> Nevertheless, all  
20 measurements were performed at the same calf level. Comparing axial and muscular calf veins,  
21 which exhibit different anatomical features, would be necessary in future studies for a more  
22 comprehensive assessment. We recruited patients with C<sub>1s</sub>, C<sub>3</sub>, and C<sub>5</sub> CEAP categories because  
23 compression is the main therapeutic option for them, whereas C<sub>2</sub> and C<sub>4</sub> categories may be more

1 representative of CVD. We included CVD patients with various etiologies, topographies, and  
2 severity of venous lesions, precluding subgroup analyses for lack of statistical power. Foot or  
3 knee deformation and body weight distribution may affect saphenous vein caliber and should be  
4 specifically studied. Evaluating leg tissues and measuring blood viscosity would be useful for  
5 thorough pathophysiological assessment.

## 6 **Conclusion:**

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

17

## 18 **Acknowledgments:**

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

## 21 **Declaration of conflicting interests**

22 The authors declare that there is no conflict of interest.

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## 1 **References**

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15  
16

1 **Figure Legends:**

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

7 **Fig 2. CEAP characteristics of the examined lower limb of patients with C<sub>1s</sub>, C<sub>3</sub>, and C<sub>5</sub>**  
8 **class of chronic venous disease.** Legend: *per* CEAP classification, C<sub>1s</sub>: *telangectasia* or reticular  
9 veins and symptoms; C<sub>3</sub>: edema; C<sub>5</sub>: healed venous ulcer; Ep: primary; Es (PTS): secondary  
10 (post-thrombotic syndrome); En: no venous cause identified but presence of several potential  
11 causes and risk factors (obesity, ankylosis, limb deformity, history of trauma...); As: disease  
12 involving superficial veins; Ad: disease involving deep veins; An: no venous location identified;  
13 Po: venous obstruction; Pr: venous reflux; Pn: no venous pathophysiology identifiable.

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

18 **Fig 4: Schematic drawing of the hysteresis loops of controls and patients.** Legend: Hysteresis  
19 loops redrawn from the median values of the small saphenous vein cross-sectional area during  
20 the compression test for normal controls and for limbs with C<sub>1s</sub>, C<sub>3</sub>, and C<sub>5</sub> CEAP category of  
21 chronic venous disease, in the supine and in the standing position.

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

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Table 1. *Hysteresis loop variables of the small saphenous vein in patients and controls.*

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

**Legend:** C<sub>15</sub>: limbs with C<sub>15</sub> CEAP class of chronic venous disease; C<sub>3&5</sub>: limbs with either C<sub>3</sub> or C<sub>5</sub> 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.

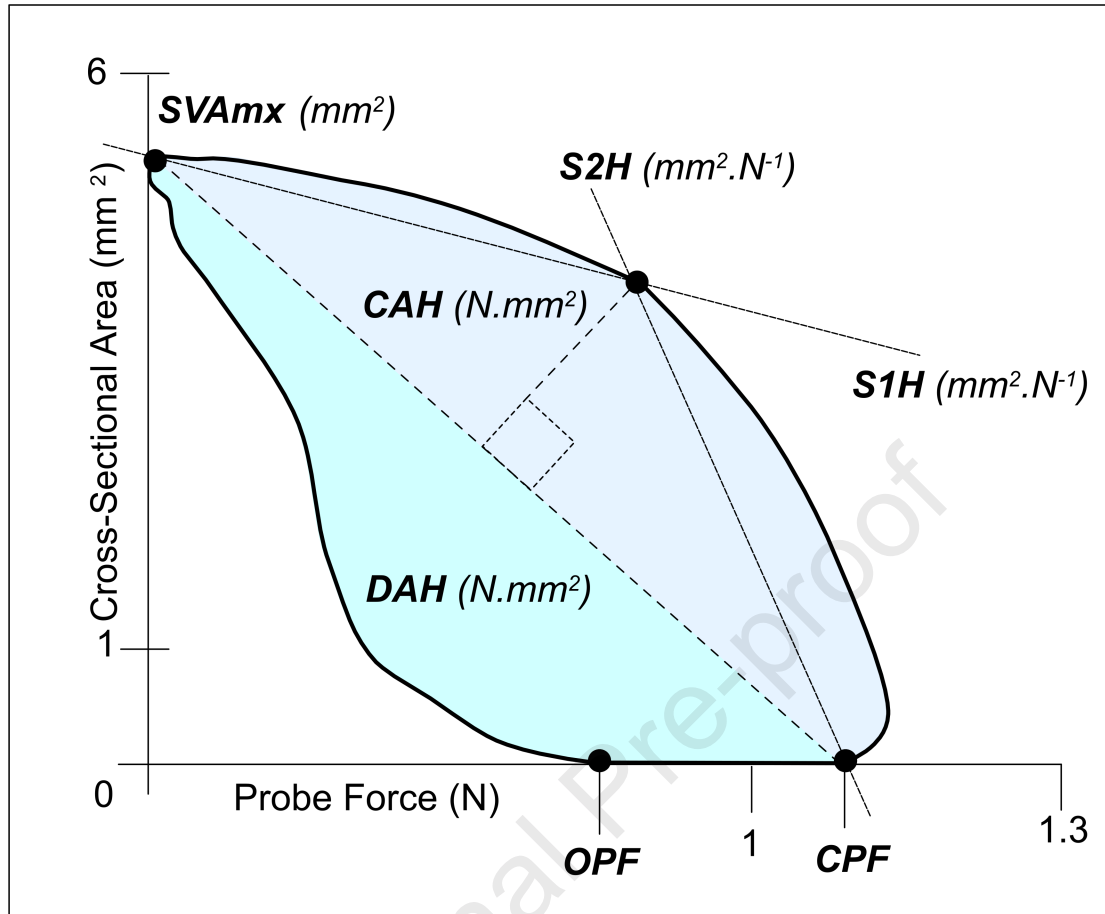
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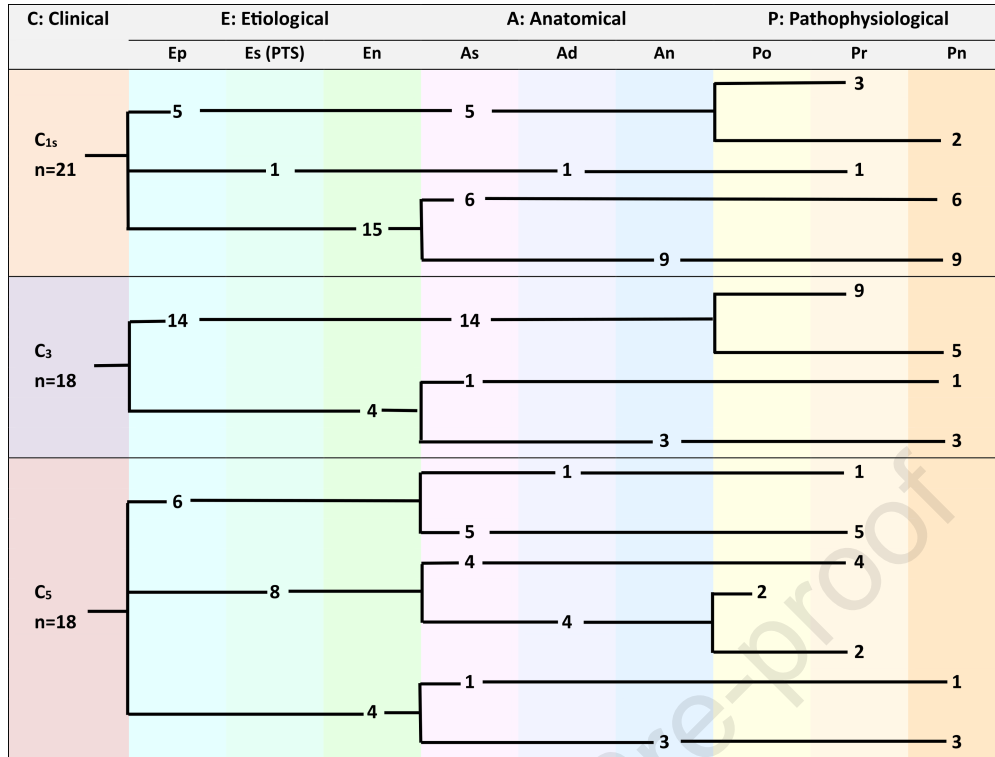


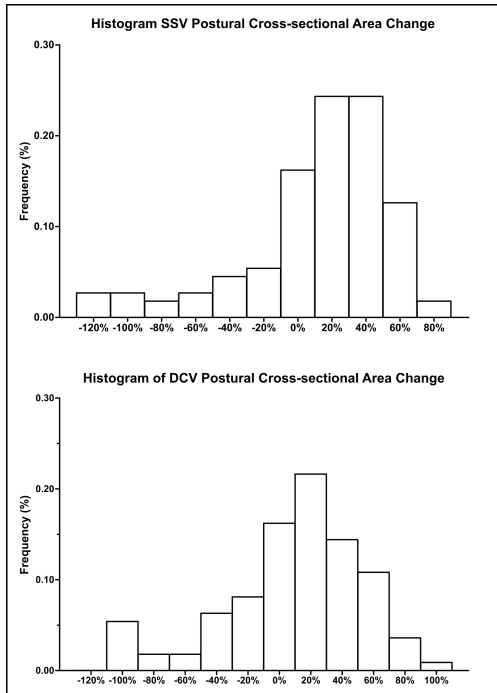
**Table II. Discriminative value of *hysteresis* variables.**

		Controls vs. CVD	Controls vs. C <sub>1s</sub>	Controls vs. C <sub>3</sub> & C <sub>5</sub>	C <sub>1s</sub> vs. C <sub>3</sub> & C <sub>5</sub>
<b>SVAmx</b>	Supine	p=.015		p=.006	p=.007
	Standing	<b>p=.007</b>		<b>p=.003</b>	<b>p=.054</b>
<b>CPF</b>	Supine				<b>p=.198</b>
	Standing	<b>p=.059</b>		<b>p=.007</b>	<b>p=.039</b>
<b>OPF</b>	Supine			<b>p=.038</b>	<b>p=.090</b>
	Standing	<b>P&lt;.001</b>	<b>p=.013</b>	<b>P&lt;.001</b>	
<b>TAH</b>	Supine	p=.022		p=.008	p=.049
	Standing	p=.035		p=.005	<b>p=.022</b>
<b>CAH</b>	Supine			p=.085	p=.113
	Standing	<b>p=.025</b>		<b>p=.004</b>	p=.027
<b>DAH</b>	Supine	<b>p=.004</b>		<b>p=.001</b>	<b>p=.045</b>
	Standing	p=.074		p=.022	p=.046
<b>S1H</b>	Supine	<b>p=.007</b>	<b>p=.032</b>	<b>p=.015</b>	
	Standing	<b>p=.078</b>	<b>p=.032</b>		
<b>S2H</b>	Supine	<b>p=.110</b>		<b>p=.085</b>	
	Standing	<b>p=.005</b>	<b>p=.056</b>	<b>p=.004</b>	
Number of variables introduced in the model		<b>9</b>	<b>4</b>	<b>9</b>	<b>6</b>
Multivariate AUC with selected variables		<b>0.796</b>	<b>0.777</b>	<b>0.826</b>	<b>0.744</b>
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

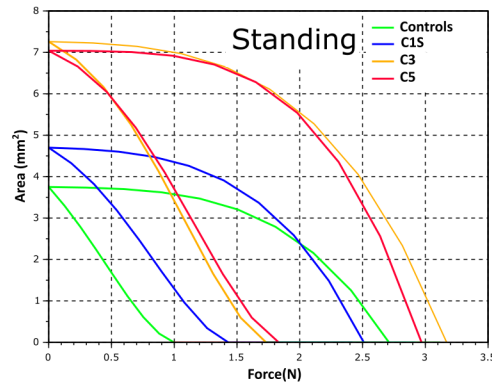
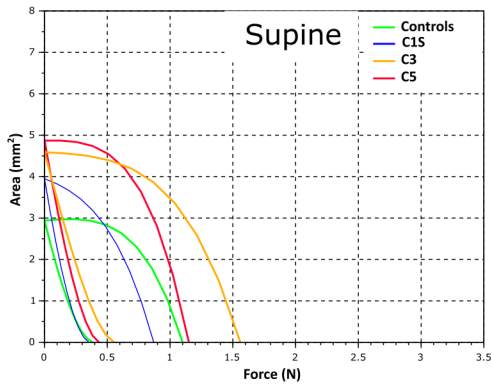
**Legend:** p-values of univariate logistic regression analysis, and multivariate logistic regression analysis of eligible *Hysteresis* variables. Variables were eligible if yielding a p value <0.2. Among strongly correlated variables, only the one with the smaller p-value was included in the multivariate model. Other variables (shaded background) were not included. CVD: chronic venous disease (all categories); C<sub>1s</sub>, C<sub>3</sub>, C<sub>5</sub>: 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.







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# 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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## 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<sup>1,2</sup> or *hysteresis*<sup>3</sup> between CVD patients and controls, and between young and elderly subjects,<sup>4</sup> 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  $\geq 26$  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  $\leq \pm 0.5\%$  of full scale, and *hysteresis*  $\leq \pm 0.5\%$  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  $\pm 0.003$  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 720x576 pixels resolution at 25 images per second, and stored as consecutive 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  $100 \times (AS - AL) / AS$ , 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.<sup>5</sup> 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.<sup>5</sup> 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:<sup>6,6</sup>

- 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 ( $\rho_c$ )

Intra-observer reading reproducibility of cross-sectional area measurements yielded  $\rho_c=0.988$  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  $\rho_c$  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  $\rho$  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<sub>3</sub> and C<sub>5</sub> 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<sub>15</sub> 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<sub>3</sub> and C<sub>5</sub> 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<sub>15</sub> from C<sub>3</sub> and C<sub>5</sub> 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<sup>7</sup>, BMI, and age.<sup>8</sup> The great saphenous vein blood pressure, although not different between groups at baseline, correlated with weight in agreement with previous reports.<sup>8</sup> Intravenous pressure increased, whereas intramuscular pressure decreased slightly, in the standing position,<sup>9</sup> 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<sup>10-12</sup> and CEAP classes.<sup>13-15</sup> 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.<sup>16</sup> 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.<sup>9</sup>

We found the expected relation between vein cross-sectional area and body weight<sup>7</sup>, BMI, and age.<sup>8</sup> The great saphenous vein blood pressure, although not different between groups at baseline, correlated with weight in agreement with previous reports.<sup>8</sup> Intravenous pressure increased, whereas intramuscular pressure decreased slightly, in the standing position,<sup>9</sup> 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<sup>17</sup> may form an inelastic sleeve around the calf, limiting vein expansion. This may be reflected by the greater calf circumference we found in C<sub>3</sub> but not in C<sub>5</sub> limbs than in controls, and the greater ankle circumference we found in C<sub>3</sub> and C<sub>5</sub> 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<sub>5</sub> 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.<sup>18</sup> 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,<sup>19</sup> 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.<sup>20</sup> 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.<sup>21,22</sup> The viscosity component of the vessel wall is mainly attributed to smooth muscle cells<sup>23</sup> 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<sup>24-28</sup> and illustrated in cardiovascular diseases, including arterial hypertension.<sup>23</sup> Viscoelasticity of venous walls has been much less studied but is nevertheless acknowledged as essential.<sup>29</sup> Most studies have been performed *in vitro*, in animal<sup>30</sup> 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.<sup>31-34</sup> *In vivo*, venous occlusion plethysmographic studies also demonstrated hysteresis,<sup>6,35</sup> 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.<sup>36,37</sup> 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.<sup>37-40</sup> These changes result in increased distensibility,<sup>2,41</sup> 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.<sup>3</sup> On the other hand, although data remain scarce, a decrease in calf muscle tissue viscoelasticity with age have been demonstrated,<sup>42</sup> and biopsy specimens showed structural and biochemical changes in the gastrocnemius muscles of patients with chronic venous disease.<sup>43</sup> 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<sup>-1</sup>).<sup>30</sup> 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.

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## Appendix: Additional Tables

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

Small saphenous vein depth (mm)			
	Supine	Standing	Supine vs. Standing
<b>Baseline</b>	7.3 [5.2–9.7]	6.8 [4.7–8.8]	p<.0001
<b>At collapse</b>	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
<b>Baseline</b>	18.9 [15.7–22.9]	18.6 [14.1–23.4]	p=.018
<b>At collapse</b>	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 <sub>1s</sub> limbs n=21	C <sub>3</sub> limbs n=18	C <sub>5</sub> limbs n=18
Small Saphenous Vein				
<b>Supine</b>				
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]
<b>Standing</b>				
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				
<b>Supine down</b>				
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]
<b>Standing</b>				
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<sub>1s</sub>, C<sub>3</sub>, and C<sub>5</sub> 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 <sub>1s</sub> (n=21)	C <sub>3</sub> (n=18)	C <sub>5</sub> (n=18)
<b>SSV</b>	22.26 [-9.40—41.66]	19.34 [-5.42—46.21]	26.97 [1.46—36.30]	20.18 [-17.52—36.86]
<b>DCV</b>	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<sub>1s</sub>, C<sub>3</sub>, and C<sub>5</sub> 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]	<b>p=0.030</b>
CVD (n=16)	14.3 [8.3—22.0]	58.0 [51.0—65.0]	<b>p=0.0001</b>
Controls vs CVD	p=0.093	<b>p=0.011</b>	

**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 test 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.

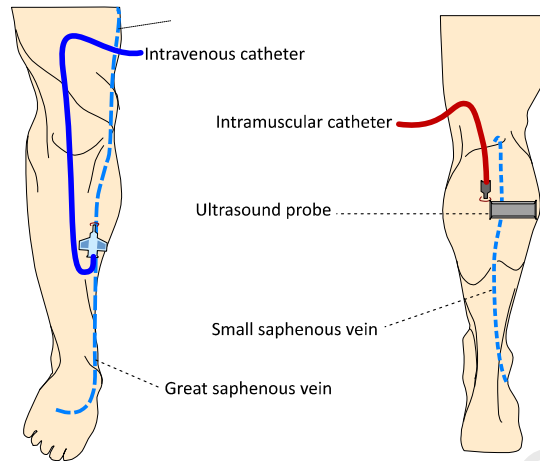
Controls	CVD Patients	p
	<b>Supine</b>	
1.5 [-2.7 — 4.13]	2.7 [-0.1 — 7.7]	p=0.523
	<b>Standing</b>	
-16.8 [-20.1 — -8.4]	-7.3 [-11.0 — -2.4]	p=0.007

**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

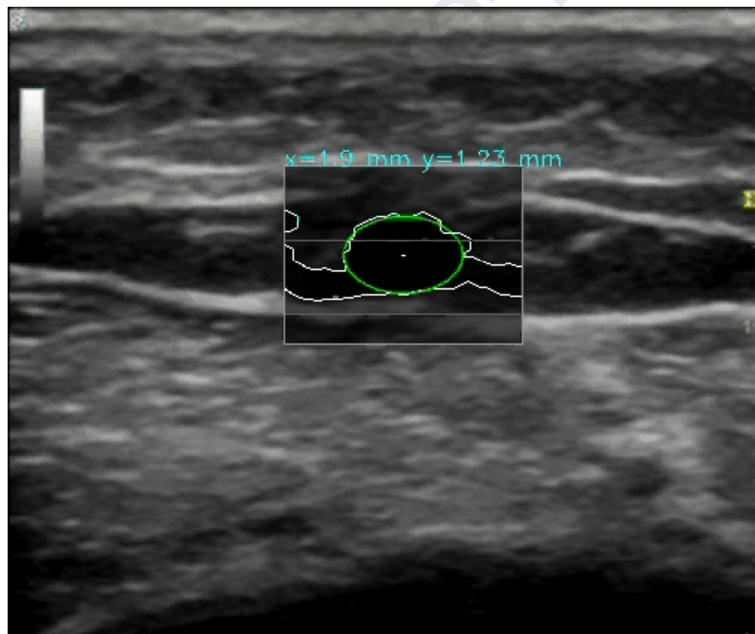
## Appendix Figure 1

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

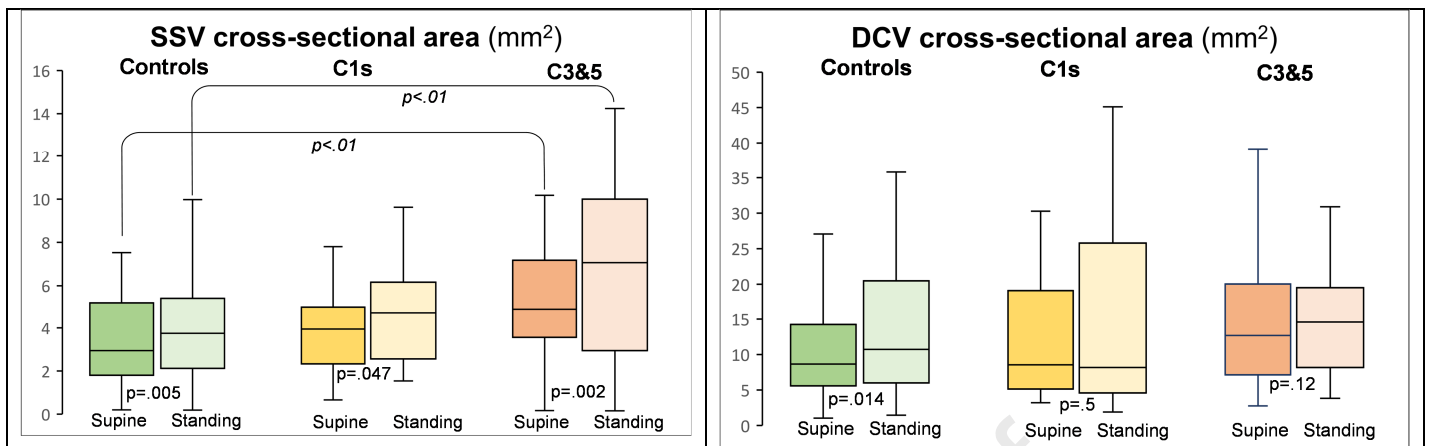


## Appendix Figure 2

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

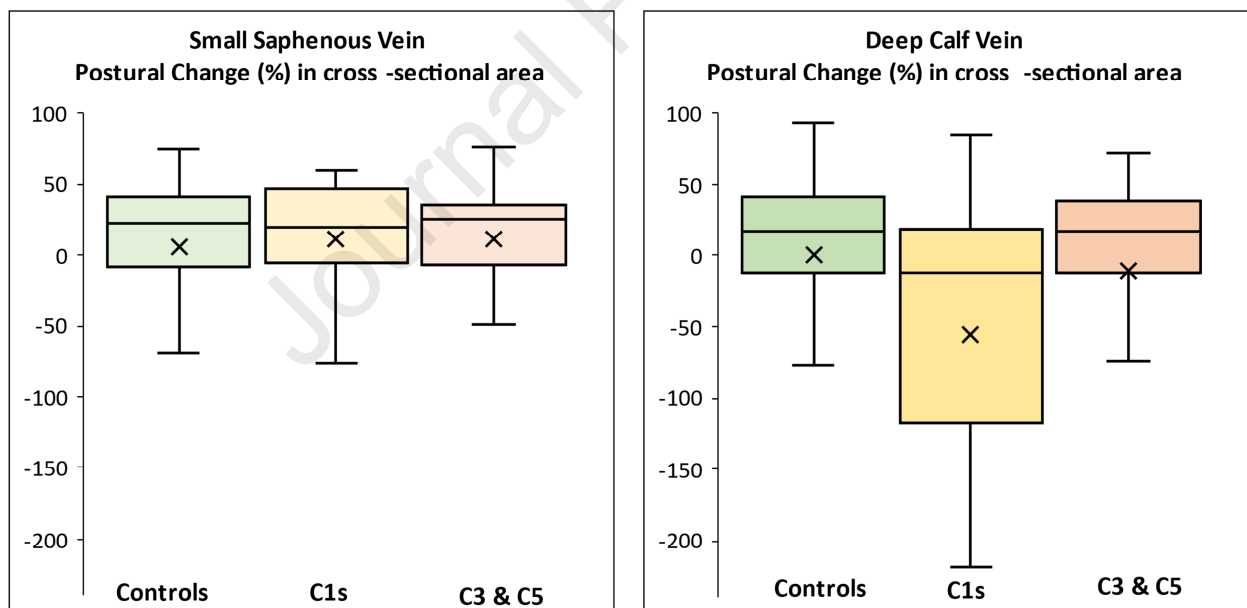


**Appendix Figure 3**  
**Cross-sectional area of the small saphenous vein and of the deep calf vein.**



**Legend:** Box-and-Whiskers plots of the cross-sectional area (in mm<sup>2</sup>) 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<sub>1s</sub>, and in limbs with C<sub>3</sub> or C<sub>5</sub> 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<sub>1s</sub>, and in limbs with C<sub>3</sub> or C<sub>5</sub> 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.

	<b>Controls</b> (n=54)	<b>C<sub>1S</sub></b> (n=21)	<b>C<sub>3</sub></b> (n=18)	<b>C<sub>5</sub></b> (n=18)
<b>Age</b> (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]
<b>Weight</b> (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]
<b>Height</b> (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]
<b>BMI</b> (kg.m <sup>-2</sup> )	24.8 [21.5—27.3]	25.6 [21.5—28.5]	29.0 [23.0—33.1]	27.3 [22.6—36.4]
<b>Leg length</b> (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]
<b>Calf Circumference</b> (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]
<b>Ankle Circumference</b> (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<sub>1S</sub>, C<sub>3</sub>, and C<sub>5</sub> 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.

	<b>Controls (n=54)</b>	<b>C<sub>1s</sub> (n=21)</b>	<b>C<sub>3</sub> &amp; C<sub>5</sub> (n=36)</b>
<b>Small Saphenous Vein cross-sectional area (mm<sup>2</sup>)</b>			
<b>Supine</b>	2.9 [1.8—5.2]	4.0 [2.3—5.0]	4.9 [3.6—7.1]
<b>Standing</b>	3.8 [2.1—5.4]	4.7 [2.6—6.2]	7.07 [3.0—9.9]
Wilcoxon signed-rank test	<b>p=.005</b>	<b>p=.047</b>	<b>p=.002</b>
<b>Deep Calf Vein cross-sectional area (mm<sup>2</sup>)</b>			
<b>Supine</b>	8.7 [5.6—14.3]	8.6 [5.1—19.1]	12.7 [7.2—20.0]
<b>Standing</b>	10.7 [6.0—20.8]	8.2 [4.6—25.8]	14.6 [8.2—19.4]
Wilcoxon signed-rank test	<b>p=.014</b>	p=.500	p=.120

**Legend:** Cross-sectional area (in mm<sup>2</sup>) of the small saphenous vein and of the deep calf vein in normal controls, in patients in C<sub>1s</sub>, and in patients with limbs in C<sub>3</sub> or C<sub>5</sub> 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
<b>All subjects and patients (n=111)</b>				
<b>Supine</b>				
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)
<b>Standing</b>				
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)
<b>Normal controls (n=54)</b>				
<b>Supine</b>				
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)
<b>Standing</b>				
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)
<b>C<sub>1s</sub> patients (n=21)</b>				
<b>Supine</b>				
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)
<b>Standing</b>				
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)
<b>C<sub>3</sub> &amp; C<sub>5</sub> patients (n=36)</b>				
<b>Supine</b>				
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)
<b>Standing</b>				
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<sub>1s</sub>, and in patients with limbs in C<sub>3</sub> or C<sub>5</sub> CEAP category of chronic venous disease.