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Changes in the wall of the great saphenous vein at consecutive stages in patients suffering from chronic vein disease of the lower limbs

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The aim of the study was to show the changes in the great saphenous vein (GSV) wall at consecutive stages in the development of chronic vein disease (CVD) in patients qualified for a surgical procedure after physical examination and Doppler ultrasonography. Four groups of patients were formed (C2, C3, C4 and C5/6) according to clinical stage of the CEAP classification (C — clinical signs, E — aetiopathology, A — anatomy and P —pathophysiology). After the surgical procedure for removal of the varicose GSVs, 40 segments were harvested from their proximal parts near the saphenofemoral junction, 10 segments for each CEAP group. The veins were sectioned transversally and stained with the resorcin-fuchsin and AZAN method to visualise the elastic end collagen fibres. Afterwards the specimens were analysed under an optical microscope and photographed. As the GSV is an elastic vessel and its wall is divided into three zones, namely the internal layer (intima), the medial layer (media) and the external layer (adventitia), we found a proliferation of the connective tissue among the smooth muscle cells inside the internal and medial layers at consecutive stages of CVD. The later stages of CVD also revealed a larger number of the elastic and collagen fibres inside the intima and media and a looser arrangement of the smooth muscle cells of the media in the GSV wall.

Key words: great saphenous vein, chronic vein disease, smooth muscle cells, connective tissue, elastic and collagen fibres, CEAP classification

INTRODUCTION

The aim of the study was to describe the changes in the great saphenous vein (GSV) wall at consecutive stages of chronic vein disease.

Chronic vein disease (CVD) of the lower limbs is caused by valve insufficiency, weakness of the vein wall and therefore an increased inflow of blood into the superficial veins of the lower limbs. Prolonged venous hypertension leads to serious changes in the vein wall and if this lasts for a long time varicose veins can appear [2, 4]. Their treatment is based on a surgical approach that relies on the removal of the insufficient GSV, which drains into the femoral vein and forms the saphenofemoral junction located in the saphenous hiatus of the

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femoral triangle. The GSV wall is divided into three zones: the internal layer (intima), the medial layer (media) and the external layer (adventitia), with a thin layer of elastic fibres, termed the internal elastic lamina, between the intima and the media, and an external elastic lamina between the media and adventitia. There are pathological changes in the structure of the intima and media during CVD, including a decrease in the number of smooth muscle cells (SMCs), an increase in the amount of collagen fibres and obliteration of the three-layer morphology. This can cause weakness and a lack of elasticity in the venous wall and lead to the formation of varicose veins [2, 14, 17].

Some authors have also noted variety in the anatomy of the tributaries of GSV [5–7].

MATERIAL AND METHODS

A total of 94 patients suffering from chronic vein insufficiency were qualified for a surgical procedure using Babcock's method after physical examination and Doppler ultrasonography. The patients were divided into four groups (C2, C3, C4, C5/6) according to the corresponding clinical stages of the CEAP classification (which itself has four divisions, namely C: clinical, E: aetiopathological, A: anatomical, P: pathophysiological), which defines seven stages of CVD: C0 — no symptoms, C1 — teleangiectasias and spider veins, C2 — varicose veins, C3 — varicose veins with oedema, C4 — varicose veins, oedema and some skin changes such as lipodermatosclerosis, C5 — healed ulceration, C6 — active ulceration. During the operation the saphenous hiatus and the great saphenous vein entrance were exposed. We harvested 40 segments, each of 1 cm in length, from the proximal parts of the GSVs near their saphenofemoral junction. We obtained 10 segments for each group examined. A further 10 segments of the GSVs were taken from cadavers that were known from their case histories not to have suffered from CVD to form a control group. The GVS samples were fixed in 8% formaldehyde in saline. After the routine histological procedures, the blocks in paraffin were sectioned transversally at 6 μm and stained with the resorcin-fuchsin and AZAN method to visualise the elastic end collagen fibres. Afterwards, the specimens were analysed under a light microscope to compare the structure of the wall in each group and obtain photographic documentation (Fig. 1–4).

The qualitative method was used to describe the number of collagen fibres. One to three pluses were used for each of five views of one specimen of the GSV wall: one plus (+) when the number of the fibres was low, two pluses (++) when it was a medium and three pluses (+++) when the number was high. This method was employed as we had difficulty in calculating precisely the number of fibres for their destruction and fragmentation.

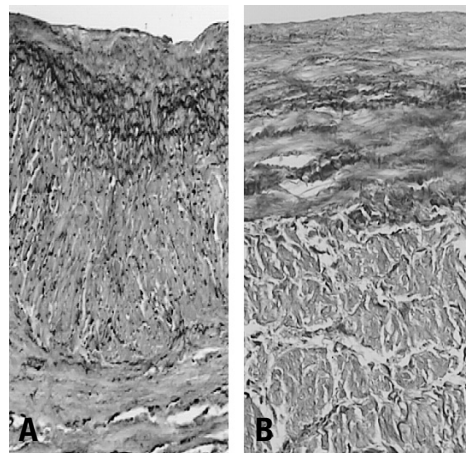

Figure 1. The wall of the great saphenous vein. **A.** Group C2 in the CEAP classification of CVD. Transverse section 6 μm. The elastic fibres of the intima and media stained with resorcin-fuchsin. Objective 10×. C2 20× RF. **B.** Group C2 in the CEAP classification of CVD. Transverse section 6 μm. The collagen fibres of the intima and media stained with AZAN. Objective 10×. C2 20× AZAN.

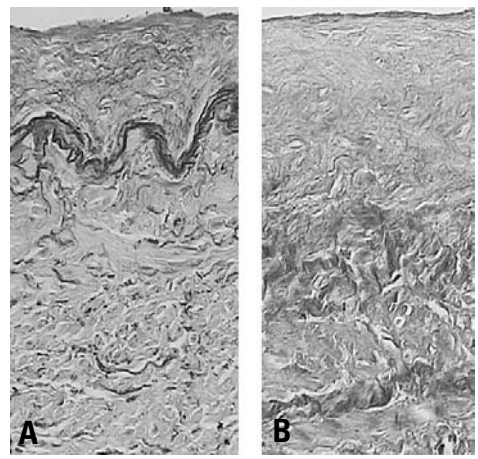

Figure 2. Group C3 in the CEAP classification of CVD. **A.** Transverse section 6 μm. The elastic fibres of the intima and media stained with resorcin-fuchsin. Objective 20×. C3 20× RF. **B.** Transverse section 6 μm. The collagen fibres of the intima and media stained with AZAN. Objective 20×. C3 20× AZAN.

Taking into consideration the arrangement of SMCs, we identified, using the same qualitative method, two types of SMC organisation: "dense" and "loose".

RESULTS

A large number of the collagen and elastic fibres were noticed inside the medial layer among the

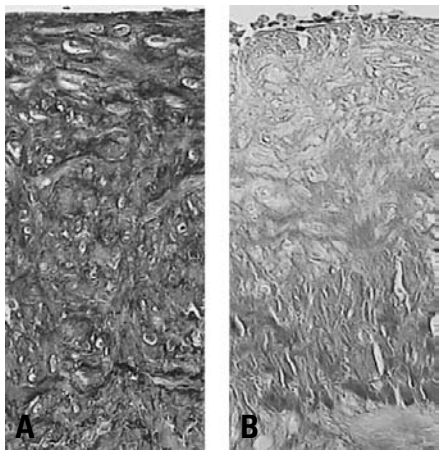

Figure 3. Group C4 in the CEAP classification of CVD. **A.** Transverse section 6 μm. The elastic fibres of the intima and media stained with resorcin-fuchsin. Objective 20×. C4 20× RF. **B.** Transverse section 6 μm. The collagen fibres of the intima and media stained with AZAN. Objective 20×. C4 20× AZAN.

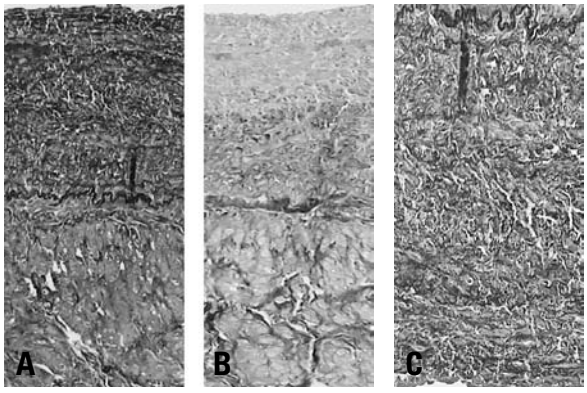

Figure 4. Group C5/6 in the CEAP classification of CVD. **A.** Transverse section 6 μm. The elastic fibres of the intima and media stained with resorcin-fuchsin. Objective 10×. C5/6 10× RF. **B.** Transverse section 6 μm. The collagen fibres of the intima and media stained with AZAN. Objective 10×. C5/6 10× AZAN. **C.** Transverse section 6 μm. The elastic fibres of the intima and media stained with resorcin-fuchsin. Objective 20×. C5/6 20× RF.

smooth muscle cells. The fibres were short and fragmented. Moreover, it was observed that there was an increase in the intima at later stages of CVD (C4 and C5/6) and that it was formed by connective tissue with collagen fibres, which formed strata (Fig. 2). The numbers of collagen fibres are presented in the Figure 5. We also found a much higher density of connective tissue components at stages C4 and C5/6 (Fig. 3, 4).

In some specimens destabilisation and fragmentation of the internal elastic lamina of the GSV wall were found (Fig. 2, 3).

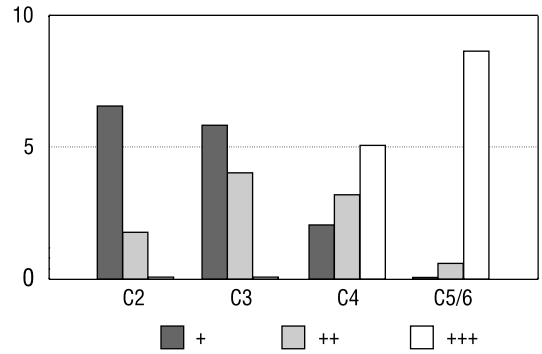

Figure 5. Correlation between the number of collagen fibres and the grade of chronic vein disease.

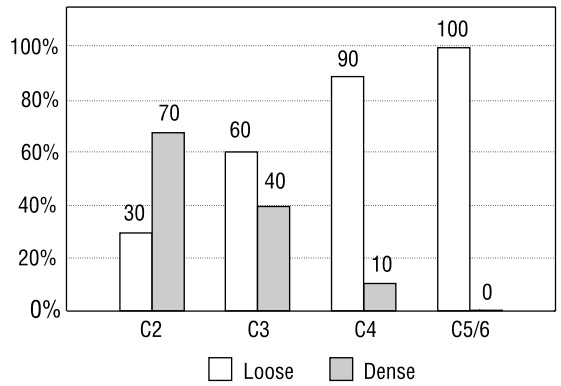

Figure 6. Correlation between the smooth muscle cells arrangement and the grade of chronic vein disease.

Proliferation of the connective tissue was also noticed between the smooth muscle cells (SMC) inside the internal and medial layers in the wall of the GSV. We thus identified two types of SMC arrangement, "dense" and "loose", a "dense" arrangement being when SMCs were attached to each other firmly, and a "loose" one when the SMCs were disconnected because of the hyperproliferation of connective tissue with collagen and elastic fibres. The loose arrangement of the SMCs inside the media occurred more often at the later stages (C4 and C5/6) of CVD, as shown in Figures 3, 4C and 6.

DISCUSSION

It was noticed that during the development of CVD there was considerable expansion of the connective tissue inside the medial and intimal layers of the GSV wall. In the last few years other authors have obtained similar results. In 2003 Wali and Eid [21, 22] observed that the number of SMCs decreased during CVD, in contrast to the widespread elastic

and, especially, collagen fibres, which increased in number during the course of the disease. They concluded that the loss of the elasticity, increasing the lumen of the GSV during CVD, was a cause of abnormalities [20]. In 1999 Jones et al. [8] obtained similar results to ours. Porto et al. [15, 16] also made similar observations in 2002. They stained the slides with resorcin-fuchsin and found that the increasing thickness of the media depended on the number of elastic and collagen fibres destroyed. In their opinion all such abnormalities could be a cause of wall stiffness in GSV. There was also a larger amount of connective tissue with elastic and collagen fibres among the SMCs at stages C4 and C5/6 of CVD in our material. In the course of CVD Bujan et al. [3] also noticed the rupture of the intimal elastic lamina and a blurring of the border between the media and the intima. Similar changes have been seen in other research [9, 10, 12, 13]. Kockx et al. [11] also suggested that the disruption of the elastic pattern can be triggered by hypertrophy of the SMCs. However, Andreotti et al. [1] in 1978 reported that the number of collagen and elastic fibres decreases during CVD. Bouissou and Maurel [2] in 1991 found that there was no change in the number of collagen fibres in the GSV wall and that this was not connected with age.

The most recent studies on the hyperproliferation of the connective tissue components refuted the previous hypothesis of Andreotti et al. and Bouissou et al. [15, 16, 18]. Urbanek et al. [19] considered the changes in GSV wall to be a kind of reaction to chronic venous hypertension in the venous vessels. Leu et al. [13] found that the changes in the intimal and medial layers of the vein wall played the main role in the pathogenesis of the disease and that valve insufficiency was secondary to wall stiffness. We therefore formed the hypothesis that early surgical treatment of CVD, as a procedure which would remove venous hypertension, could probably slow down the processes taking place in the vein wall.

CONCLUSIONS

1. A greater density of collagen fibres, a loose arrangement of the smooth muscle cells and proliferation of the connective tissue inside the intima and media of the great saphenous vein are characteristics of grades C4 and C5/6 in the CEAP classification.
2. Changes in the intimal and medial layer of the veins wall probably play the main role in the pathogenesis of chronic vein disease.
3. Early surgical treatment of chronic vein disease, as a procedure which would remove venous hypertension, could probably slow down the processes taking place in the wall of the great saphenous vein.

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REFERENCES

1. Andreotti L, Cammelli D, Banchi G, Guarnieri M, Serantoni C (1978) Collagen, elastin and sugar content in primary varicose veins. Ric Clin Lab, 8: 273–285.
2. Bouissou H, Maurel E (1991) Collagens of the internal saphenous vein, normal and varicose, as a function of age. Bull Acad Natl Med, 175: 603–606.
3. Bujan J, Gimeno MJ, Jimenez JA, Kielty CM, Mecham RP, Bellon JM (2003) Expression of elastic components in healthy and varicose veins. World J Surg, 27: 901–905.
4. Clarke GH, Vasdekis SN, Hobbs JT (1992) Venous wall function in the pathogenesis of varicose veins. Surgery, 111: 402–408.
5. Czarniawska-Grzesinska M, Bruska M (2003) The structure of the cusps of valves in the human foetal great saphenous vein. Folia Morphol, 62: 275–276.
6. Flisinski P, Szpinda M, Flisinski M (2004) Perforating veins of the shin in human foetuses. Folia Morphol, 63: 445–448.
7. Janowski K, Topol M (2004) Types of outlet of the major saphenous vein tributaries in patients with chronic vein insufficiency of the lower limbs. Folia Morphol, 63: 473–479.
8. Jones GT, Solomon C, Moaveni A, van Rij AM, Thomson IA, Galvin I (1999) Venous morphology predicts class of chronic venous insufficiency. Eur J Vasc Endovasc Surg, 18: 349–354.
9. Khan AA, Eid RA, Hamdi A (2000) Structural changes in the tunica intima of varicose veins: a histopathological and ultrastructural study. Pathology, 32: 253–257.
10. Kirsch D, Wahl W, Bottger T, Junginger T (2000) Primary varicose veins — changes in the venous wall and elastic behaviour. Chirurg, 71: 300–306.
11. Kockx MM, Knaapen MW, Bortier HE, Cromheeke KM, Boutherin-Falson O, Finet M (1998) Vascular remodeling in varicose veins. Angiology, 49: 871–877.
12. Lengyel I, Acsady G (1990) Histomorphological and pathobiochemical changes of varicose veins. A possible explanation of the development of varicosis. Acta Morphol Hung, 38: 259–67.
13. Leu HJ, Vogt M, Pfrunder H (1979) Morphological alterations of non-varicose and varicose veins. A morphological contribution to the discussion on the pathogenesis of varicose veins. Basic Res Cardiol, 74: 435–444.
14. Niebes P (1996) Vessel wall modification in venous pathology. Application to the study of phlebotonic drugs. Int Angiol, 15: 88–92.

15. Porto LC, Azizi MA, Pelajo-Machado M, Matos da SP, Lenzi HL (2002) Elastic fibers in saphenous varicose veins. Angiology, 53:131–40.
16. Porto LC, da Silveira PR, de Carvalho JJ, Panico MD (1995) Connective tissue accumulation in the muscle layer in normal and varicose saphenous veins. Angiol, 46: 243–249.
17. Sansilvestri-Morel S. (2002) Synthesis of collagen is dysregulated in cultured fibroblasts derived from skin of subjects with varicose veins as it is in venous smooth muscle cells. Circulation, 106: 479–483.
18. Travers JP, Brookes CE, Evans J, Baker DM, Kent C, Makin GS, Mayhew TM (1996) Assessment of wall structure and composition of varicose veins with reference to collagen, elastin and smooth muscle content. Eur J Vasc Endovasc Surg, 11: 230–237.
19. Urbanek T, Skop B, Wiaderkiewicz R, Wilczok T, Ziaja K, Lebda-Wyborny T, Pawlicki K (2004) Smooth muscle cell apoptosis in primary varicose veins. Eur J Vasc Endovasc Surg, 28: 600–611.
20. Wali MA, Dewan M, Eid RA (2003) Histopathological changes in the wall of varicose veins. Int Angiol, 22: 188–193.
21. Wali MA, Eid RA (2002) Changes of elastic and collagen fibers in varicose veins. Int Angiol, 21: 337––343.
22. Wali MA, Eid RA (2001) Smooth muscle changes in varicose veins: an ultrastructural study. J Smooth Muscle Res, 37: 123–135.