



HAL
open science

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

Sandrine Mestre, Jean Triboulet, Christophe Demattei, Florent Veye, Monira Nou, Antonia Pérez-Martin, Michel Dauzat, Isabelle Quéré

► 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-03228579v1>

Submitted on 27 May 2021

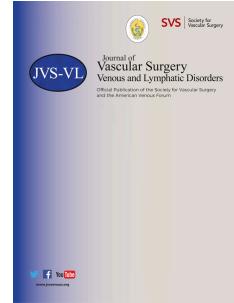
HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC-ND 4.0 - Attribution - Non-commercial use - No Derivative Works - International License

Journal Pre-proof



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

Sandrine Mestre, MD, PhD, Jean Triboulet, PhD, Christophe Demattei, PhD, Florent Veye, PhD, Monira Nou, MD, Antonia Pérez-Martin, MD, PhD, Michel Dauzat, MD, PhD, Isabelle Quéré, MD, PhD

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>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Copyright © 2020 Published by Elsevier Inc. on behalf of the Society for Vascular Surgery.

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

4 Sandrine Mestre, MD, PhD^{1,2*}, Jean Triboulet, PhD³, Christophe Demattei, PhD⁴, Florent Veye,
5 PhD³, Monira Nou, MD¹, Antonia Pérez-Martin, MD, PhD^{2,5¶}, Michel Dauzat, MD, PhD^{2,5},
6 Isabelle Quéré, MD, PhD^{1,2¶}

7
8 ¹ Vascular Medicine, Montpellier University Hospital, France

9 ² EA2992, Montpellier University, France

10 ³ LIRMM, Montpellier University, France

11 ⁴ BESPIM, Nimes University Hospital, France

12 ⁵ Vascular Medicine, Nimes University Hospital, France

13
14 *** Corresponding author:**

15 E-mail: s-mestre@chu-montpellier.fr (SM)

16 ORCID 0000-0002-4711-2996

17 Médecine Vasculaire – CHU St-Eloi

18 80 avenue Augustin-Fliche

19 34295 Montpellier – France

20 Tel: +33 467.337 028

21 Fax: +33 467 337 023

22

23 ¶ These authors contributed equally to this work

1

2 Sandrine Mestre ORCID 0000-0002-4711-2996 s-mestre@chu-montpellier.fr

3 Jean Triboulet ORCID 0000-0003-0151-1446 triboule@lirmm.fr

4 Christophe Demattei ORCID 0000-0001-6955-9281 christophe.demattei@chu-

5 nimes.fr

6 Florent Veye ORCID 0000-0002-0855-4687 florent.veye@free.fr

7 Monira Nou ORCID 0000-0002-2440-7804 m-nou@chu-montpellier.fr

8 Antonia Pérez-Martin ORCID 0000-0002-8527-7783 antonia.perez.martin@chu-

9 nimes.fr

10 Michel Dauzat ORCID 0000-0002-9496-3857

11 michel.dauzat@gmail.com

12 Isabelle Quéré ORCID 0000-0002-1492-9764 i-quere@chu-montpellier.fr

13

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_{1s}, C₃, or C₅ 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 C₃ or C₅ disease while not different from C_{1s}. 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 C₃
16 and C₅ limbs (median 2.40 [lower–upper quartile 1.65–3.88] N.mm²) than in controls (1.24
17 [0.64–2.14] N.mm²) and C_{1s} limbs (1.15 [0.71–2.97] N.mm²). 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 C₃ and C₅ 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.¹ 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,^{2,3} together with Ca^{2+} mobilization,⁴ while post-receptor contraction mechanisms
6 are preserved.⁵ Such changes in smooth muscle tone may alter the biomechanical properties of
7 the vessel wall.⁶ Chronic venous wall stress and inflammation, notably with TGF- β 1 activation,
8 result in an imbalance between matrix metalloproteases and their tissue inhibitors and lead to
9 wall remodeling.⁷ 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.⁸⁻¹¹ These structural changes also alter the
12 vein biomechanical characteristics.^{12,13} 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.¹⁴ 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).¹⁵ 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 C₄-C₆ than in patients
2 with C₀-C₁ or C₂-C₃ CEAP category.¹⁶

3 Blood vessel walls are viscoelastic, combining features of elastic solids and viscous
4 fluids.¹⁷ 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.¹⁸ 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,¹⁹⁻²¹ although viscoelasticity may be as essential for veins as it is for
13 arteries.¹¹

14 Venous distensibility increases in patients with CVD,^{22,23} even in unaffected veins.^{13,24}
15 However, the smaller postural diameter changes that has been reported in C₄-C₆ than in C₀-C₁ or
16 C₂-C₃ patients, and in enlarged than in unaffected veins,¹⁶ 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^{13,22} while others compared veins with and without reflux^{16,25,26} or limbs with different
21 CEAP categories.²⁷

22 Our aim was to assess non-invasively the biomechanics of normal and diseased lower
23 limb veins. Measuring, with B-mode ultrasonography (US),²⁸ 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),²⁹ 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_{1S}, C₃, or C₅ 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_{1S} 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 C₃ group patients with bilateral
21 leg edema as the prominent sign of CVD, and in the C₅ 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 $100 \times (AS - AL) / AS$, with AL and AS = cross sectional area respectively in the supine and the standing
19 position. SSV and DCV depth (US probe-to-vein distance) was measured at null PF and at vein
20 collapse.
21

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.²⁸ 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 extracted³⁰ 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_{1s}, and pooled C₃ and C₅ patients (C_{3&5}) 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_{1S} patients, so that the population
7 sample comprised 57 CVD patients (41 females) with 21 C_{1S}, 18 C₃, and 18 C₅ (**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 C₅ patients than in controls and C_{1S} patients. Calf circumference was greater in
11 C₃ patients than in controls, whereas ankle circumference was greater in C₃ and C₅ 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_{3&5} 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_{3&5} 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_{3&5} 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_{1S}, and C_{3&5} 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 C₃ and C₅
2 patients, 0.78 for differentiating controls from C_{1S} patients, and 0.75 for differentiating C_{1S} from
3 C₃ and C₅ 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_{1S} patients and from
9 C₃ and C₅ 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^{15,25,26,31} and CEAP categories.^{27,32,33} We
12 found no difference in DCV cross-sectional area. Deep calf veins are thought to be supported by
13 surrounding tissues and muscles,³⁴ but intramuscular pressure decreased in the standing position,
14 in our study as in another.³⁵

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 pressure¹⁵ 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,¹⁵ 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.^{2,3} Van der Velden *et al.* found a negative postural
2 diameter change in 10% of their subjects, but dismissed it as measurement error.¹⁶ 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.³⁶ Observing calf veins
10 with US through a modified pneumatic cuff, Partsch *et al.*³⁷ 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 C₅ than in C₁₅ patients or in controls. In the standing position, the force
15 at which SSV reopened was greater in C₃ and C₅ 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,³⁸ Valsalva maneuver,¹³ or posture.³⁹
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.^{14,40,41} 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,¹³ and of endothelium and smooth muscle abnormalities in CVD patients,⁴² even in non-
9 varicose veins,⁴³ suggesting systemic alteration of venous wall resistance to stress.¹⁹ Such
10 abnormalities should affect viscoelasticity.⁴⁴ It is plausible that, beside or before remodeling,
11 changes in smooth muscle cells contractility^{2-4,45} alter the venous wall viscoelasticity.¹² 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 measurements²⁴ and plethysmography.^{30,46} However, viscoelasticity is
15 frequency-dependent,³⁶ 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_{1S} from controls and from C_{3&5} patients. As
20 telangiectasias or spider-veins are the only objective signs in C_{1S} patients, such quantitative data
21 should help characterizing this distinct entity, which may have some features in common with
22 C_{0s} patients described by Andreozzi *et al.* as suffering from ‘hypotonic phlebopathy’.⁴⁷ 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.^{13,24,38}

3

4 **Limitations:**

5 CVD also involves skin and soft tissues.^{19,48} 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).²⁹ 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_{1s}, C₃, and C₅ CEAP categories because
23 compression is the main therapeutic option for them, whereas C₂ and C₄ 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.

1

2

Journal Pre-proof

1 **References**

2 Reference List

3

4 (1) Lu D, Kassab GS. Role of shear stress and stretch in vascular mechanobiology. *J R Soc*
5 *Interface* 2011;8:1379-85.

6 (2) Barber DA, Wang X, Gloviczki P, Miller VM. Characterization of endothelin receptors in
7 human varicose veins. *J Vasc Surg* 1997;26:61-9.

8 (3) Rizzi A, Quaglio D, Vasquez G, Mascoli F, Amadesi S, Calo G, et al. Effects of
9 vasoactive agents in healthy and diseased human saphenous veins. *J Vasc Surg*
10 1998;28:855-61.

11 (4) Schuller-Petrovic S, Stessel H, Brunner F. Ca²⁺ mobilization in saphenous vein smooth
12 muscle cells derived from patients with primary varicosity. *Eur J Clin Invest*
13 2002;32:649-56.

14 (5) Raffetto JD, Qiao X, Beauregard KG, Tanbe AF, Kumar A, Mam V, et al. Functional
15 adaptation of venous smooth muscle response to vasoconstriction in proximal, distal, and
16 varix segments of varicose veins. *J Vasc Surg* 2010;51:962-71.

17 (6) Roca F, Iacob M, Remy-Jouet I, Bellien J, Joannides R. Evidence for a Role of Vascular
18 Endothelium in the Control of Arterial Wall Viscosity in Humans. *Hypertension*
19 2018;71:143-50.

- 1 (7) Serralheiro P, Soares A, Costa Almeida CM, Verde I. TGF-beta1 in Vascular Wall
2 Pathology: Unraveling Chronic Venous Insufficiency Pathophysiology. *Int J Mol Sci*
3 2017;18.
- 4 (8) Venturi M, Bonavina L, Annoni F, Colombo L, Butera C, Peracchia A, et al. Biochemical
5 assay of collagen and elastin in the normal and varicose vein wall. *J Surg Res*
6 1996;60:245-8.
- 7 (9) Wali MA, Dewan M, Eid RA. Histopathological changes in the wall of varicose veins. *Int*
8 *Angiol* 2003;22:188-93.
- 9 (10) Vekilov DP, Grande-Allen KJ. Mechanical Properties of Diseased Veins. *Methodist*
10 *Debaquey Cardiovasc J* 2018;14:182-7.
- 11 (11) Zamboni P, Tavoni V, Sisini F, Pedriali M, Rimondi E, Tessari M, et al. Venous
12 compliance and clinical implications. 7 ed. 2018. p. 50-5.
- 13 (12) Jeanneret C, Baldi T, Hailemariam S, Koella C, Gewaltig J, Biedermann BC. Selective
14 loss of extracellular matrix proteins is linked to biophysical properties of varicose veins
15 assessed by ultrasonography. *Br J Surg* 2007;94:449-56.
- 16 (13) Jeanneret C, Jager KA, Zaugg CE, Hoffmann U. Venous reflux and venous distensibility
17 in varicose and healthy veins. *Eur J Vasc Endovasc Surg* 2007;34:236-42.
- 18 (14) Meissner MH, Moneta G, Burnand K, Gloviczki P, Lohr JM, Lurie F, et al. The
19 hemodynamics and diagnosis of venous disease. *J Vasc Surg* 2007;46 Suppl S:4S-24S.

- 1 (15) Zamboni P, Portaluppi F, Marcellino MG, Manfredini R, Pisano L, Liboni A.
2 Ultrasonographic assessment of ambulatory venous pressure in superficial venous
3 incompetence. *J Vasc Surg* 1997;26:796-802.
- 4 (16) van der Velden SK, De Maeseneer MG, Pichot O, Nijsten T, van den Bos RR. Postural
5 Diameter Change of the Saphenous Trunk in Chronic Venous Disease. *Eur J Vasc*
6 *Endovasc Surg* 2016;51:831-7.
- 7 (17) Wang Z, Golob MJ, Chesler NC. Viscoelastic Properties of Cardiovascular Tissues.
8 <https://www.intechopen.com/books/viscoelastic-and-viscoplastic-materials> 2016 [cited
9 2019 Jul 11];(7):141-163.
- 10 (18) Vincent J. Basic elasticity and viscoelasticity. *Structural biomaterials*. Third edition ed.
11 Princeton: Princeton University Press; 2012. p. 1-28.
- 12 (19) Elsharawy MA, Naim MM, Abdelmaguid EM, Al-Mulhim AA. Role of saphenous vein
13 wall in the pathogenesis of primary varicose veins. *Interact Cardiovasc Thorac Surg*
14 2007;6:219-24.
- 15 (20) Lim CS, Davies AH. Pathogenesis of primary varicose veins. *Br J Surg* 2009;96:1231-42.
- 16 (21) Krasinski Z, Biskupski P, Dzieciuchowicz L, Kaczmarek E, Krasinska B, Staniszewski
17 R, et al. The influence of elastic components of the venous wall on the biomechanical
18 properties of different veins used for arterial reconstruction. *Eur J Vasc Endovasc Surg*
19 2010;40:224-9.

- 1 (22) Zsoter T, Cronin RF. Venous distensibility in patients with varicose veins. *Can Med*
2 *Assoc J* 1966;94:1293-7.
- 3 (23) Raffetto JD, Khalil RA. Mechanisms of varicose vein formation: valve dysfunction and
4 wall dilation. *Phlebology* 2008;23:85-98.
- 5 (24) Zsoter T, Moore S, Keon W. Venous distensibility in patients with varicosities: in vitro
6 studies. *J Appl Physiol* 1967;22:505-8.
- 7 (25) Navarro TP, Delis KT, Ribeiro AP. Clinical and hemodynamic significance of the greater
8 saphenous vein diameter in chronic venous insufficiency. *Arch Surg* 2002;137:1233-7.
- 9 (26) Joh JH, Park HC. The cutoff value of saphenous vein diameter to predict reflux. *J Korean*
10 *Surg Soc* 2013;85:169-74.
- 11 (27) Konoeda H, Yamaki T, Hamahata A, Ochi M, Sakurai H. Quantification of superficial
12 venous reflux by duplex ultrasound-role of reflux velocity in the assessment the clinical
13 stage of chronic venous insufficiency. *Ann Vasc Dis* 2014;7:376-82.
- 14 (28) Veye F, Mestre S, Berron N, Perez-Martin A, Triboulet J. Evaluation of lower limb vein
15 biomechanical properties and the effects of compression stockings, with an instrumented
16 ultrasound probe. *Conf Proc IEEE Eng Med Biol Soc* 2014;2014:74-7.
- 17 (29) Meissner MH. Lower extremity venous anatomy. *Semin Intervent Radiol* 2005;22:147-
18 56.

- 1 (30) Journo HJ, Chanudet XA, Pannier BM, Laroque PL, London GM, Safar ME. Hysteresis
2 of the venous pressure-volume relationship in the forearm of borderline hypertensive
3 subjects. *Clin Sci (Lond)* 1992;82:329-34.
- 4 (31) Mendoza E, Blattler W, Amsler F. Great saphenous vein diameter at the saphenofemoral
5 junction and proximal thigh as parameters of venous disease class. *Eur J Vasc Endovasc*
6 *Surg* 2013;45:76-83.
- 7 (32) Gibson K, Meissner M, Wright D. Great saphenous vein diameter does not correlate with
8 worsening quality of life scores in patients with great saphenous vein incompetence. *J*
9 *Vasc Surg* 2012;56:1634-41.
- 10 (33) Lane TRA, Varatharajan L, Fiorentino F, Shepherd AC, Zimmo L, Gohel MS, et al.
11 Truncal varicose vein diameter and patient-reported outcome measures. *Br J Surg*
12 2017;104:1648-55.
- 13 (34) Eberhardt RT, Raffetto JD. Chronic venous insufficiency. *Circulation* 2014;130:333-46.
- 14 (35) Alimi YS, Barthelemy P, Juhan C. Venous pump of the calf: a study of venous and
15 muscular pressures. *J Vasc Surg* 1994;20:728-35.
- 16 (36) Goto M, Kimoto Y. Hysteresis and stress-relaxation of the blood vessels studied by a
17 universal tensile testing instrument. *Jpn J Physiol* 1966;16:169-84.
- 18 (37) Partsch B, Partsch H. Calf compression pressure required to achieve venous closure from
19 supine to standing positions. *J Vasc Surg* 2005;42:734-8.

- 1 (38) De Groot PC, Bleeker MW, Hopman MT. Ultrasound: a reproducible method to measure
2 conduit vein compliance. *J Appl Physiol* (1985) 2005;98:1878-83.
- 3 (39) Molnar AA, Apor A, Kristof V, Nadasy GL, Preda I, Huttli K, et al. Generalized changes
4 in venous distensibility in postthrombotic patients. *Thromb Res* 2006;117:639-45.
- 5 (40) Norgren L, Thulesius O, Gjores JE, Soderlundh S. Foot-volumetry and simultaneous
6 venous pressure measurements for evaluation of venous insufficiency. *Vasa* 1974;3:140-
7 7.
- 8 (41) Norgren L, Widmer LK. Venous function, evaluated by foot volumetry, in patients with a
9 previous deep venous thrombosis, treated with streptokinase. Preliminary results. *Vasa*
10 1978;7:412-4.
- 11 (42) Golledge J, Quigley FG. Pathogenesis of varicose veins. *Eur J Vasc Endovasc Surg*
12 2003;25:319-24.
- 13 (43) Meissner MH, Gloviczki P, Bergan J, Kistner RL, Morrison N, Pannier F, et al. Primary
14 chronic venous disorders. *J Vasc Surg* 2007;46 Suppl S:54S-67S.
- 15 (44) Zocalo Y, Bia D, Cabrera FE, Wray S, Galli C, Armentano RL. Structural and functional
16 properties of venous wall: relationship between elastin, collagen, and smooth muscle
17 components and viscoelastic properties. 2013 ed. Hindawi Publishing Corporation; 2016.
18 p. 1-9.

- 1 (45) Raffetto JD, Qiao X, Beauregard KG, Tanbe AF, Kumar A, Mam V, et al. Functional
2 adaptation of venous smooth muscle response to vasoconstriction in proximal, distal, and
3 varix segments of varicose veins
4 RAFFETTO2010A. *J Vasc Surg* 2010;51:962-71.
- 5 (46) Halliwill JR, Minson CT, Joyner MJ. Measurement of limb venous compliance in
6 humans: technical considerations and physiological findings. *J Appl Physiol*
7 1999;87:1555-63.
- 8 (47) Andreozzi GM, Signorelli S, Di PL, Garozzo S, Cacciaguerra G, Leone A, et al. Varicose
9 symptoms without varicose veins: the hypotonic phlebopathy, epidemiology and
10 pathophysiology. The Acireale project. *Minerva Cardioangiol* 2000;48:277-85.
- 11 (48) Sansilvestri-Morel P, Fioretti F, Rupin A, Senni K, Fabiani JN, Godeau G, et al.
12 Comparison of extracellular matrix in skin and saphenous veins from patients with
13 varicose veins: does the skin reflect venous matrix changes? *Clin Sci (Lond)*
14 2007;112:229-39.
- 15
16

1 **Figure Legends:**

2 **Fig 1. A typical hysteresis loop of the short saphenous vein.** Legend: Cross-sectional area (in
3 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_{1s}, C₃, and C₅**
8 **class of chronic venous disease.** Legend: *per* CEAP classification, C_{1s}: *telangectasia* or reticular
9 veins and symptoms; C₃: edema; C₅: 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_{1s}, C₃, and C₅ 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.**

Journal Pre-proof

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

	Controls	C _{1s}	C ₃ & C ₅	Controls vs C _{1s}	Controls vs C _{3&5}	C _{1s} vs C _{3&5}
SVAmx (mm²)						
Supine	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	
Standing	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			
CPF (N)						
Supine	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			
Standing	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			
OPF (N)						
Supine	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			
Standing	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			
DPF (N)						
Supine	0.64[.38—.94] AUC=0.54	0.50[.32—.90] AUC=0.53	0.65[.42—1.02] AUC=0.57			
Standing	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			
TAH (N.mm²)						
Supine	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	
Standing	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			
CAH (N.mm²)						
Supine	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			
Standing	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			
DAH (N.mm²)						
Supine	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	
Standing	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			
S1H (mm².N⁻¹)						
Supine	-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	
Standing	-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			
S2H (mm².N⁻¹)						
Supine	-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			
Standing	-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			

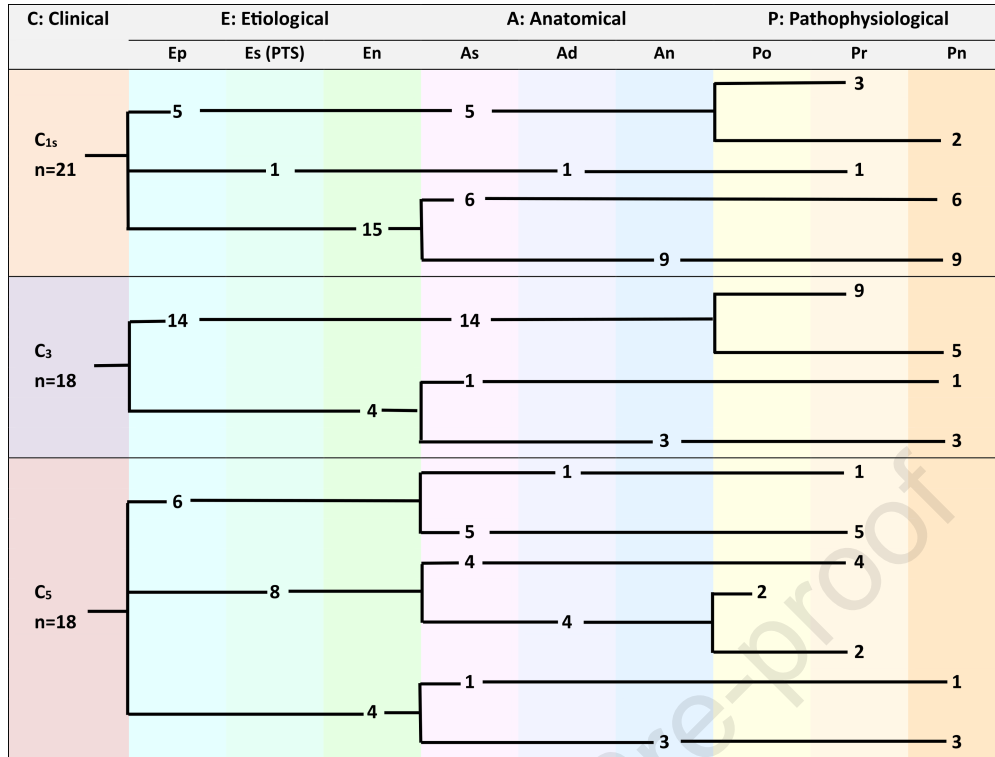
Legend: C₁₅: limbs with C₁₅ CEAP class of chronic venous disease; C_{3&5}: limbs with either C₃ or C₅ class of chronic venous disease; **p** = p-value (when significant) of Dunn's multiple comparison post-Kruskal-Wallis test for group comparison, and of paired t-test for supine versus standing position. 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.

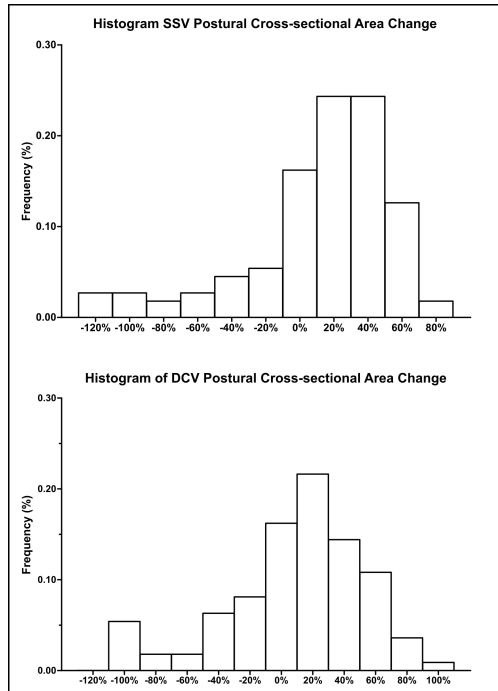
Journal Pre-proof

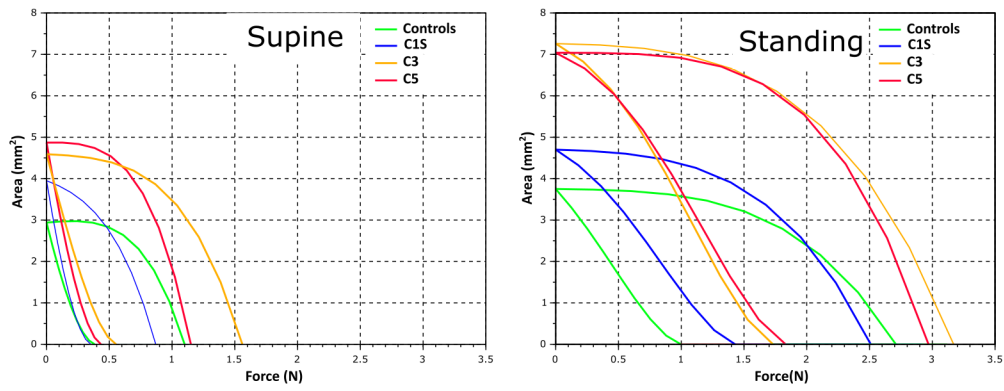
Table II. Discriminative value of *hysteresis* variables.

		Controls vs. CVD	Controls vs. C _{1s}	Controls vs. C ₃ & C ₅	C _{1s} vs. C ₃ & C ₅
SVAmx	Supine	p=.015		p=.006	p=.007
	Standing	p=.007		p=.003	p=.054
CPF	Supine				p=.198
	Standing	p=.059		p=.007	p=.039
OPF	Supine			p=.038	p=.090
	Standing	P<.001	p=.013	P<.001	
TAH	Supine	p=.022		p=.008	p=.049
	Standing	p=.035		p=.005	p=.022
CAH	Supine			p=.085	p=.113
	Standing	p=.025		p=.004	p=.027
DAH	Supine	p=.004		p=.001	p=.045
	Standing	p=.074		p=.022	p=.046
S1H	Supine	p=.007	p=.032	p=.015	
	Standing	p=.078	p=.032		
S2H	Supine	p=.110		p=.085	
	Standing	p=.005	p=.056	p=.004	
Number of variables introduced in the model		9	4	9	6
Multivariate AUC with selected variables		0.796	0.777	0.826	0.744
IC95% AUC (Delong method)		0.710—0.882	0.662—0.892	0.739—0.9141	0.614—0.873
IC95% AUC (boot-strap 10000)		0.707—0.878	0.657—0.884	0.731—0.908	0.609—0.866

Legend: p-values of univariate logistic regression analysis, and multivariate logistic regression analysis of eligible *Hysteresis* variables. Variables were eligible if yielding a p value <0.2. Among strongly correlated variables, only the one with the smaller p-value was included in the multivariate model. Other variables (shaded background) were not included. CVD: chronic venous disease (all categories); C_{1s}, C₃, C₅: 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.







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

Sandrine Mestre, MD, PhD^{1,2*}, Jean Triboulet, PhD³, Christophe Demattei, PhD⁴, Florent Veye, PhD³, Monira Nou, MD¹, Antonia Pérez-Martin, MD, PhD^{2,5¶}, Michel Dauzat, MD, PhD^{2,5}, Isabelle Quéré, MD, PhD^{1,2¶}

Additional Material

Material and Methods: additional information	2
Determination of population sample size	2
Detailed methods	2
Detailed measurements and calculations	3
Additional Results	3
Reproducibility	3
Hysteresis Variables	4
Supplemental Discussion	4
Intravenous blood pressure	4
Vein cross-sectional area	4
Postural changes in cross-sectional area	4
Vessel wall viscoelasticity	5
Appendix: Additional Tables	7
Appendix Table I. Small saphenous and deep calf vein depth.	7
Appendix Table II. Depth of the small saphenous vein according to CEAP class.	7
Appendix Table III. Relative postural changes in cross-sectional area of the small saphenous vein and of the deep calf vein.	8
Appendix Table IV. Mean intravenous blood pressure in the great saphenous vein.	8
Appendix Table V. Mean calf intramuscular pressure.	8
Appendix: Additional Figures	9
Appendix Figure 1	9
Appendix Figure 2	9
Appendix Figure 3	10
Appendix Figure 4	10
References	11

Material and Methods: additional information

Determination of population sample size

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

Detailed methods

US examinations were performed with a Logiq-e system and its 12L-RS linear probe (GE Ultrasound, Chicago, IL, USA). Settings were harmonics mode, 75 dB dynamic range, and one focal zone. We adjusted emitting frequency, depth, gain, time-gain compensation, and focus to obtain the best image of the vein. Frame rate was ≥ 26 images per second. The ultrasound probe was mounted on a berth gliding on a rail and instrumented with a XFTC300 sensor (Measurement Specialties, Hampton, VI, USA), with range 2-2000 N, linearity $\leq \pm 0.5\%$ 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 ± 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.⁵ Within the rectangular area of interest (ROI) drawn by the observer to enclose the observed vein on the first image of the recorded sequence, the software automatically adjusted the grey scale threshold for image binarization, then proceed to morphology adjustment for edge smoothing.⁵ This allowed the detection of the venous wall along the horizontal (X) and vertical (Y) axes, and the computation of the X and Y lengths for ellipse approximation. The calculated ellipse was then overlaid on the initial B-mode image for visual control. The ROI center was calculated for each approximated ellipse, allowing to track automatically the movements of the vein all along the sequence.

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

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

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

Additional Results

Reproducibility

Reproducibility was evaluated on two independent readings of the same recorded image or signal by Lin concordance correlation coefficient (ρ_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 ρ_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 ρ 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 C₃ and C₅ 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 C₁₅ 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 C₃ and C₅ 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 C₁₅ from C₃ and C₅ 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 weight⁷, BMI, and age.⁸ The great saphenous vein blood pressure, although not different between groups at baseline, correlated with weight in agreement with previous reports.⁸ Intravenous pressure increased, whereas intramuscular pressure decreased slightly, in the standing position,⁹ but intramuscular pressure remained higher in CVD patients than in controls.

Vein cross-sectional area

The greater SSV, but not DCV, cross-sectional area we found in CVD patients than in controls is in agreement with previous studies about GSV diameter¹⁰⁻¹² and CEAP classes.¹³⁻¹⁵ However, these studies included no controls, while we included normal controls and measured unaffected superficial but also deep calf veins. Saphenous veins are thought to be more prone to dilation because they are not supported by surrounding tissues and muscles, contrary to deep veins.¹⁶ However, the contribution of surrounding tissues to the limitation of transmural pressure of deep calf veins at rest appears limited since intramuscular pressure decreases in the standing position, as shown by our study and another.⁹

We found the expected relation between vein cross-sectional area and body weight⁷, BMI, and age.⁸ The great saphenous vein blood pressure, although not different between groups at baseline, correlated with weight in agreement with previous reports.⁸ Intravenous pressure increased, whereas intramuscular pressure decreased slightly, in the standing position,⁹ but intramuscular pressure remained higher in CVD patients than in controls.

Postural changes in cross-sectional area

Our most striking result is the extent of interindividual differences in PAC, independently of the healthy or CVD *status*, since the vein area increased in some subjects, staid unchanged or even decreased in others in the standing position. As we took care to avoid residual muscle contraction, the absence, in some subjects, of vein area increase in spite of greater hydrostatic blood pressure could be due to multiple, possibly opposite factors such as greater venous wall stiffness and/or stronger venous tone and/or higher

interstitial pressure and/or lower distensibility of skin and/or of surrounding soft tissues. For instance, edema and lipodermatosclerosis¹⁷ may form an inelastic sleeve around the calf, limiting vein expansion. This may be reflected by the greater calf circumference we found in C₃ but not in C₅ limbs than in controls, and the greater ankle circumference we found in C₃ and C₅ 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 C₅ limbs) could lead to the same results by limiting vein expansion. Increased venous tone could explain the negative PAC we observed in a noticeable proportion of subjects. Each of these mechanisms should be specifically investigated.

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

Vessel wall viscoelasticity

Although viscosity is a characteristic of fluids and a major feature of blood, the walls of arteries and veins do present viscoelastic characteristics, combining features of elastic solids and viscous fluids.²⁰ The elastic component represents the amount of energy stored during loading, while the viscous component is responsible for energy dissipation. The ratio of the viscous to elastic component increases with strain and strain rate.^{21,22} The viscosity component of the vessel wall is mainly attributed to smooth muscle cells²³ 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 demonstrated²⁴⁻²⁸ and illustrated in cardiovascular diseases, including arterial hypertension.²³ Viscoelasticity of venous walls has been much less studied but is nevertheless acknowledged as essential.²⁹ Most studies have been performed *in vitro*, in animal³⁰ or human specimens, especially for the evaluation of saphenous veins used as homografts since their viscoelastic properties are essential for proper function when implanted in the arterial system.³¹⁻³⁴ *In vivo*, venous occlusion plethysmographic studies also demonstrated hysteresis,^{6,35} which implies viscosity.

Ex vivo biomechanical and immuno-histochemical studies have clearly demonstrated the presence of structural changes in the venous wall of patients with chronic venous disease, with subsequent alteration of vein biomechanics.^{36,37} Loss of elastin and type III collagen has been found in varicose veins, together with disorganization of the extracellular matrix, disturbed expression of matrix remodeling enzymes, and loss of smooth muscle cells.³⁷⁻⁴⁰ These changes result in increased distensibility,^{2,41} which means decreased elastic modulus. This also applies to plethysmography, which allowed to record *hysteresis* loops and showed greater leg veins distensibility in patients with varicose veins than in controls.³ On the other hand, although data remain scarce, a decrease in calf muscle tissue viscoelasticity with age have been demonstrated,⁴² and biopsy specimens showed structural and biochemical changes in the gastrocnemius muscles of patients with chronic venous disease.⁴³ Therefore, our results, obtained noninvasively *in vivo*, are consistent with previous *in vitro* and *ex vivo* findings demonstrating altered venous viscoelasticity in patients with chronic venous disease (CVD). However, most of these studies were performed on varicose veins whereas we studied

unaffected veins of CVD patients, and we could not specifically identify the role of the venous wall in the viscoelasticity differences we observed.

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

Journal Pre-proof

Appendix: Additional Tables

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

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

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

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

	Controls n=54	C _{1s} limbs n=21	C ₃ limbs n=18	C ₅ limbs n=18
Small Saphenous Vein				
Supine				
Baseline	7.6 [6.0–10.1]	7.1 [4.4–8.5]	7.0 [4.3–11.2]	6.9 [5.2–9.9]
At collapse	7.3 [5.0–8.7]	7.6 [6.0–9.9]	6.8 [4.4–9.9]	8.5 [5.4–10.3]
Standing				
Baseline	6.8 [5.6–8.8]	6.3 [4.4–8.0]	7.0 [4.3–11.2]	6.7 [3.9–9.3]
At collapse	6.6 [5.0–8.3]	7.4 [5.6–9.8]	6.8 [4.4–9.9]	7.4 [5.4–10.3]
Deep Calf Vein				
Supine down				
Baseline	18.9 [15.2–23.6]	18.7 [16.0–26.6]	19.2 [14.8–21.9]	18.9 [15.2–23.2]
At collapse	17.0 [14.5–20.8]	18.5 [16.2–24.8]	17.2 [14.0–24.3]	17.7 [13.4–22.0]
Standing				
Baseline	18.9 [13.9–23.72]	18.3 [15.9–26.5]	18.3 [12.3–21.6]	17.7 [13.8–23.0]
At collapse	16.6 [13.2–19.9]	17.8 [13.9–22.8]	17.0 [12.5–24.1]	16.8 [14.2–20.4]

Legend: Depth, in mm, of the small saphenous and of the deep calf vein at baseline (with void compression force) and at vein collapse, in normal controls, and in limbs with C_{1s}, C₃, and C₅ CEAP category of chronic venous disease. Values are reported as median [lower—upper quartile].

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

	Controls (n=54)	C _{1s} (n=21)	C ₃ (n=18)	C ₅ (n=18)
SSV	22.26 [-9.40—41.66]	19.34 [-5.42—46.21]	26.97 [1.46—36.30]	20.18 [-17.52—36.86]
DCV	17.34 [-13.31—41.34]	-12.18 [-117.20—18.19]	19.73 [-72.84—44.43]	18.09 [-11.84—36.04]

Legend: Relative (%) postural changes in cross-sectional area of the small saphenous vein (**SSV**) and of the deep calf vein (**DCV**) in normal controls and in limbs with C_{1s}, C₃, and C₅ CEAP category of chronic venous disease. Values are provided as median [lower-upper quartile].

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

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

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

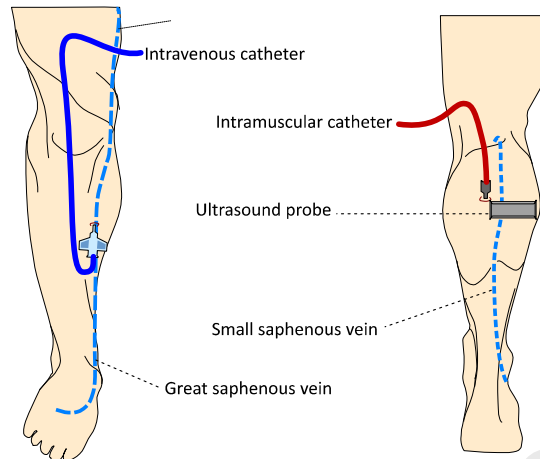
Controls	CVD Patients	p
	Supine	
1.5 [-2.7 — 4.13]	2.7 [-0.1 — 7.7]	p=0.523
	Standing	
-16.8 [-20.1 — -8.4]	-7.3 [-11.0 — -2.4]	p=0.007

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

Appendix: Additional Figures

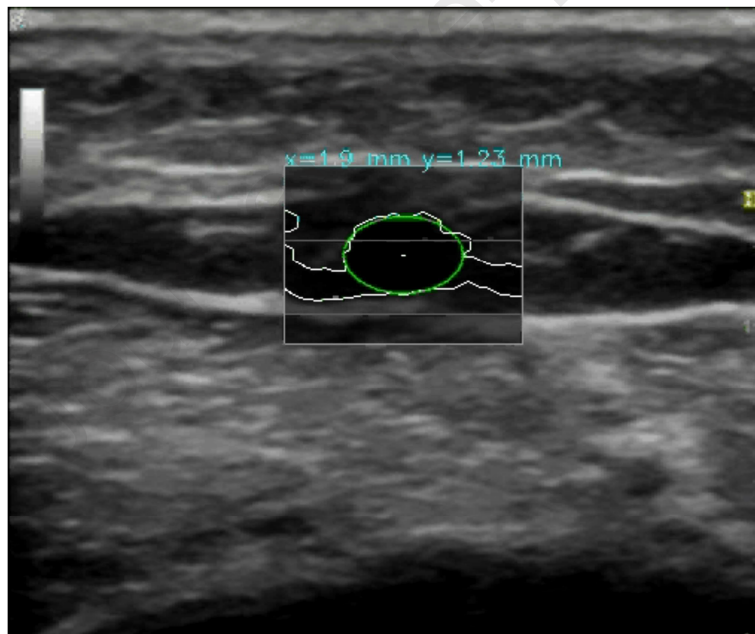
Appendix Figure 1

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

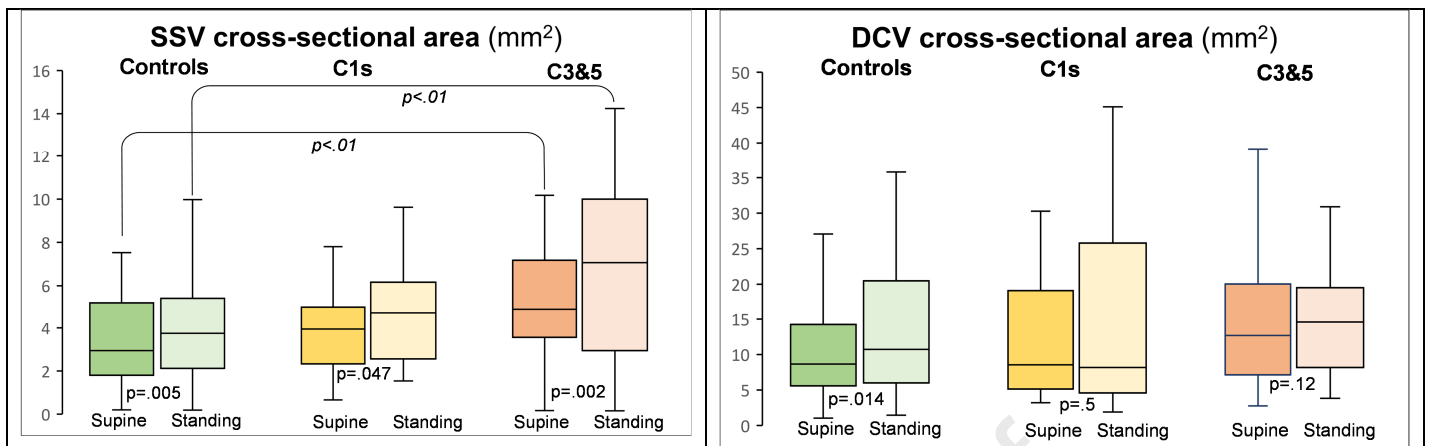


Appendix Figure 2

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

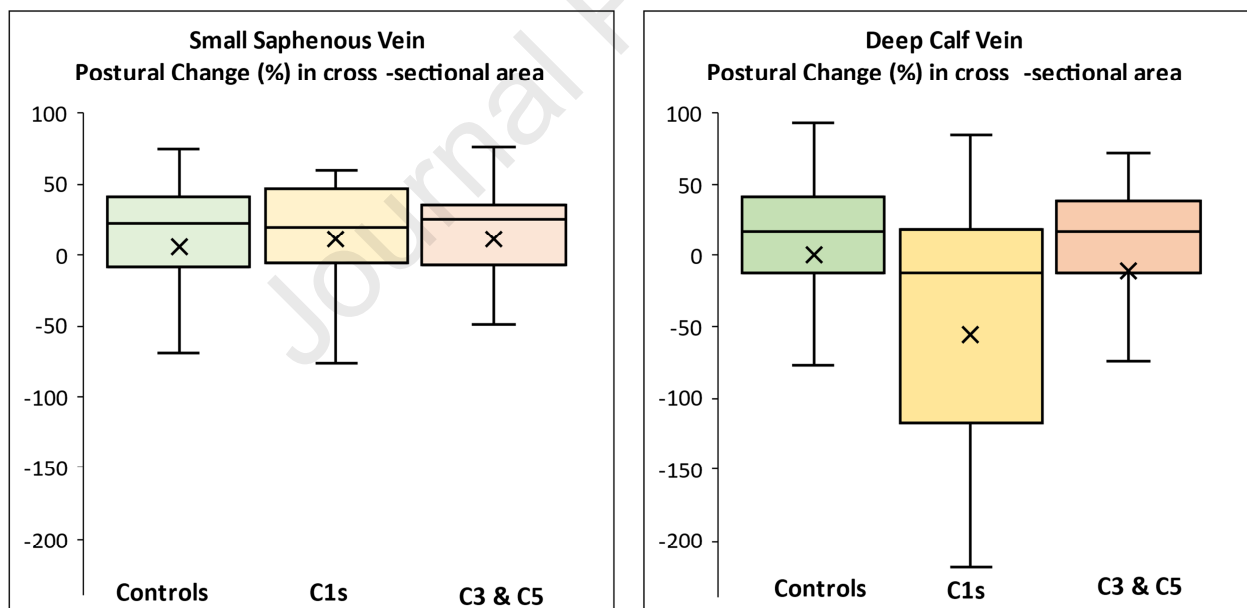


Appendix Figure 3
Cross-sectional area of the small saphenous vein and of the deep calf vein.



Legend: Box-and-Whiskers plots of the cross-sectional area (in mm²) of the small saphenous vein (SSV) and of the deep calf vein (DCV) in the supine and in the standing position, in normal controls, in limbs with C_{1s}, and in limbs with C₃ or C₅ CEAP category of chronic venous disease. p: p-value of comparison between the supine and the standing position. Comparison between groups are shown, when significant, as horizontal brackets with p-value.

Appendix Figure 4
Relative postural change in cross-sectional area of the small saphenous and of the deep calf vein.



Legend: Box-and-Whiskers plots of relative (%) postural changes of vein cross-sectional area in normal controls, in limbs with C_{1s}, and in limbs with C₃ or C₅ category of chronic venous disease. The horizontal line dividing the boxes represents the median value, and "X" represents the mean.

References

- (1) Molnar AA, Apor A, Kristof V, Nadasy GL, Preda I, Huttl K, et al. Generalized changes in venous distensibility in postthrombotic patients. *Thromb Res* 2006;117:639-45.
- (2) Jeanneret C, Jager KA, Zaugg CE, Hoffmann U. Venous reflux and venous distensibility in varicose and healthy veins. *Eur J Vasc Endovasc Surg* 2007;34:236-42.
- (3) Pointel JP, Petit B, Walrant P, Chicaud P, Drouin P, Debry G. [Automatic recording of venous hysteresis. Results in the normal subject and in varicose patients]. *J Mal Vasc* 1983;8:51-4.
- (4) Zachrisson H, Lindenberger M, Hallman D, Ekman M, Neider D, Lanne T. Diameter and compliance of the greater saphenous vein - effect of age and nitroglycerine. *Clin Physiol Funct Imaging* 2011;31:300-6.
- (5) Veye F, Mestre S, Berron N, Perez-Martin A, Triboulet J. Evaluation of lower limb vein biomechanical properties and the effects of compression stockings, with an instrumented ultrasound probe. *Conf Proc IEEE Eng Med Biol Soc* 2014;2014:74-7.
- (6) Journo HJ, Chanudet XA, Pannier BM, Laroque PL, London GM, Safar ME. Hysteresis of the venous pressure-volume relationship in the forearm of borderline hypertensive subjects. *Clin Sci (Lond)* 1992;82:329-34.
- (7) Willenberg T, Clemens R, Haegeli LM, Amann-Vesti B, Baumgartner I, Husmann M. The influence of abdominal pressure on lower extremity venous pressure and hemodynamics: a human in-vivo model simulating the effect of abdominal obesity. *Eur J Vasc Endovasc Surg* 2011;41:849-55.
- (8) van Rij AM, De Alwis CS, Jiang P, Christie RA, Hill GB, Dutton SJ, et al. Obesity and impaired venous function. *Eur J Vasc Endovasc Surg* 2008;35:739-44.
- (9) Alimi YS, Barthelemy P, Juhan C. Venous pump of the calf: a study of venous and muscular pressures. *J Vasc Surg* 1994;20:728-35.
- (10) Navarro TP, Delis KT, Ribeiro AP. Clinical and hemodynamic significance of the greater saphenous vein diameter in chronic venous insufficiency. *Arch Surg* 2002;137:1233-7.
- (11) Mendoza E, Blattler W, Amsler F. Great saphenous vein diameter at the saphenofemoral junction and proximal thigh as parameters of venous disease class. *Eur J Vasc Endovasc Surg* 2013;45:76-83.
- (12) Joh JH, Park HC. The cutoff value of saphenous vein diameter to predict reflux. *J Korean Surg Soc* 2013;85:169-74.
- (13) Konoeda H, Yamaki T, Hamahata A, Ochi M, Sakurai H. Quantification of superficial venous reflux by duplex ultrasound-role of reflux velocity in the assessment the clinical stage of chronic venous insufficiency. *Ann Vasc Dis* 2014;7:376-82.
- (14) Gibson K, Meissner M, Wright D. Great saphenous vein diameter does not correlate with worsening quality of life scores in patients with great saphenous vein incompetence. *J Vasc Surg* 2012;56:1634-41.
- (15) Lane TRA, Varatharajan L, Fiorentino F, Shepherd AC, Zimmo L, Gohel MS, et al. Truncal varicose vein diameter and patient-reported outcome measures. *Br J Surg* 2017;104:1648-55.
- (16) Eberhardt RT, Raffetto JD. Chronic venous insufficiency. *Circulation* 2014;130:333-46.
- (17) Caggiati A. Ultrasonography of Skin Changes in Legs with Chronic Venous Disease. *Eur J Vasc Endovasc Surg* 2016;52:534-42.

- (18) van der Velden SI, De Maesseneer MG, Fiebert O, Nijsten T, van den Bos RR. Postural Diameter Change of the Saphenous Trunk in Chronic Venous Disease. *Eur J Vasc Endovasc Surg* 2016;51:831-7.
- (19) Zamboni P, Portaluppi F, Marcellino MG, Manfredini R, Pisano L, Liboni A. Ultrasonographic assessment of ambulatory venous pressure in superficial venous incompetence. *J Vasc Surg* 1997;26:796-802.
- (20) Wang Z, Golob MJ, Chesler NC. Viscoelastic Properties of Cardiovascular Tissues. <https://www.intechopen.com/books/viscoelastic-and-viscoplastic-materials> 2016 [cited 2019 Jul 11];(7):141-163.
- (21) Li JK, Cui T, Drzewiecki GM. A nonlinear model of the arterial system incorporating a pressure-dependent compliance. *IEEE Trans Biomed Eng* 1990;37:673-8.
- (22) Nichols WW, O'Rourke MF, Vlachopoulos C. McDonald's Blood Flow in Arteries. Theoretical, experimental, and clinical principles. 6th ed. London: Hodder Arnold; 2011.
- (23) Armentano RL, Graf S, Barra JG, Velikovskiy G, Baglivo H, Sanchez R, et al. Carotid wall viscosity increase is related to intima-media thickening in hypertensive patients. *Hypertension* 1998;31:534-9.
- (24) Bia D, Aguirre I, Zocalo Y, Devera L, Cabrera FE, Armentano R. [Regional differences in viscosity, elasticity and wall buffering function in systemic arteries: pulse wave analysis of the arterial pressure-diameter relationship]. *Rev Esp Cardiol* 2005;58:167-74.
- (25) Tian L, Wang Z, Lakes RS, Chesler NC. Comparison of approaches to quantify arterial damping capacity from pressurization tests on mouse conduit arteries. *J Biomech Eng* 2013;135:54504.
- (26) Taniguchi R, Hosaka A, Miyahara T, Hoshina K, Okamoto H, Shigematsu K, et al. Viscoelastic Deterioration of the Carotid Artery Vascular Wall is a Possible Predictor of Coronary Artery Disease. *J Atheroscler Thromb* 2015;22:415-23.
- (27) Ghigo AR, Wang XF, Armentano R, Fullana JM, Lagree PY. Linear and Nonlinear Viscoelastic Arterial Wall Models: Application on Animals. *J Biomech Eng* 2017;139.
- (28) Xiao H, Tan I, Butlin M, Li D, Avolio AP. Arterial viscoelasticity: role in the dependency of pulse wave velocity on heart rate in conduit arteries. *Am J Physiol Heart Circ Physiol* 2017;312:H1185-H1194.
- (29) Zocalo Y, Bia D, Lluberas S, Armentano RL. Regional differences in veins wall viscosity, compliance, energetics and damping: analysis of the pressure-diameter relationship during cyclical overloads. *Biol Res* 2008;41:227-33.
- (30) Rossmann JS. Elastomechanical properties of bovine veins. *J Mech Behav Biomed Mater* 2010;3:210-5.
- (31) Mavrilas D, Tapikouni T, Mikroulis D, Bitzikas G, Didilis V, Tsakiridis K, et al. Dynamic mechanical properties of arterial and venous grafts used in coronary bypass surgery. 2 ed. 2002. p. 329-37.
- (32) Bia D, Zocalo Y, Pessana F, Armentano R, Perez H, Cabrera E, et al. [Viscoelastic and functional similarities between native femoral arteries and fresh or cryopreserved arterial and venous homografts]. *Rev Esp Cardiol* 2006;59:679-87.
- (33) Darjani M, Esteki A, Hassantash SA. Measuring and Modeling the Viscoelastic Properties of the Human Saphenous Vein Using the Pressure-Diameter Test. 16 ed. 2016. p. 27-35.
- (34) Wise ES, Hocking KM, Evans BC, Duvall CL, Cheung-Flynn J, Brophy CM. Unregulated saphenous vein graft distension decreases tissue viscoelasticity. *Perfusion* 2017;32:489-94.
- (35) Halliwill JR, Minson CT, Joyner MJ. Measurement of limb venous compliance in humans: technical considerations and physiological findings. *J Appl Physiol* 1999;87:1555-63.

- (36) Krasinski Z, Biskupski T, Dzieciuchowicz L, Kaczmarek E, Krasinska B, Stanszewski N, et al. The influence of elastic components of the venous wall on the biomechanical properties of different veins used for arterial reconstruction. *Eur J Vasc Endovasc Surg* 2010;40:224-9.
- (37) Vekilov DP, Grande-Allen KJ. Mechanical Properties of Diseased Veins. *Methodist Debaque Cardiovasc J* 2018;14:182-7.
- (38) Venturi M, Bonavina L, Annoni F, Colombo L, Butera C, Peracchia A, et al. Biochemical assay of collagen and elastin in the normal and varicose vein wall. *J Surg Res* 1996;60:245-8.
- (39) Wali MA, Dewan M, Eid RA. Histopathological changes in the wall of varicose veins. *Int Angiol* 2003;22:188-93.
- (40) Zamboni P, Tavoni V, Sisini F, Pedriali M, Rimondi E, Tessari M, et al. Venous compliance and clinical implications. 7 ed. 2018. p. 50-5.
- (41) Jeanneret C, Baldi T, Hailemariam S, Koella C, Gewaltig J, Biedermann BC. Selective loss of extracellular matrix proteins is linked to biophysical properties of varicose veins assessed by ultrasonography. *Br J Surg* 2007;94:449-56.
- (42) Olsen H, Lanne T. Reduced venous compliance in lower limbs of aging humans and its importance for capacitance function. *Am J Physiol* 1998;275:H878-H886.
- (43) Qiao T, Liu C, Ran F. The impact of gastrocnemius muscle cell changes in chronic venous insufficiency. *Eur J Vasc Endovasc Surg* 2005;30:430-6.

Supplemental Table I. Biometrics of the population sample.

	Controls (n=54)	C_{1S} (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]

Legend: age, body weight, height, body mass index (BMI), leg length, calf circumference, and ankle circumference of the examined lower limbs in normal controls and in patients with limbs in C_{1S}, C₃, and C₅ CEAP category of chronic venous disease. Values are reported as median [lower—upper quartile].

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

	Controls (n=54)	C_{1s} (n=21)	C₃ & C₅ (n=36)
Small Saphenous Vein cross-sectional area (mm²)			
Supine	2.9 [1.8—5.2]	4.0 [2.3—5.0]	4.9 [3.6—7.1]
Standing	3.8 [2.1—5.4]	4.7 [2.6—6.2]	7.07 [3.0—9.9]
Wilcoxon signed-rank test	p=.005	p=.047	p=.002
Deep Calf Vein cross-sectional area (mm²)			
Supine	8.7 [5.6—14.3]	8.6 [5.1—19.1]	12.7 [7.2—20.0]
Standing	10.7 [6.0—20.8]	8.2 [4.6—25.8]	14.6 [8.2—19.4]
Wilcoxon signed-rank test	p=.014	p=.500	p=.120

Legend: Cross-sectional area (in mm²) of the small saphenous vein and of the deep calf vein in normal controls, in patients in C_{1s}, and in patients with limbs in C₃ or C₅ CEAP category of chronic venous disease in the supine and in the standing position. Values are provided as median [lower-upper quartile]. p: p-value of the comparison between the supine and the standing position by Wilcoxon signed-rank test.

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

	Age	Body mass	Height	BMI
All subjects and patients (n=111)				
Supine				
SSV	0.277 (p=.003)	0.382 (p<.001)	0.101 (p=.300)	0.326 (p<.001)
DCV	0.305 (p=.001)	0.191 (p=.047)	0.140 (p=.150)	0.170 (p=.080)
Standing				
SSV	0.181 (p=.060)	0.412 (p<.001)	0.148 (p=.130)	0.357 (p<.001)
DCV	0.083 (p=.390)	0.080 (p=.410)	0.070 (p=.470)	0.057 (p=.560)
Normal controls (n=54)				
Supine				
SSV	0.336 (p=.010)	0.224 (p=.110)	-0.118 (p=.410)	0.232 (p=.100)
DCV	0.251 (p=.070)	0.134 (p=.340)	0.020 (p=.890)	0.126 (p=.380)
Standing				
SSV	0.248 (p=.070)	0.255 (p=.070)	-0.124 (p=.390)	0.322 (p=.020)
DCV	0.117 (p=.400)	0.130 (p=.350)	-0.001 (p=.990)	0.141 (p=.330)
C_{1s} patients (n=21)				
Supine				
SSV	0.292 (p=.200)	0.308 (p=.190)	0.161 (p=.500)	0.181 (p=.450)
DCV	0.305 (p=.180)	0.128 (p=.590)	0.310 (p=.180)	-0.063 (p=.790)
Standing				
SSV	0.513 (p=.017)	0.236 (p=.320)	0.365 (p=.110)	0.012 (p=.960)
DCV	-0.214 (p=.350)	-0.291 (p=.210)	-0.071 (p=.770)	-0.275 (p=.240)
C₃ & C₅ patients (n=36)				
Supine				
SSV	0.106 (p=.540)	0.514 (p=.001)	0.213 (p=.210)	0.407 (p=.010)
DCV	0.305 (p=.070)	0.055 (p=.750)	-0.021 (p=.900)	0.139 (p=.420)
Standing				
SSV	-0.054 (p=.750)	0.398 (p=.020)	0.248 (p=.150)	0.298 (p=.080)
DCV	0.235 (p=.170)	0.002 (p=.990)	0.227 (p=.180)	-0.017 (p=.920)

Legend: Spearman r and significance (p) of the cross-sectional area in the supine and in the standing position, of the small saphenous vein (SSV) and of the deep calf vein (DCV) correlation with age, weight, height, and body mass index (BMI), in the whole population sample, in normal controls, in patients with limbs in C_{1s}, and in patients with limbs in C₃ or C₅ CEAP category of chronic venous disease.