Venous intima-media thickness increases both in deep and superficial systems in patients with great saphenous vein reflux

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ABSTRACT

Objective: To evaluate by Doppler ultrasound (DUS) the venous intima-media thickness (vIMT) in patients with or without great saphenous vein (GSV) incompetence.

Methods: A prospective vIMT measurement was performed by DUS in an outpatient cohort. Patients were divided in two groups: group A, patients without GSV reflux; and group B, patients with at least one refluxing GSV. Group B was further divided in group B1, patients with monolateral refluxing GSV; and group B2, patients with bilateral GSV reflux. The vIMT was measured in the femoral vein (FV), 3 to 5 cm distal to the saphenofemoral junction (vIMT[FV]), and in the GSV, 3 to 5 cm from saphenofemoral junction (vIMT[R-] or vIMT[R+]) in the case of a nonrefluxing or a refluxing GSV, respectively. Only one limb per patient was considered for vIMT analysis: in group A, the limb with the greater vIMT(R-), in subgroup B1 the limb with a refluxing GSV, and in subgroup B2 the limb with the lower vIMT(R+). The primary outcome was the difference of vIMT of GSV between groups A and B. Secondary outcomes were differences in vIMT(FV) among groups and the correlation between vIMT of GSV and demographic or clinical parameters. A subgroup analysis of vIMT in GSV was conducted in B1 patients, describing vIMT variations in both limbs.

Results: Forty-four patients were enrolled. In the group A (26 patients), vIMT of the GSV was lower than in the group B (18 patients; 0.31 ± 0.01 mm vs 0.49 ± 0.02 mm; $P < .001$). The difference was significant also for vIMT(FV) (group A, 0.67 ± 0.02 mm vs group B, 0.77 ± 0.03 mm; $P < .014$). No statistical correlation between age, body mass index, family history, or use of elastic stockings and vIMT(FV) or vIMT(R+ or R-) was detected. Considering the whole population, vIMT of GSV was higher in patients with Clinical, Etiology, Anatomy and Pathophysiology (CEAP) class C of 2 or greater than in classes C 0 and 1 (0.43 ± 0.02 mm vs 0.32 ± 0.02 mm; $P < .0002$). The difference was significant also for vIMT(FV) in patients with class a class C of 2 or greater and C of 0 to 1 (0.77 ± 0.02 mm vs 0.64 ± 0.03 mm; $P < .0008$, respectively). In group B1, vIMT(R+) was higher than vIMT(R-) (0.50 ± 0.02 mm vs 0.32 ± 0.02 mm, respectively; $P < .0001$). The difference was not significant for vIMT(FV).

Conclusions: vIMT seems to be an indirect marker of saphenous insufficiency. In GSV incompetence, an augmented wall thickening is visible in the FV as well. Further studies are needed to assess the accuracy of DUS measurements of vIMT. Longitudinal studies are also needed to evaluate possible GSV and FV vIMT variations related to disease progression or treatment. (J Vasc Surg: Venous and Lym Dis 2019;■:1-7.)

Keywords: Venous insufficiency; Varicose veins; Color Doppler ultrasonography; Venous intima-media thickness; Vein wall

The etiology and pathophysiology of venous reflux and varicose veins (VVs) of the lower limbs include a series of macroscopic and microscopic mechanisms not yet fully clarified.[1,2] The vein wall plays a crucial role in the development of valvular dysfunction and venous hypertension; it is universally recognized as the main pathophysiologic mechanisms of chronic venous disease (CVD) progression.[3,4] Several studies described the genetic, physical, and biomechanical factors responsible of wall changes in VV disease.[5,6] These changes have been investigated histologically,[7] but very few data concerning vein wall modification in vivo are available.[8]

The aim of this article was to evaluate variations of the venous intima-media thickness (vIMT) in both superficial and deep venous systems in patients with or without great saphenous vein (GSV) reflux.

METHODS

We prospectively collected data from patients who underwent lower limb venous color Doppler ultrasound (DUS) scanning between January 1 and May 31, 2018, in an outpatient setting. Exclusion criteria were defined as age less than 18 and greater than 80 years; pregnancy or lactation; immobilization; active malignant disease; coagulation or blood cell disorders; obesity

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Author conflict of interest: none.

Presented at the vWINter International Meeting in Phlebology, Lymphology & Aesthetics, Cortina D'Ampezzo, Italy, January, 22-26, 2019.

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The editors and reviewers of this article have no relevant financial relationships to disclose per the Journal policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

2213-333X

https://doi.org/10.1016/j.jvsv.2019.07.005

(defined as a body mass index of >35); a history of deep venous thrombosis (DVT), superficial venous thrombosis, and/or pulmonary embolism; venous surgery or other lower limbs invasive treatments; previous limbs trauma; deep venous system incompetence; any kind of active or healed leg ulcers; incompetence of thigh GSV collaterals; and small saphenous vein incompetence. Exclusion criteria were restrictive to select a homogeneous population afflicted by isolated GSV disease.

The examination of each patient included four steps: (1) a medical history and clinical evaluation, (2) color DUS analysis for venous reflux assessment, (3) vIMT and vein calibers measurement, and (4) informed consent acquisition and group allocation.

Medical history and clinical evaluation. Primarily, clinical history, including a family history of CVD and the use of compressive stockings, was collected. A clinical evaluation was performed in an orthostatic position to evaluate for signs of CVD or other pathologies, such as active or previous superficial venous thrombosis and DVT, erysipelas, lipodermatosclerosis, and active or healed ulcers. The ankle-brachial index was calculated to exclude peripheral artery disease. The severity of CVD was graded by the C class of the Clinical, Etiology, Anatomy and Pathophysiology (CEAP) classification.[9] Referred venous symptoms were also recorded, according to the SYM Vein Consensus statement.[10]

DUS analysis for reflux assessment. The upright position was preferred to investigate both the superficial and deep venous systems. Reflux was investigated according to recommendations from the Society for Vascular Surgery[11] and Italian Society for Diagnostic Vascular Investigation[12] guidelines. In particular, a reflux of greater than 0.5 seconds was considered significant for GSV and a reflux of greater than 1 second for deep veins.

vIMT and vein calibers measurement. A Conformité Européenne (CE) and U.S. Food and Drug Administration mark approved for cardiovascular imaging ultrasound machine (MyLab Seven; Esaote S.p.A., Florence, Italy) was used for all the examinations, with a linear, multifrequency 3- to 13-mHz array transducer (SL1543). A dedicated setting for venous system was employed. DUS evaluation was performed by a single expert operator (S.O.), a specialist in venous surgery and instrumental diagnosis of venous disease.

The vIMT measurement protocol was similar to that adopted by Labropoulos et al.[8] The supine position was preferred to avoid patient movements and to stretch the inguinal region in case of overweight patients. The vIMT measurements were performed by positioning the probe parallel to the target vein segment to optimize the longitudinal view. The B mode and time gain compensation were set to make the vein lumen dark.

ARTICLE HIGHLIGHTS

- **Type of Research:** Single-center, case-control, retrospective analysis of prospectively collected data
- **Key Findings:** Venous intima-media thickness (vIMT) measured in great saphenous vein (GSV) and femoral vein was greater in 18 patients with isolated GSV incompetence than in 26 patients without GSV incompetence. Patients with unilateral GSV incompetence showed a significant higher value of vIMT in the limb affected by reflux.
- **Take Home Message:** vIMT seems to be an indirect marker of vein wall stress in both superficial and deep venous systems.

The posterior wall was preferred for measurement. Once obtained, high-quality images were frozen. Wall measurements were performed in zoom modality, placing the first caliber on the luminal margin, and the second caliber on the parietal (media-adventitial) wall boundary (Fig 1, *A-D*). The vIMT measurement did not include the adventitial stripe, often combined with the posterior flap of the saphenous fascia (Fig 1, *B* and *D*).

In all groups the vIMT was measured in the GSV 3 to 5 cm from the safenofemoral junction (SFJ). In case of a nonrefluxing GSV, the vIMT was defined as vIMT(R-). In case of a refluxing GSV, the vIMT was defined as vIMT(R+). The vIMT was also measured in a femoral vein (FV) segment 3 to 5 cm caudal to the SFJ. It was named as vIMT(FV). Vein calibers were measured using the transversal view, at the same level that the vIMT(R-), vIMT(R+), and vIMT(FV) were evaluated.

For both the vIMT and vein caliber, three measurements were obtained, and the average value was used for comparative analysis.

Study enrollment and group allocation. All patients signed an informed consent form. Patients were divided in two groups: group A, patients without GSV reflux; and group B, patients with at least one refluxing GSV. In group B a further division was made: group B1, patient with only one refluxing GSV; and group B2, patient with both GSV reflux. Only one limb for each patient was considered in the comparative vIMT analysis: in group A, the limb with the greater vIMT(R-), in subgroup B1, the refluxing GSV, and in subgroup B2, the limb with the lower vIMT(R+). No distinction was made between patients with or without visible VVs, but only between patients with or without GSV reflux at the groin. Fig 2 summarizes the study protocol and group allocation details.

Primary and secondary outcomes. The primary outcome was to describe differences of vIMT between groups A and B using the vIMT(R-) and vIMT(R+) measurements. Secondary outcomes were to evaluate differences between group A and group B in vIMT(FV)

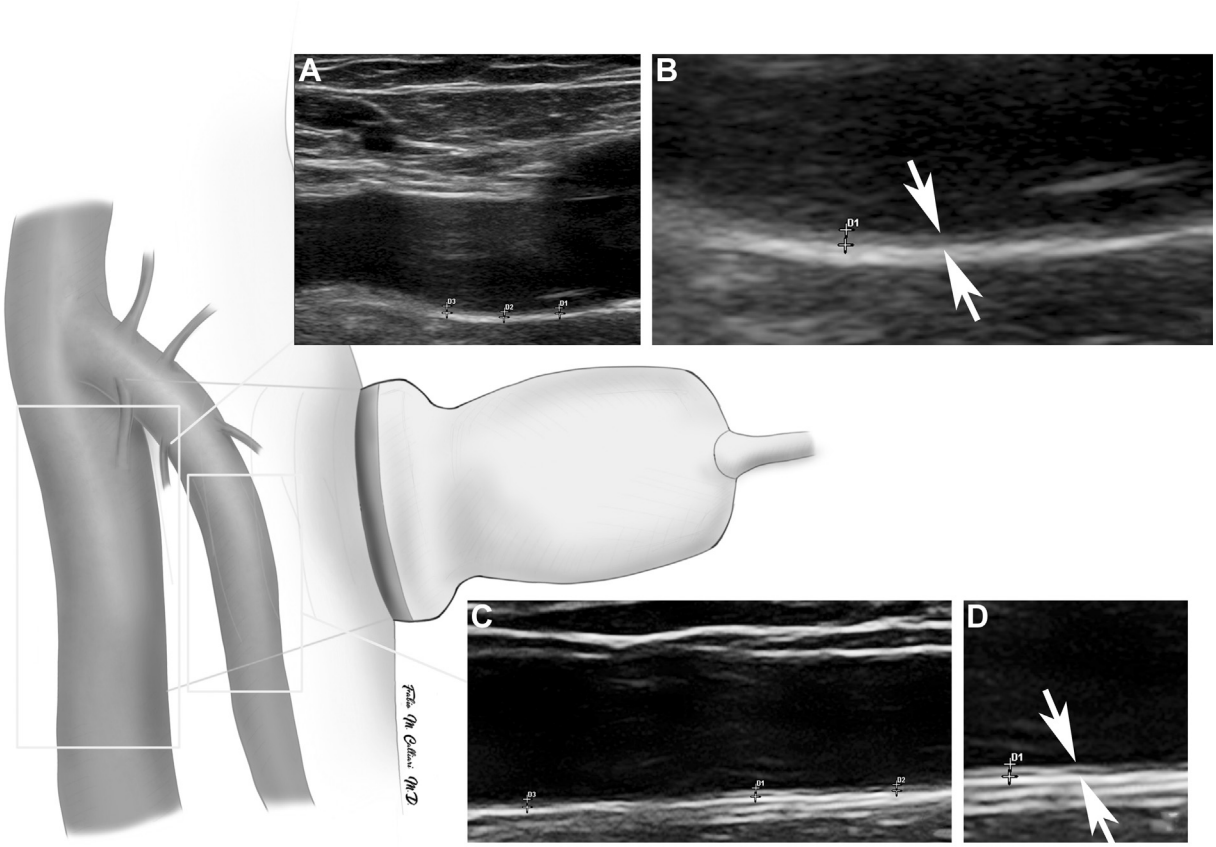

Fig 1. Venous intima-media thickness (vIMT) measurement of femoral vein (FV) **(A** and **B)** and great saphenous vein (GSV) **(C** and **D)**. The *white arrows* indicate where calipers should be positioned.

measurement, and between vIMT(R-) and vIMT(R+) in group B1. Finally, we evaluated the correlation between vIMT and demographic or clinical patients' characteristics (eg, age, CEAP C class, and family history).

Statistical analysis. Data analysis was performed using JMP 14.0.1 (SAS Institute Inc, Cary, NC). Continuous variables were presented in an average ± standard deviation form; nominal and cardinal variables were presented in a percentages form.

A two-tailed Student *t*-test was used to test statistically significant average vIMT measurement differences among groups. A *P* of .05 or less was considered statistically significant.

RESULTS

During the study period, 278 patients were screened through color DUS examination. Forty-four patients (15.8%) matched our inclusion criteria and were enrolled in the study. The mean patients age was 61 ± 12 years with a female to male ratio of 2.0 (28 vs 16). The Table describes the cohort characteristics. There were 26 patients (59.1%) in group A and 18 in group B (40.9%). Group B was further divided into subgroup B1, which included 14 patients (31.8%) and subgroup B2, which included 4 patients (9.1%). Fig 3 reports the main primary and secondary outcomes. In particular, the vIMT of the GSV was found to be thinner in group A compared with group B (0.31 ± 0.01 mm vs 0.49 ± 0.02 mm, respectively; $P < .001$; Fig 3, *A*). The difference was significant also for vIMT(FV) measurement (group A, 0.67 ± 0.02 mm vs group B, 0.77 ± 0.03 mm; $P < .014$; Fig 3, *B*). A univariate analysis revealed no statistical correlations between age, body mass index, family history, use of elastic stockings, or vIMT in the FV or GSV. Considering the whole population, vIMT(GSV) was higher in patients with a CEAP class C of 2 or greater than in patients with a class CEAP class C of 0 or 1 (0.43 ± 0.02 mm vs 0.32 ± 0.02 mm; $P < .0002$; Fig 3, *C*); results were significant also for difference in vIMT(FV) measurements (0.77 ± 0.02 mm vs 0.64 ± 0.03 mm; $P < .0008$ in patients with a CEAP class C of 2 or greater and with a class C of 0 or 1, respectively; Fig 3, *D*). In group B1 (patients with one refluxing and one nonrefluxing GSV), vIMT(R+) resulted significantly higher than vIMT(R-) (0.50 ± 0.02 mm vs 0.32 ± 0.02 mm, respectively; $P < .0001$). A small difference was detected for vIMT(FV) measurement (0.74 ± 0.04 mm vs 0.78 ± 0.04 mm in nonrefluxing and refluxing GSV limbs, respectively; $P = .535$) as well, but it did not reach statistical significance.

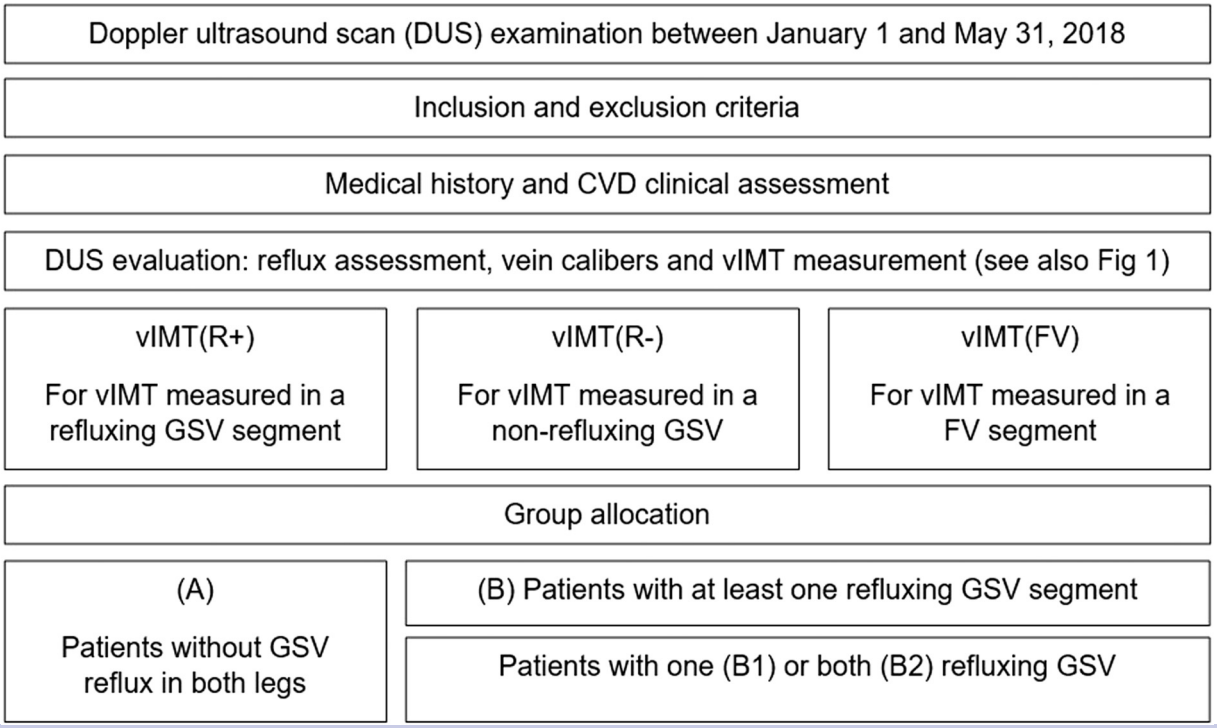

Fig 2. Summary of study protocol and group details. *CVD,* Chronic venous disease; *FV,* femoral vein; *GSV,* great saphenous vein; *(R+),* with reflux; *(R-),* without reflux; *VIMT,* venous intima-media thickness.

DISCUSSION

Venous wall modification has been recognized as a primary change in the development of venous disease and related to endothelial cell, fibroblast, and smooth muscle cell (SMC) dysfunction.[3,6] GSV wall thickening was macroscopically and microscopically described in VVs specimens[13] and senile subjects.[14] In particular, it consists of a marked increase of collagen and muscular fibers in all the three tunicae of the vessel. The subendothelial collagen fibers proliferate and the connective laminae

Table. Demographic, clinical, and ultrasound scan characteristics

Characteristic	Population	Group A	Group B	*P* value
Age, years	61.6 ± 12.0	63.7 ± 2.3	58.5 ± 2.8	.163
Sex, female	28 (63.6)	17 (65.3)	11 (61.1)	.772
BMI, kg/m^2	25.6 ± 4.3	25.5 ± 0.8	25.8 ± 1.0	.826
Height, cm	169 ± 13	166 ± 12	173 ± 9	.054
Family history	26 (59.1)	15 (57.7)	11 (61.1)	.820
Use of elastic stockings	7 (15.9)	3 (11.5)	4 (22.2)	.344
CEAP class C				
C0	14 (31.8)	13 (50.0)	1 (5.5)	
C1	6 (13.6)	5 (19.2)	1 (5.5)	
C2	21 (47.7)	7 (26.9)	14 (77.8)	.004
C3	2 (4.5)	1 (3.8)	1 (5.5)	
C4a	1 (2.3)	0 (0)	1 (5.5)	
Index limb GSV diameter, mm	3.8 ± 1.1	3.1 ± 0.2	4.9 ± 0.3	<.001
Contralateral limb GSV diameter, mm	2.9 ± 1.0	2.6 ± 0.2	3.4 ± 0.2	.014
Total	44 (100)	26 (59.9)	18 (40.1)	

BMI, Body mass index; *CEAP,* Clinical, Etiology, Anatomy and Pathophysiology; *GSV,* great saphenous vein.
Values are mean ± standard deviation or number (%).

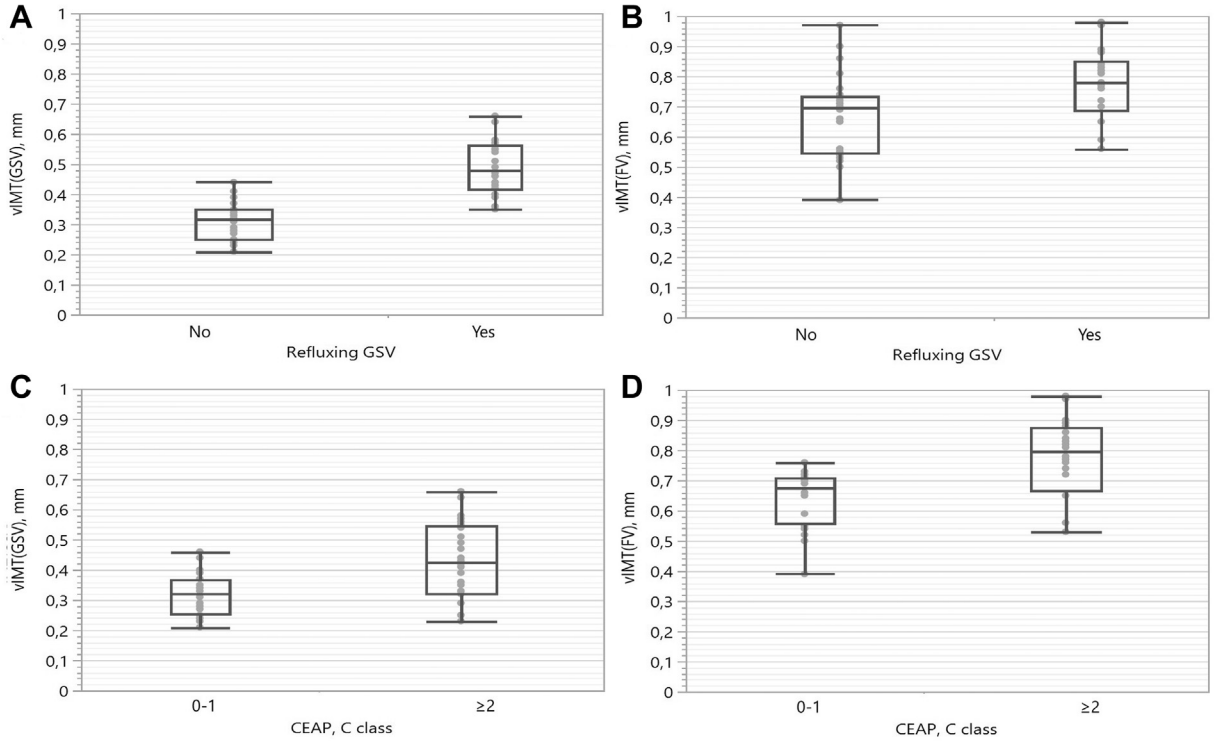

Fig 3. Main results derived from venous intima-media thickness (*vIMT*) measurements. A**,** Differences in vIMT of great saphenous vein (*GSV*) between patients without and with GSV incompetence ($P < .001$). B**,** Difference remained significant also for vIMT of competent femoral vein (*FV*; $P < .014$). vIMT demonstrated significant differences also between patients Clinical, Etiology, Anatomy and Pathophysiology (*CEAP*) C class 0 or 1 or ≥2, for vIMT of GSV ($P < .0002$) **(C)** and for vIMT of FV ($P < .0008$) **(D)**, irrespective of great saphenous vein (GSV) incompetence.

of the media thickens. Scattered longitudinal SMCs appear in the thickened intima and longitudinal clusters of SMCs in the adventitia.[3,7] Venous wall modifications during thrombotic events have already been investigated in ex vivo and in vivo models in several studies. Deatrick et al[15] demonstrated an increased vein wall thickness in both resolving and nonresolving DVT at 6 months, with a greater thickness in patients who had total resolution of the DVT rather than in patients who had persistent chronic thrombus. Chandrashekar et al[16] recently demonstrated by DUS vein wall remodeling in patients with acute DVT or postthrombotic syndrome. They found an increase in venous wall thickness in acute (mean, 0.63 mm) and postthrombotic (mean, 0.85 mm) venous segments, compared with controls (mean, 0.37 mm). In these studies, ipsilateral, contralateral, and unaffected control vein segments revealed no difference in terms of wall thickness, although ipsilateral segments were thicker than controls in patients with the postthrombotic syndrome, but not in patients with acute DVT.

Changes occurring in the wall of VVs and incompetent GSV were well-demonstrated in ex vivo models[7]; very few data from wall evaluation of in vivo models are available.

Labropoulos et al[8] first performed similar vIMT measurements and an analysis of healthy controls and patients with primary VVs (CEAP C class of 2 or 3). Measurements were obtained only in GSV in both the proximal thigh, at least 5 cm below the SFJ, and the distal third of the calf. They found a difference in vIMT at these two sites between healthy young controls (0.30 ± 0.03 mm), healthy old controls (0.40 ± 0.05 mm), nonrefluxing segments (0.45 ± 0.07 mm), and refluxing segments (0.58 ± 0.10 mm) in patients with GSV incompetence. A correlation with age was also found, with elderly patients demonstrating a greater vIMT than younger ones.

In this study, the vIMTs of the GSV and FV were measured by DUS examination in the same limb and at the same level. Furthermore, vIMTs of the FV and GSV were compared between legs with or without isolated GSV reflux and deep venous system competence. Patients with GSV reflux demonstrated not only a greater GSV thickness, but also a greater thickness of ipsilateral FV wall. Furthermore, patients with a CEAP C class of 2 or greater had a thicker GSV wall compared with patients with a CEAP C class of 0 or 1. Finally, a subgroup analysis performed in patients with only one refluxing

GSV confirmed that the refluxing GSV and the ipsilateral FV walls were thicker than in the contralateral limb.

In our cohort, only a CEAP C class of 2 or greater correlated with an increase in the vIMT. In contrast with the study from Labropoulos et al,[8] no correlation between aging and vIMT was demonstrated in this study. This difference is probably due to the fact that the mean age in groups A and B were quite similar (64 vs 58 years, respectively), unlike the groups in the study by Labropoulos et al (21 vs 64 years in young and old control group, respectively).[8]

The cause of wall thickening in both GSV and FV can only be hypothesized. It is known that the transduction of hemodynamic forces induced by venous hypertension may stimulate mechanosensors and mechanotransducers in the vein wall, involving intracellular pathways in endothelial, connective, and muscular cells.[17] A refluxing GSV is directly subject to increased hemodynamic and biomechanical stress, leading to pathologic quantitative and qualitative modifications in the wall. The hemodynamic overload of the FV related to GSV reflux probably triggers wall remodeling pathways also in the deep system. In other words, FV and GSV wall thickening revealed by DUS examination should not be the cause of VVs development, but the effects on the walls of the GSV and FV of direct and indirect hemodynamic overload owing to reflux. Indeed, Labropoulos et al[8] demonstrated a thinner wall in competent segments of the GSV if compared with the refluxing ones in the same vessel. Alternative explanation may be the diffusion of wall stimulating factors from the refluxing GSV and VVs to the deep veins.[18,19] This possible mechanism may explain the vIMT increasing of the FV in CEAP C class 0 or 1 limbs with a moderately dilated GSV.

The limitations of our study include the vIMT measurement protocol and data interpretation. Measurement of the vIMT can be difficult in both the GSV and FV, particularly in overweight patients. The vIMT must be calculated on a vein segment parallel to the probe, by focusing the posterior vein wall and excluding the hyperechoic stripe (the adventitial layer), often combined with the muscular fascia (Fig 1, *A-D*). Second, this study does not provide an interobserver or intraobserver analysis, as proposed by Labropoulos et al.[8] We aimed to minimize measurement related bias by some precautions: a shared protocol derived from the scientific literature, measurements performed by only one specialist with the same duplex machine, and an averaged vIMT from three separate measurements. Finally, all images were reviewed and discussed by all authors and repeated or excluded in case of discordance.

CONCLUSIONS

The vIMT seems to be an indirect but easily evaluable marker of hemodynamically relevant GSV insufficiency. An increase of the vIMT in the FV may demonstrate that also deep veins are sensible to the pathologic hemodynamic changes affecting the superficial system of varicose limbs.

Present findings encourage to improve venous wall thickness measurements and morphologic evaluations of both superficial and deep veins to possibly improve our knowledge of the hemodynamic changes in patients with venous insufficiency.

The authors would like to thank Fabio Calliari, MD, for article illustration, and Marco Baia, MD, for English revision.

AUTHOR CONTRIBUTIONS

Conception and design: DB, SO
Analysis and interpretation: DB, SO, DK, AC
Data collection: DB, SO
Writing the article: DB, AC
Critical revision of the article: DB, SO, DK, AC
Final approval of the article: DB, SO, DK, AC
Statistical analysis: DB
Obtained funding: Not applicable
Overall responsibility: DB

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Submitted Mar 13, 2019; accepted Jul 11, 2019.