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Inhibitory Effect of TIMP Influences the Morphology of Varicose Veins

B. Aravind [a,b], B. Saunders [a,b], T. Navin [a,b], A. Sandison [c], C. Monaco [a,b], E.M. Paleolog [a,b], A.H. Davies [a,*]

[a] *Department of Surgery and Cancer, Faculty of Medicine, Imperial College, London, UK*
[b] *Kennedy Institute of Rheumatology, Faculty of Medicine, Imperial College, London, UK*
[c] *Department of Histopathology, Charing Cross Hospital, London, UK*

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Abstract *Objectives:* Imbalance of matrix metalloproteinase enzymes (MMP) and their inhibitors (TIMPs) may contribute to the development of varicose veins. We hypothesised that, histological changes in varicose vein wall correlate with alterations in expression of MMP/TIMP.
Methods: Varicose veins ($n = 26$) were compared with great saphenous vein (GSV) segments ($n = 11$) from arterial bypass, and with arm and neck veins from fistula and carotid operations ($n = 13$). Varicose vein wall thickness was measured, enabling categorisation as atrophic and hypertrophic. MMP-2, MT1-MMP, TIMP-2, and TIMP-3 expression were quantitatively analysed by immunohistochemistry.
Results: There was significantly higher expression of TIMP-2 (immunopositive area 4.34% *versus* 0.26%), linked with connective tissue accumulation in the tunica media of varicose veins as compared with arm and neck vein controls. TIMP-2 and TIMP-3 expression was higher in hypertrophic than atrophic segments (3.2% *versus* 0.99% for TIMP-2, 1.7% *versus* 0.08% for TIMP-3). Similarly, TIMP-2 and TIMP-3 had elevated expression in the thicker proximal varicose vein segments compared to distal (4.3% *versus* 1.3% for TIMP-2 and 0.94% *versus* 0.41% for TIMP-3).
Conclusions: This study linked morphological changes in varicose vein walls with MMP/TIMP balance. A higher TIMP expression favours deposition of connective tissue and thus thicker vein wall, reducing matrix turnover by suppression of protease activity.

* Corresponding author at: Department of Vascular Surgery, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK. Tel.: +44 02087461234; fax: +44 02087467362.
E-mail address: a.h.davies@imperial.ac.uk (A.H. Davies).

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Introduction

Primary dilatation of the vein wall,[1] causing weakness and resultant separation of valve cusps leading to reflux, has gained wide acceptance as a cause of varicose vein. The primary basis for this theory, are the abnormalities found on the structural matrix components of the vein wall, such as collagen and elastin.[2–4] Remodelling of tissue is a normal dynamic process and abnormalities of the extracellular matrix, as above, suggest a defect in this process. It is already known that remodelling is a fine balance between proteases and its inhibitors, the most prominent of which are matrix metalloproteinase enzymes (MMP) and tissue inhibitors of MMP (TIMP).[5] An increase in the protease activity will promote matrix degradation, while an increase in its inhibitors would have the reverse effect. The resultant change in matrix components will also be reflected in morphology as it changes the basic structural elements. Thus, the observed morphology of varicose vein wall at any time of its development will be the result of such a matrix imbalance, and may be reflected in the expression of MMP and TIMP and *vice versa*. The latter assertion can be proved only by comparing the changes in proteases with that of morphology. In the current study, the alteration of MMP and TIMP are correlated to morphological feature of thickness of vein wall.

In this study, it was hypothesised that the observed alteration in varicose vein morphology was the result of imbalance in MMP/TIMP expression. Of the several MMP and TIMP, we analysed MMP-2, an important gelatinase with unique ability to degrade collagen and elastin[6] and MT1-MMP, which interacts with TIMP-2 in activation of MMP-2. TIMP-2 was analysed for the same reason. TIMP-3 was analysed considering its ability to inhibit all MMP and its unique anti-apoptotic activity.

Several MMPs and TIMPs have been analysed in published literature on varicose veins, with most considering GSV from bypass patients as control. However, these vein segments showed intimal and smooth muscle cell (SMC) hypertrophy, increase in intervening connective tissue, fragmentation of elastic tissue and changes of phlebosclerosis from old age.[7–12] Hence, in this study, arm and neck veins were considered as an alternative control for varicose veins, in addition to GSV from bypass grafts.

Materials and Methods

Tissue acquisition and processing

All patients were assessed and investigated in Charing Cross Hospital, London. Ethical approval was obtained from the Riverside Research Ethics Committee (RREC3092), London. The data collected from recruited subjects were anonymised and protected according to the Human Tissue Bill (2004).

A total of 26 patients with varicose veins underwent duplex ultrasound assessment to confirm the diagnosis of varicose veins, prior to "High tie and stripping of the GSV with multiple avulsions". Informed consent and clinical details were obtained, including history of deep vein thrombosis, diabetes mellitus, rheumatoid arthritis, connective tissue disorders and cancers. Drug history was obtained, to include statins, doxycycline and steroids. Patients with a history of DVT were excluded from the study, considering the risk of cellular infiltration on the vein wall from inflammation.[13] CEAP (Clinical, Etiologic, Anatomic, and Pathophysiology) classification system was used to standardize the assessment of severity of disease.[14]

Vein wall changes along the length of the varicose vein were compared by harvesting 2–3 cm long vein segments, in pairs, one from sapheno-femoral junction and the other from the thigh end of the stripped varicose vein segment, designated proximal and distal, respectively. To compare varicose veins, GSVs from primary arterial bypass operations were collected from the distal end of the harvested length of vein ($n = 11$). Neck veins from carotid endarterectomy (common facial vein ligated and excised for safe access to bifurcation of carotid artery) and arm veins from segments left after fistula formation for haemodialysis ($n = 13$), served as additional controls.

Special staining of vein segments

Vein segments preserved in 4% formalin were used for histological analysis and comparison with special stains namely, haematoxylin and eosin (H&E), Masson's Trichrome and Elastic Van Gieson (EVG).

Table 1 Demographic features of venous tissue: The average age of the arm and neck vein group was closer to those of varicose veins than GSV from bypass. More patients of the latter groups had co-morbidities than that of varicose veins.

Parameters measured	Varicose veins	GSV from bypass	Arm and neck veins
Number of patients	26	11	13
Age (range)*	51 (29–81)	76 (62–88)	63 (22–82)
Smokers	9	8	4
Coronary artery disease	0	3	3
Diabetes	0	3	2
Peripheral vascular disease	0	5	8
Hypertension	0	5	3
Statin	0	2	3
Aspirin	0	8	4

*$P < 0.01$.
Varicose vein versus GSV from bypass, by 1-way ANOVA with Bonferroni's Multiple Comparison Test.

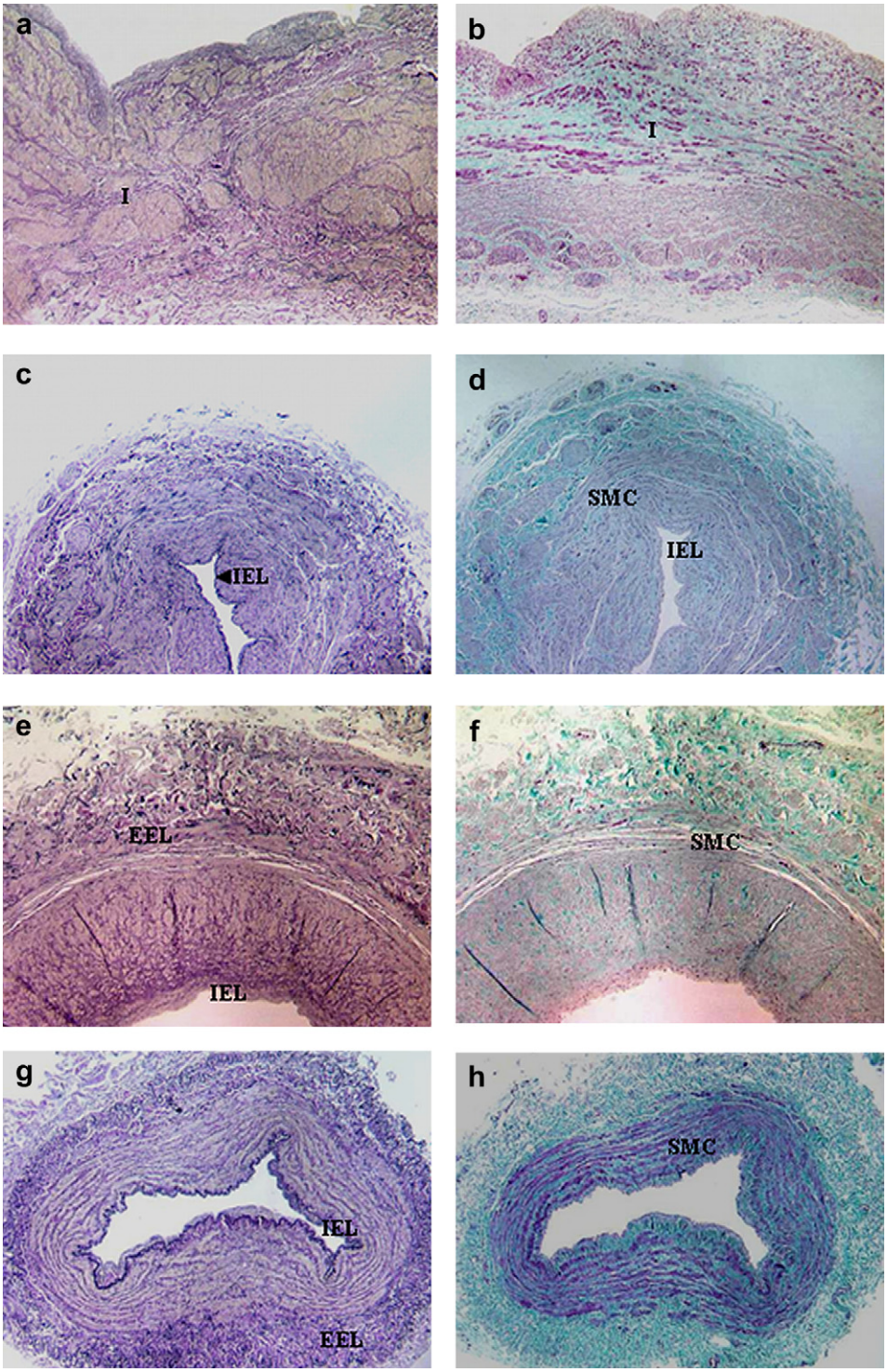


Figure 1 Varicose vein histology: comparison with GSV and basilic veins. (a—d) Representative EVG-stained (a, c) and Trichrome stained (b, d) varicose vein sections, showing connective tissue infiltration **(I)** in tunica media (a, b). Smooth muscle cell hypertrophy is seen as well-defined inner circular **(SMC)** and outer longitudinal bundles (d), with internal elastic lamina **(IEL)** in apposition to endothelium lining the lumen (c, d). (e, f) Bypass GSV, showing a well-defined circular smooth muscle cell **(SMC)** layer stained red with Trichrome (f) with scattered staining in tunica intima. EVG staining (e) demonstrates disrupted external elastic lamina **(EEL)** stained black. The well-defined internal elastic lamina **(IEL)** highlights the intimal hyperplasia (distance between luminal endothelium and **IEL**). (g, h) Basilic (arm) vein, with Trichrome stained slide (h), it was possible to clearly differentiate between connective tissue and smooth muscle cell **(SMC)** layer. EVG-stained section (g) shows well-demarcated external elastic lamina **(EEL)** and internal elastic lamina **(IEL)**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 2 Vein wall thicknesses: Measured as distance between intimal lining and external elastic lamina, this compared paired segments of proximal and distal varicose veins.

Vein type	Range of thickness (μm)	Median (μm)
Varicose veins	271—1841	755
Proximal varicose vein	406—1841	767*
Distal varicose vein	271—1588	574
GSV from bypass	274—1114	848**
Arm and neck veins	115—758	308

*$P = 0.07$, versus distal varicose vein segments.
**$P < 0.001$ versus varicose veins and $P < 0.01$ versus arm and neck veins.

H&E stained slides were used for screening of sections, and for cross-referencing with immunostained slides. EVG special stain showed the smooth muscle cell layer as red and elastin as black, helping delineation of elastic lamina on the vein wall aiding measurement of vein wall thickness. Masson's Trichrome stained connective tissue green, while the smooth muscle cell layer was stained red/purple, and was used in the visual assessment of changes in architecture.

Staining techniques used in this study were used and standardised in Charing Cross Hospital, Pathology Department, London. Tissue collected in formalin were set in paraffin blocks and cut into 4 μm sections. Routine H&E staining was performed and Masson's Trichrome with Chromazone Red (Surgipath Europe Limited) and Trichrome mixture (2 parts of 1% Chromazone Red in 1% acetic acid, 4 parts 1% phosphomolybdic acid and 6 parts 1% Light Green in 1% acetic acid). EVG staining was performed with Millers elastic stain followed by Van Gieson stain [saturated picric acid and 1% acid fuchsin (Surgipath Europe Limited)].

Vein wall thickness

Thickness of the vein wall was considered as the index of morphology as it was easily measured, and reproducible (see Fig. 2 a—b). It was measured as the distance between endothelium and external elastic lamina using AnalySIS software (Soft Imaging Software GmbH, Munster; Germany). The adventitia was excluded to reduce potential inconsistency resulting from dissection close to the vein wall at surgery. Intimal thickness was measured using the same principle, as the distance between endothelium and internal elastic lamina. To further correlate the extremes of thicknesses of varicose vein wall, they were sub-divided into atrophic, which was inside the 25th quartiles and hypertrophic, which was beyond the 75th quartile. Bland Altman analysis was performed for inter-observer variance (bias 19.9 SD 15.2 μm, $r^2 = 0.14$, P=NS).

Immunohistochemistry for MMP and TIMP and image analysis

Immunohistochemical staining was performed for MMP-2, MT1-MMP, TIMP-2, and TIMP-3 on paraffin sections. Non-specific antibody sites were blocked with normal horse serum (Serotec, Oxford, UK). Tissue sections were incubated with mouse monoclonal anti-human antibody recognising MMP-2, TIMP-2, MT1-MMP and TIMP-3 (Chemicon, Chandlers Ford, Hampshire, UK). Biotinylated horse anti-mouse antibody (Chemicon, Chandlers Ford, Hampshire, UK) followed by streptavidin biotin with HRP (horseradish peroxidase) was used, and sections were developed with DAB (3,3′-di aminobenzidine) and counterstained with haematoxylin.

Colour images were captured (at 4× magnification using a JVC KY-F55BE) using a video camera (Victor Company of Japan, Tokyo, Japan) connected to the microscope and projected to a computer screen (GATEWAY 2000, North Sioux City, USA). The immunopositive areas were sampled for red, blue, green, hue, saturation and intensity using AnalySIS software (Soft Imaging Software GmbH, Munster; Germany). Minimum and maximum values were further assigned by the software for each of the characteristics of the identified positive pixels. For uniformity, these measurements were repeated in 10 projections each on five different tissue immunostained sections. Bland Altman analysis was performed for inter-observer variability (bias −13.3 SD 8.9, $r^2 = 0.02$, P=NS).

For quantitative analysis of immunostained sections, the software binarises the immunopositive areas to grey scale, accounts for erosion and dilatation of pixels and provides the area of immunopositivity in the defined area of interest as a percentage. The mean of all such reading on a given section of vein was considered as % immunopositivity.

Statistical analysis

Data were analysed using the Graph Pad Prism software package (Graph Pad Software, CA, USA). For 2 groups of data, Wilcoxon rank test was used to compare non-parametrically distributed paired data, and paired-test for normally distributed paired data. Mann—Whitney test was used for comparison of normally distributed unpaired data. For 3 or more groups of data, one-way analysis of variance (ANOVA) with Bonferroni post-test for multiple comparisons was used to compare different groups of normally distributed data. For non-parametrically distributed data, Kruskal—Wallis ANOVA with Dunn's post-test for multiple comparisons was used. Correlations were assessed using Spearman's co-efficient for non-parametrically distributed data. Bland Altman analysis was performed to analyse inter-observer variation.

Results

Patient demographics

Patients with varicose veins were significantly younger than those who contributed GSV from bypass operations (Table 1), but similar to that of arm and neck vein group. There was no significant gender difference (by χ^2 test) between the groups (varicose vein 11 male, 15 female; GSV from bypass 7 male 4 female; arm and neck vein 10 male, 3 female).

Venous tissue harvested from non-varicose control groups was variably exposed to diabetes, statins, and aspirin unlike

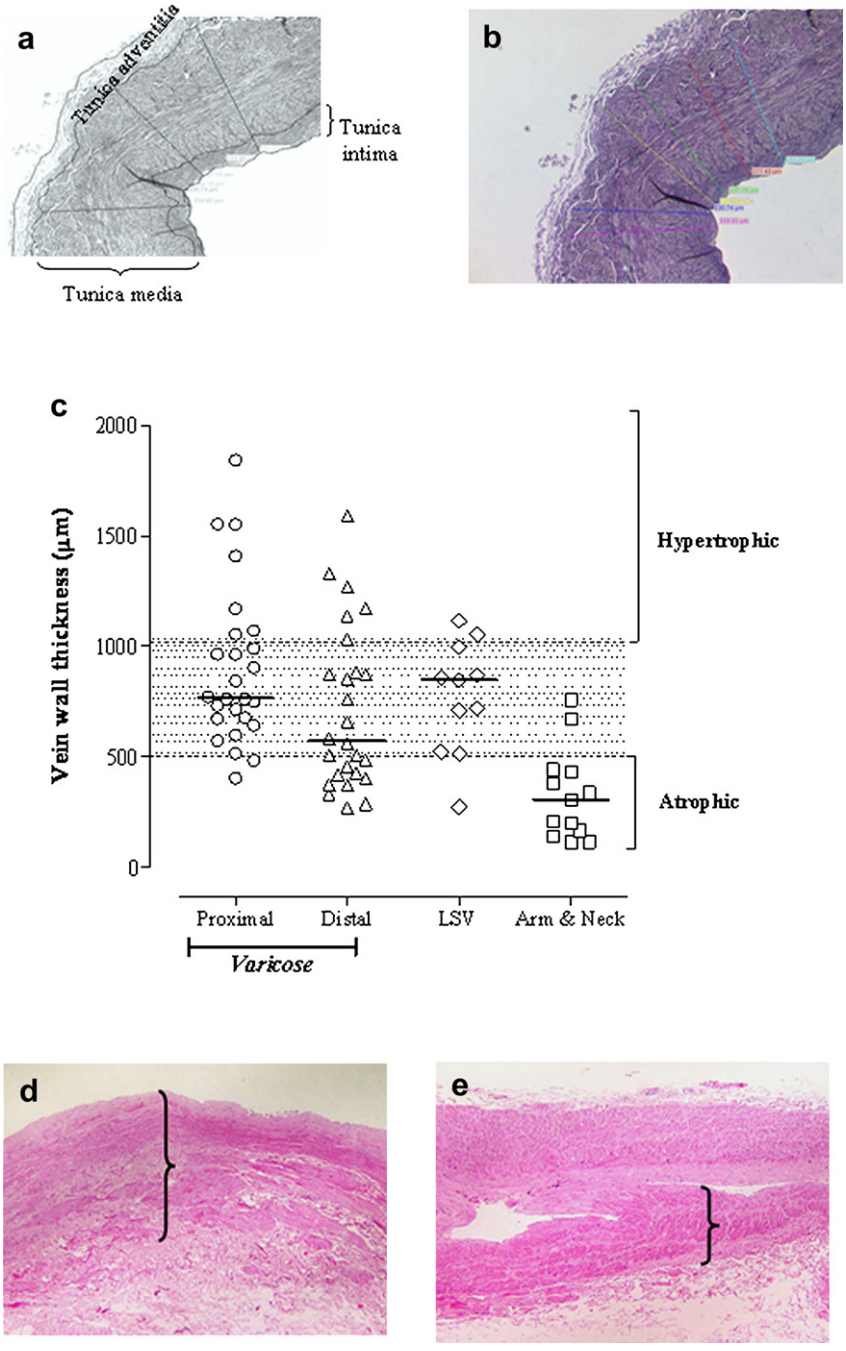


Figure 2 Measurement of vein wall thickness. Definitions of vein wall thickness and measurements. (a) The outer boundary for measurement of vein wall thickness was defined as external elastic lamina, which limits the tunica media. (b) The distance between the intimal endothelium and the external elastic lamina, which spans the tunica intima and tunica media, was measured as the vein wall thickness. An average of 6 measurements per section were obtained, and a mean was calculated. (c) Thickness (μm) of proximal and distal varicose veins ($n = 26$ paired segments), compared to bypass GSV ($n = 11$), and to arm and neck veins ($n = 13$). Veins were sub-divided further into atrophic (thickness <507 μm based on 25th percentile of varicose vein wall thickness) and hypertrophic (>1022 μm based on 75th percentile of varicose vein wall thickness). Shaded area shows region between 25th and 75th percentiles. (d—e) The variation in relative thickness in a representative pair of proximal (d) and distal (e) segments of varicose vein, shown in sections at same magnification, stained with haematoxylin/eosin.

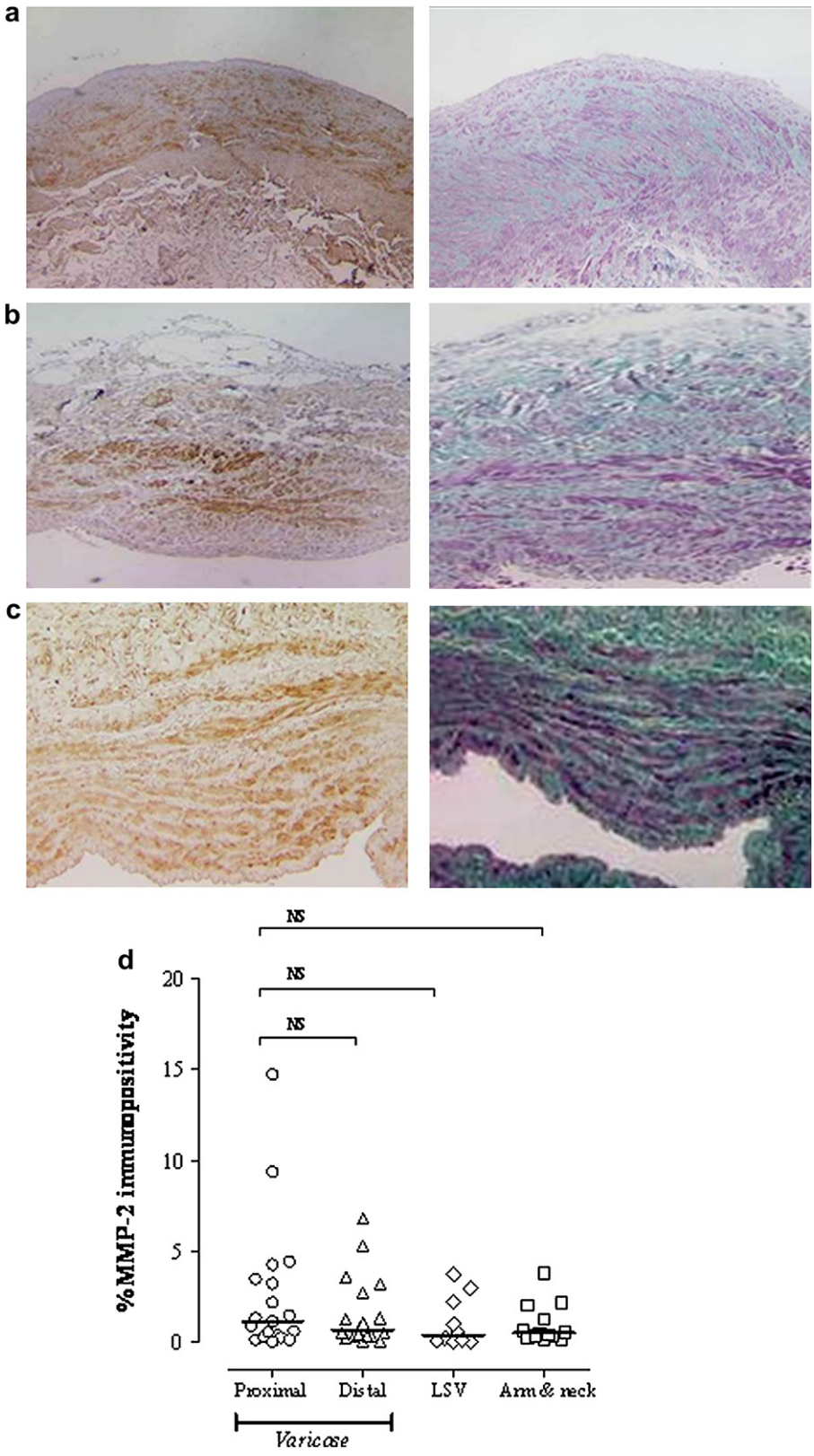


Figure 3 MMP-2 expression in varicose veins. Left panel: Proximal varicose vein segments (a) were stained for MMP-2, and compared with GSV segments from arterial bypass operations (b) and with arm veins (c). Right panel: Corresponding Trichrome stained slides are shown for defining smooth muscle (red/purple) and connective tissue (green). (d) Percentage immunopositivity of MMP-2 expression in proximal and distal varicose vein segments ($n = 19$), compared with GSV from bypass ($n = 10$), and neck and arm veins ($n = 13$). Data were analysed by 1-way ANOVA (Kruskal—Wallis) with Dunn's post-hoc test for multiple comparisons. Bars indicate median values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

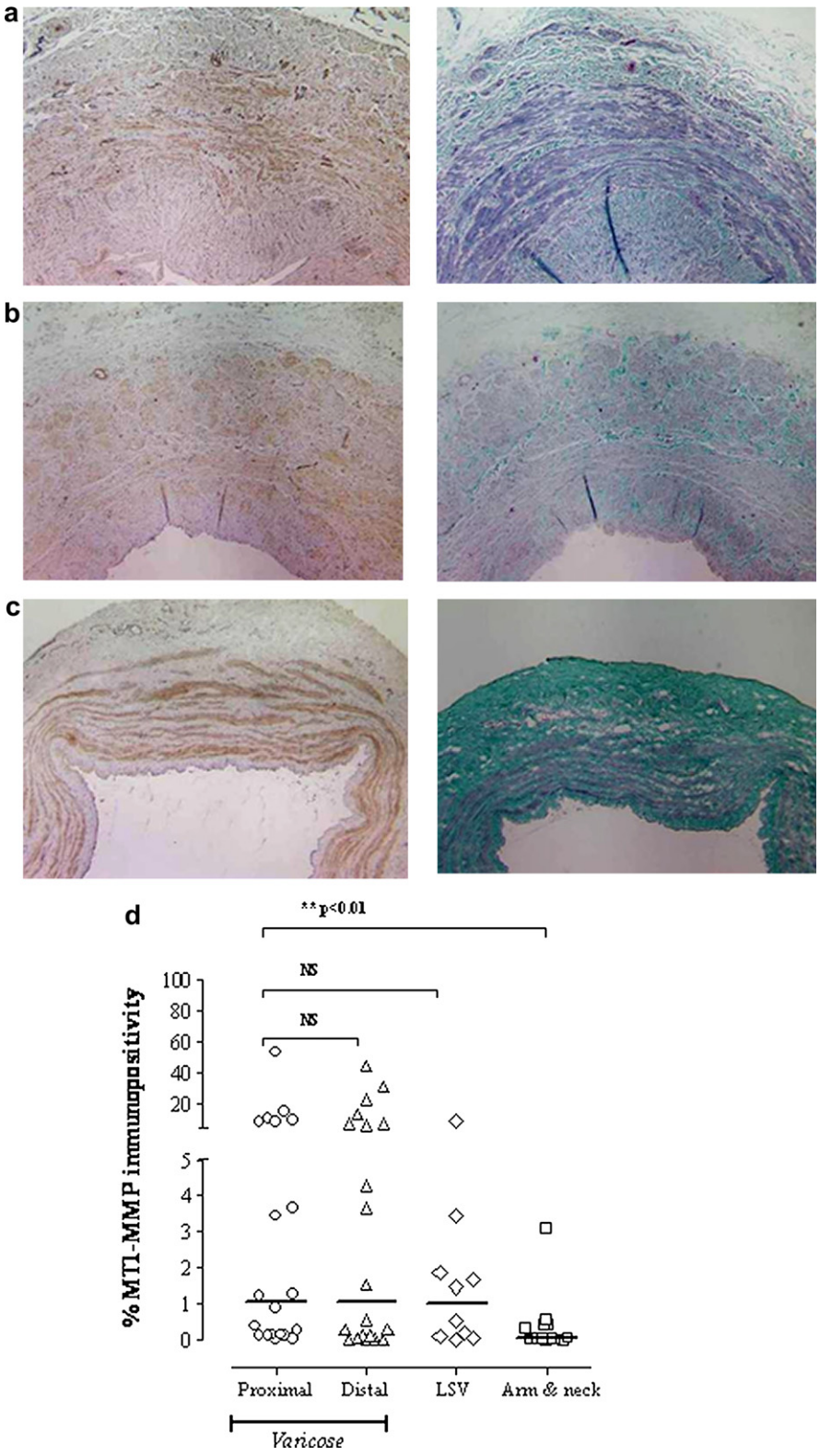


Figure 4 MT1-MMP expression in varicose veins. Left panel: Proximal varicose vein segments (a) were stained for MT1-MMP, and compared with arterial bypass GSV segments (b) and with arm veins (c). Right panel: Corresponding Trichrome stained slides are shown for defining smooth muscle (red/purple) and connective tissue (green). (d) Percentage immunopositivity of MT1-MMP expression in proximal and distal varicose vein segments ($n = 20$), compared with GSV from bypass ($n = 10$), and neck and arm veins ($n = 13$). Data were analysed by 1-way ANOVA (Kruskal—Wallis) with Dunn's post-hoc test for multiple comparisons. Bars indicate median values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

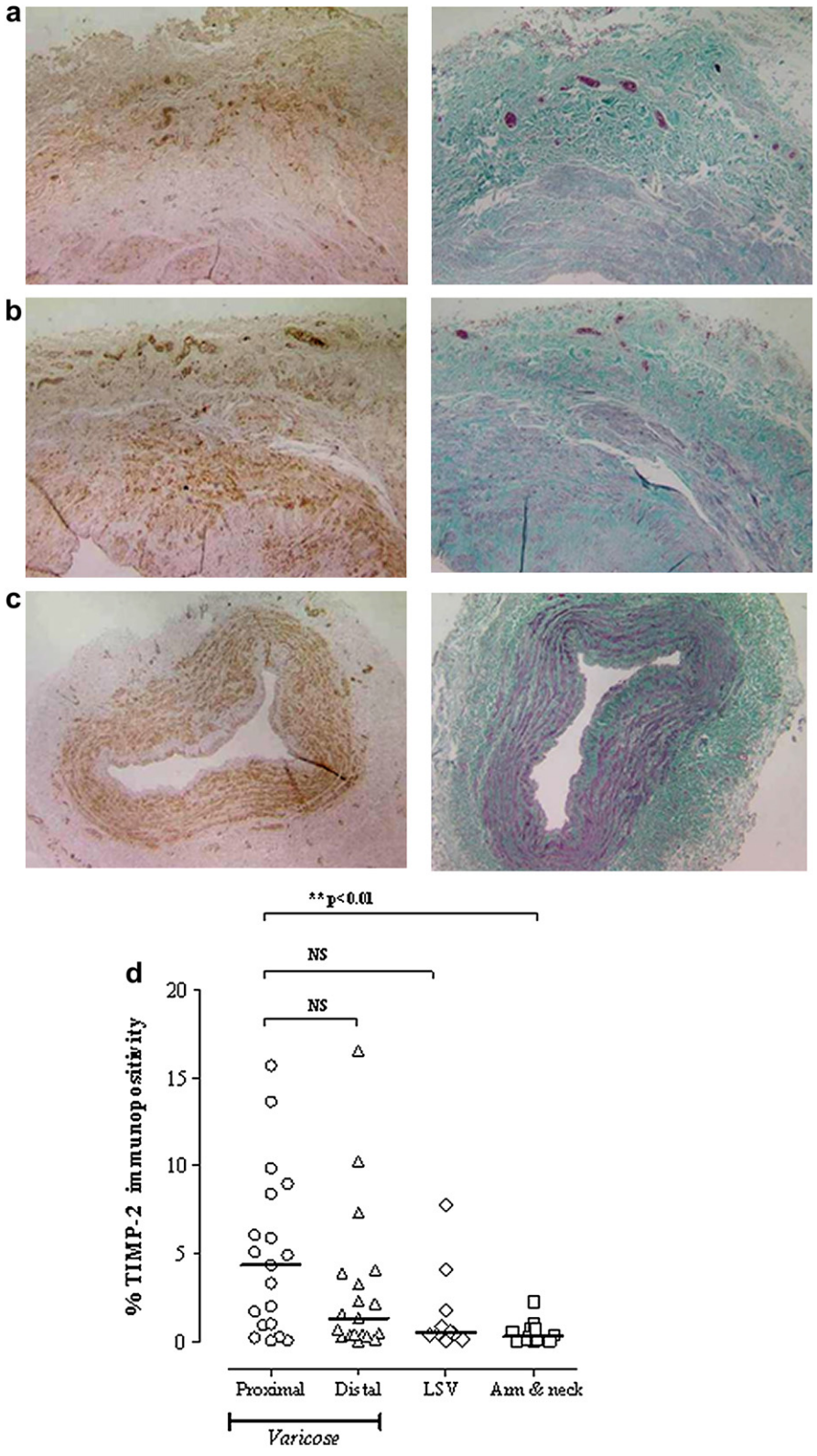


Figure 5 TIMP-2 expression in varicose veins. Left panel: Proximal varicose vein segments (a) were stained for TIMP-2, and compared with arterial bypass GSV segments (b) and with arm veins (c). Right panel: Corresponding Trichrome stained slides are shown for defining smooth muscle (red/purple) and connective tissue (green). (d)Percentage immunopositivity of TIMP-2 expression in proximal and distal varicose vein segments ($n = 19$), compared with GSV from bypass ($n = 10$), and neck and arm veins ($n = 13$). Data were analysed by 1-way ANOVA (Kruskal—Wallis) with Dunn's post-hoc test for multiple comparisons. Bars indicate median values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

those in varicose group (Table 1). When varicose veins were classified for clinical severity (C of CEAP scoring devised by Porter and Moneta), 82% belonged to C2 (simple varicose vein only), with the remainder classified as C4 (skin pigmentation) and C6 (venous ulcer).

Histology of varicose *versus* non-varicose veins

The most conspicuous and consistent changes were noted in tunica media of varicose veins. Histology sections of all 26 patients showed loss of architecture of this layer due to infiltration of connective tissue (green in Trichrome). Tunica intima showed hypertrophy due to similar infiltration of connective tissue in nearly half of the sections (12 of 26). In all the sections, there was extensive disruption of external elastic lamina, the outer limiting layer between tunica media and adventitia. The internal elastic lamina, however, was relatively well-preserved (Fig. 1 a—d).

The above changes in the varicose veins contrasted with those observed in GSV from bypass surgery, which showed predominant smooth muscle hypertrophy of tunica media (Fig. 1 e—f). The arm and neck veins, however, showed no such changes of tunica intima or tunica media with well-preserved elastic lamina and general architecture in all the sections (Fig. 1 g—h for arm and neck veins).

Measurement of vein wall thickness

GSV from bypass was thicker than both varicose and arm and neck vein segments (Table 2, Fig. 2c and e). Comparison made between paired proximal and distal varicose vein segments showed that the distal segments were thinner than proximal segments (Fig. 2 c—e and Table 2). Further, there were more atrophic segments (42%, thickness <507 μm based on 25th percentile of mean varicose vein wall thickness) among these distal varicose vein segments than in their proximal counterparts (Fig. 2c). The proximal segments, on the other hand, showed predominantly hypertrophic changes (27%, thickness >1022 μm based on 75th percentile).

There was no histological difference in the constitution of tunica media between distal and proximal or atrophic and hypertrophic varicose vein segments. There was infiltration of connective tissue in all these sections. The relative thickness of the tunica media is proportionately lower in distal and atrophic.

Intimal thickness was measured to find the contribution of tunica intima and media separately to the observed thickness of the vein wall, and was expressed as a percentage of total vein wall thickness. Tunica intima accounted for 5.6% of the total thickness of the proximal vein walls, while it was 7.6% for distal segments. Similarly, 4.3% of the wall thickness of GSV from bypass was its intima, while arm and neck vein, had no significant measurable contribution from the intima.

MMP and TIMP expression in varicose and non-varicose veins

Immunohistochemical staining of the vein wall showed predominant localisation of MMP-2, MT1-MMP, TIMP-2, and TIMP-3 in the smooth muscle of the tunica media of vein wall. MMP-2, MT1-MMP and TIMP-2 were, however, not identifiable in the intervening connective tissue of tunica media or the endothelium of the vein wall (Figs. 3—5 a—c). Vein segments also showed TIMP-2 immunopositivity on endothelium lining the blood vessels in tunica adventitia, but not those lining the lumen of the vein. TIMP-3, in addition to its presence in smooth muscle, was identified on the elastic tissue of the vein wall. This was confirmed by cross-referencing with EVG-stained corresponding sections where the elastic tissue was stained as black (Fig. 6a—c). There was no difference in the immunohistochemical staining pattern between the proximal or distal varicose veins or between different types of non-varicose vein sections.

Quantitative analysis showed a significantly higher expression of MT1-MMP and TIMP-2 in varicose veins than arm and neck vein controls (Figs. 4—6 d and; Table 3). A similar trend was observed for MMP-2, but was not statistically significant. TIMP-2 expression was significantly higher in varicose veins compared to neck and arm veins. Expression of TIMP-3 was comparable between all three groups.

The expression of the analysed MMP and TIMP were similar in proximal and distal varicose vein segments although there was a trend of higher expression of TIMP-2 (4.3% *versus* 1.3%) and TIMP-3 (0.94% *versus* 0.41%) in proximal segments.

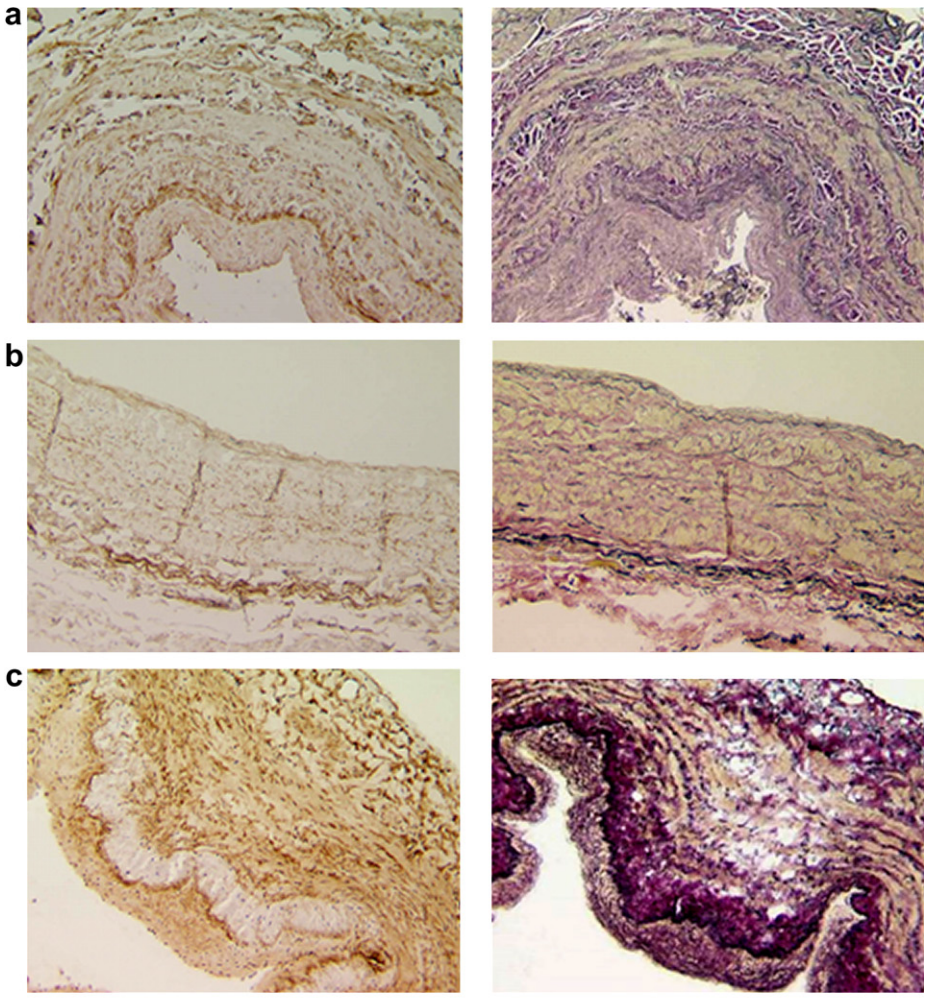
Comparison of expression between hypertrophic and atrophic varicose vein segments

Expression of the protease MT1-MMP was higher in the thinner, atrophic segments compared to the hypertrophic varicose vein segments. However, in hypertrophic segments, the expression of protease inhibitors TIMP-2 and TIMP-3 was higher than atrophic varicose vein segments (Table 4).

Discussion

The key histological finding in this study was infiltration of connective tissue in the tunica media of varicose veins. Most of the change was noted in tunica media, especially the smooth muscles, which also localised the analysed MMP and TIMP, emphasising their importance in the matrix turnover. Moreover, the split measurement suggested that the thickness of tunica media determine the overall morphology of the vein wall.

Quantitative analysis in previous studies have showed elevated amount of collagen while elastin was degraded and reduced on varicose vein wall.[15—17] The accumulation of connective tissue on varicose vein wall in our study could account for this elevated amount of collagen. TIMP-2 expression of varicose veins was found to be significantly higher than in arm and neck veins, with both TIMP-2 and TIMP-3 expression being higher than in GSV harvested from bypass. The elevated inhibitor will favour the accumulation of matrix components, like collagen, by suppressing the proteases. On the other hand, MT1-MMP, a protease, was significantly elevated in the varicose vein segments as well. However, it is possible that MT1-MMP, being a weak collagenase, with known ability to degrade collagen, fibronectin, laminin, fibrin and aggrecans, does not have a dominant role



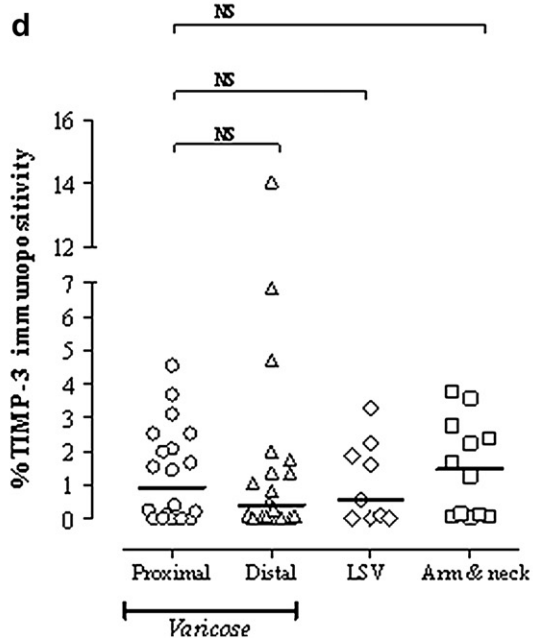


Figure 6 TIMP-3 expression in varicose veins. Left panel: Proximal varicose vein segments (a) were stained for TIMP-3, and compared with arterial bypass GSV segments (b) and with arm veins (c). Right panel: Corresponding EVG-stained slides are shown, to demonstrate expression of TIMP-3 predominantly in elastic tissue of internal and external elastic lamina. (d)Percentage immunopositivity of TIMP-3 expression in proximal and distal varicose vein segments ($n = 20$), compared with GSV from bypass ($n = 9$), and neck and arm veins ($n = 12$). Data were analysed by 1-way ANOVA (Kruskal—Wallis) with Dunn's post-hoc test for multiple comparisons. Bars indicate median.

Table 3 Quantitative analysis of MMP and TIMP of vein segments: The values were expressed as percentage immunopositivity, which is the average area in percentage showing uptake of antibody per analysed field.

MMP/TIMP[a]	Varicose Vein	GSV from bypass	Arm and Neck
	Median	Median	Median
	25—75 percentile	25—75 percentile	25—75 percentile
	95% CI	95% CI	95% CI
MMP-2	**1.14**	**0.37**	**0.50**
	0.27—3.46	0.03—2.57	0.23—1.64
	0.76 to 4.36	0.08 to 2.05	0.28 to 1.6
MT1-MMP	**1.05**[b]	**0.99**	**0.04**
	0.13—8.05	0.07—2.65	0.02—0.42
	2.71 to 10.36	0.12 to 3.76	0.12 to 0.89
TIMP-2	**4.34**[c]	**0.50**	**0.26**
	0.91—8.39	0.22—2.90	0.03—0.70
	2.60 to 7.10	0.13 to 3.38	0.08—0.83
TIMP-3	**0.94**	**0.57**	**1.48**
	0.05—2.31	0.03—2.05	0.10—2.58
	0.65 to 1.98	0.15 to 2.01	0.61 to 2.42

[a] All values as percentage immunopositivity.
[b] $P < 0.01$ versus MT1-MMP expression of arm and neck veins.
[c] $P < 0.01$ versus TIMP-2 expression of arm and neck veins.

in matrix turnover.[18,19] Its major role, on the other hand, is in the activation of a much powerful collagenase, MMP-2, by forming a ternary complex with TIMP-2.[20]

Measurement of vein wall thickness was performed to allow correlation of morphology with protease homeostasis. Previous studies proved that changes in structural proteins such as collagen and elastin were linked to vein wall thickness.[16,21,22] Thus a thinner vein wall could reflect a reduction in connective tissue content by a shift in the balance in matrix turnover to favour protease activity and *vice versa*. Distal varicose vein segments were overall thinner and showed dominant atrophic changes, as against proximal which were relatively thicker. This regional variation has been observed in previous studies which showed increased collagen, smooth muscle cells and elastin content at the sapheno-femoral junction end of varicose vein.[16] In this study, there was a three-fold increase in TIMP-2 and a two-fold increase in TIMP-3 expression in proximal segments. Thus, a reduced protease activity could explain the thicker proximal wall compared to that of distal varicose veins. In keeping with this trend, hypertrophic segments of varicose veins showed higher expression of TIMP-2 and TIMP-3, while MT1-MMP expression was elevated in atrophic segments.

TIMP-2 and TIMP-3, like others in their group, can inhibit all MMP, with the exception of TIMP-1, which is a poor inhibitor of several MT-MMPs.[23] TIMP-3 is unique in its ability to be sequestered in extracellular matrix, possibly

Table 4 Regional variation of MMP and TIMP on varicose vein wall: Varicose vein segments were paired for proximal and distal. Hypertrophic segments were those varicose vein segments with thickness more than 75th percentile and atrophic, less than the 25th percentile.

MMP/TIMP	Proximal varicose vein	Distal varicose vein	Hypertrophic segments	Atrophic segments
	Median	Median	Median	Median
	25—75 percentile	25—75 percentile	25—75 percentile	25—75 percentile
	95% CI	95% CI	95% CI	95% CI
MMP-2	**1.14**	**0.77**	**1.04**	**0.88**
	0.27—3.45	0.33—2.71	0.26—2.88	0.33—3.19
	0.76 to 4.36	0.61 to 2.45	0.07 to 5.35	0.37 to 2.75
MT1-MMP	**1.05**	**1.05**	**1.05**	**1.90**
	0.14—8.51	0.09—7.56	0.13—7.55	0.06—24.40
	0.21 to 11.53	1.52 to 12.87	0.04 to 11.67	4.24 to 28.28
TIMP-2	**4.34**	**1.29**	**3.24**	**0.99**
	0.91—8.39	0.36—3.89	0.27—6.05	0.38—3.67
	2.60 to 7.10	0.85 to 4.96	1.26 to 7.14	0.78 to7.19
TIMP-3	**0.94**	**0.41**	**1.66**	**0.08**
	0.05—2.31	0.06—1.56	0.15—3.83	0.03—1.21
	0.65 to 1.98	0.17 to3.35	0.68 to 3.55	0.10 to 1.27

explaining expression of TIMP-3 on elastic tissue on vein wall in this study. It is also unique in its ability to inhibit the ADAM and ADAMTS group of proteases. TIMP-2 has other phenotypic effects including promoting apoptosis and fibroblast growth, distinct from its ability to inhibit MMP.[24] Thus, these two TIMP are able to alter the morphology of vein wall by more than one mechanism. The dominant nature of inhibitors in the varicose vein wall was suggested previously in two studies, which showed an elevated TIMP-1 expression.[25,26] This inhibitor influence can cause accumulation of connective tissue by suppression of proteases. The current study gives further evidence to link these changes in MMP and TIMP to the morphology of varicose vein wall.

Besides being a cross sectional descriptive analysis, this study has the limitation that the immunohistochemical techniques employed detect both active and bound forms of these proteins. The enzyme activity or the active form of these proteases and its inhibitors should be further analysed to study aspects like the influence of high TIMP-2 expression on MMP-2 and its interaction with MT1-MMP.

In summary, this study was able to link the morphological changes of varicose vein wall to the alteration of MMP and TIMP. While we demonstrated a significant expression of TIMP-2 and MT1-MMP in varicose vein, the dominant influence of TIMP was reflected in histology by the accumulation of connective tissue in vein wall. There was correlation between vein wall thickness and expression of proteases and their inhibitors, proving the latter's role in moulding the morphology of the vein wall. Although the trigger for the changes in MMP and TIMP are not known, it appears that they could potentially work through elements in tunica media, especially the smooth muscles of this layer.

Conflict of Interest/Funding

None.

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