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Intimal fibrosis (phlebosclerosis) in the saphenous vein of the lower limb: a quantitative analysis

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Summary. The influence of pre-existing phlebosclerosis on the patency rate of aorto-coronary bypasses is uncertain. To examine this influence, extensive quantitative histological investigations of the intimal thickness of the left saphenous vein were made in 30 cases. In almost all veins the intima was thickened by collagen and elastic fibres as well as by fusiform cells which were assumed to be smooth muscle cells. The innermost layers also contained increased collagen adding to the intimal thickening. Three different methods to measure the intimal thickness were tested morphometrically: planimetric, a four-point method and a so-called method of estimate. The latter is the most time-saving and effective method. The average intimal thickness showed considerable deviations, but the intimal thickness in individual veins did not deviate greatly. As a rule, a specimen with an intimal thickness of less than 100 μm belonged to a vein with mild or moderate intimal thickening, but specimens with an average intimal thickness of 100–250 μm usually derived from a vein with moderate or pronounced intimal fibrosis. However, extreme values allowed a more precise statement to be made. A specimen with a non-sclerotic intima suggested at best a mild intimal fibrosis of the vein in the lower limb, whereas a specimen with marked intimal thickening derived from a vein with severe phlebosclerosis.

Key words: Saphenous vein – Histology – Coronary bypass

Introduction

The saphenous vein is used extensively for aorto-coronary bypass grafts. Numerous morphological investigations have been performed to determine the extent of changes in autologous bypass veins. Several investigations have focussed on pre-existing fibrous thickening or phlebosclerosis of the potential bypass veins (Leu et al. 1971, 1973, 1980; Milroy et al. 1989; Seydewitz and Staubesand 1990; Stolte 1977; Thiene et al. 1980; Waller and Roberts 1985) but few investigations have tried to elucidate the influence of phlebosclerosis on the patency rate systematically; a matter of controversy (Cheanvechai et al. 1975; Eschenbruch et al. 1974; Langes et al. 1984; Szilagy 1979). Usually, only a small segment from the transplanted vein is investigated morphologically and it is important to know if this segment is representative.

In the present study we compared three different methods to determine the severity of phlebosclerosis and its range in the saphenous vein and on the basis of these measurements we discuss whether one venous specimen is representative.

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Patients and methods

The non-varicose saphenous vein of the left lower limb of 16 men and 14 women (aged 55 ± 10 years) was investigated post mortem. Each vein was taken from the medial malleolus to the lower medial popliteal space. After fixation with 4% formaldehyde the vein was cut into three segments, each 7.5 cm long. The segments were completely divided in steps of 2–3 mm, resulting in a mean of 22 venous rings (Fig. 1). The distal segment was labelled I, the middle II and the proximal III. The specimens were embedded in paraffin and stained with haematoxylin and eosin or elastic van Gieson.

To evaluate the mean intimal thickness and to determine the differences within a single ring (circumference) or between several rings and segments, the following methods were used (Fig. 2):

In the *four-point method* a total of 132 rings of three randomly selected veins were investigated. With the help of an ocular micrometer the intimal thickness (the thickness of the fibrous inner layer) was measured at four points that were arranged in a rectangular pattern (Fig. 2). From these values the mean intimal thickness and the standard deviation of the arithmetic mean were calculated.

For *planimetry*, the intima of the same segments mentioned above was measured. The area of the lumen was subtracted from the area surrounded by the internal elastic lamina (Fig. 2). Assum-

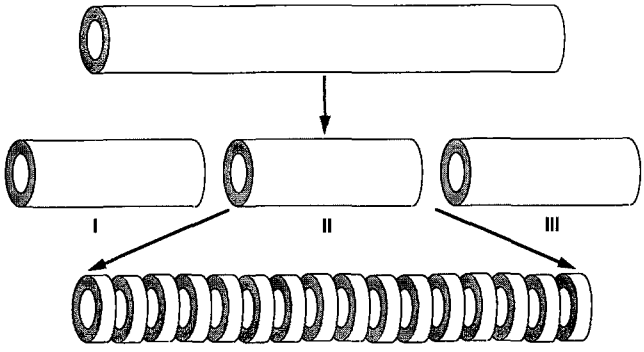

Fig. 1. Scheme of the distal (I), middle (II) and proximal (III) segment of the saphenous vein and specimens at a distance of 2–3 mm from each other

ing these areas to be circular, the radii of the two areas were calculated and subtracted from each other.

Since the pathologist normally receives only one specimen from the distal saphenous vein during surgery, the distal segments of a further 27 veins were investigated. From each segment the rings with running numbers 1, 6, 11, 16, 21 and 26 (distal to proximal) were evaluated using the same planimetric method. The measurements were performed with MOP-AMO3 (Kontron). In the estimates a total of 593 rings of segments I–III of ten randomly selected veins were evaluated microscopically at a magnification of $\times 25$. The intima (the fibrous thickened intimal layer) was measured at only one place, which was assumed to be the mean intimal thickness (Fig. 2). The mean and standard deviation of the mean were calculated. From the other 20 veins, 6 rings with the numbers mentioned above were investigated. Sometimes only 5 rings were taken due to the poor quality of the microscopic specimen.

Results

The values of the three veins were compared quantitatively, using the methods mentioned above. All suitable rings of the three segments were investigated (at least 26, maximum 68 rings). The mean values and standard deviation of the mean are given in Table 1. Figure 3 shows the values determined by planimetry.

The mean values of the segments obtained with the help of the three methods were close together (Table 1). A comparison of the nine groups of values (three segments of each vein) reveals the following mean deviations: estimate versus planimetry: 13.2%; planimetry versus four-point method: 14.3%; and estimate versus four-point method: 9.3%.

The standard deviations of the mean, calculated by the three methods, were not significantly different. The best concordance existed between the estimate and the four-point method. Moreover, in several rings we determined the difference of each specimen between the different methods. The values only showed minor deviations (mean deviation = 10%), possibly due to the experience of the investigator. The estimate is the quickest method and presents only minor deviations compared with the others and was therefore used in most cases.

The mean values of the different segments in the three veins, described above, were compared (Table 1). The fibrous thickening of the intima in the middle segment of two veins (no. 1 and 2) was half as pronounced as in the distal and proximal segments. This difference is statistically significant ($p < 0.01$) and reveals large deviations in the intimal thickness. Nevertheless, systematic deviation depending on localization were not established in this rather limited cohort. The evident deviation of the intimal thickness is also expressed by the single values established by the four-point method. These values are depicted in Fig. 3.

Figures 4 and 5 show all values obtained by estimate and by planimetry. The order of the veins depends on their median value. The findings were very similar when

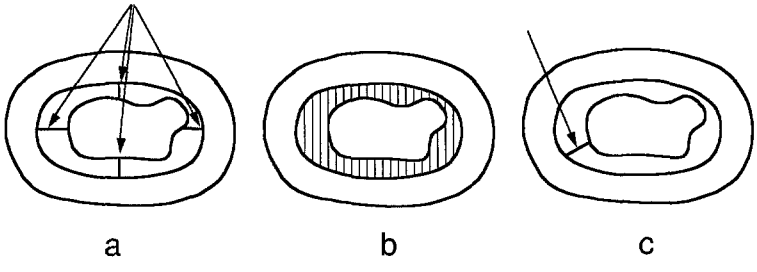

Fig. 2. Methods to determine the mean intimal thickness: **a** four-point method, **b** planimetry, **c** estimate

Table 1. Mean intimal thickness (μm) in the distal (I), middle (II) and proximal (III) segment of three veins by means of different methods

Vein no	No. of specimens	Segment			Method
		I	II	III	
1	37	143±46	56±14	110±51	Estimate
	35	106±14	66±11	112±38	Planimetry
	37	133±19	74±14	129±47	4-Point method
2	26	114±37	51±11	108±65	Estimate
	26	140±33	65±7	120±57	Planimetry
	26	98±30	51±10	100±38	4-Point method
3	69	154±48	290±83	269±59	Estimate
	68	176±61	264±64	262±43	Planimetry
	69	173±38	289±36	257±36	4-Point method

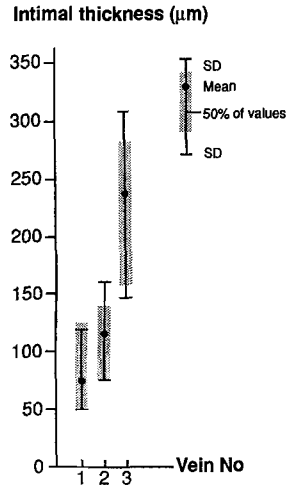

Fig. 3. Values of three veins by means of the four-point method

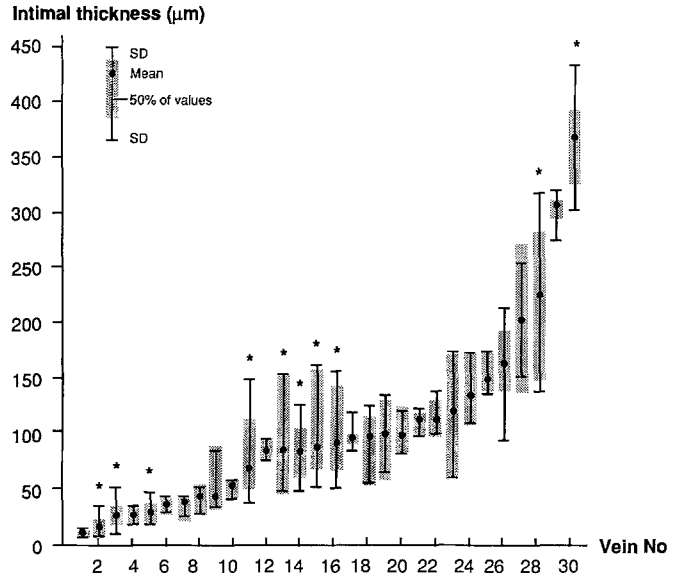

Fig. 4. Values of 30 veins by means of planimetry. *, Veins with 25 or more specimens investigated

using both methods, but the values measured by estimate were slightly lower than those obtained by planimetry. In the two graphs the same veins do not always take the same place; on average they are shifted by two places which is not surprising in the view of the minor differences in the means of adjacent veins. Because the values in the two figures were similar, we examined them together. We did not discriminate between sexes because no significant differences were observed. All 30 veins presented marked differences concerning the extent of intimal fibrosis (Fig. 4), ranging from minimal to severe intimal thickening. The deviation was also marked when many values were determined. Veins with 25 or more specimens are identified by a cross in Fig. 4. From the others only 5 to 6 specimens were investigated. This explains why extreme values were sometimes near the 50% limit.

Clear deviation of values does not allow a precise separation into groups but there were extreme values.

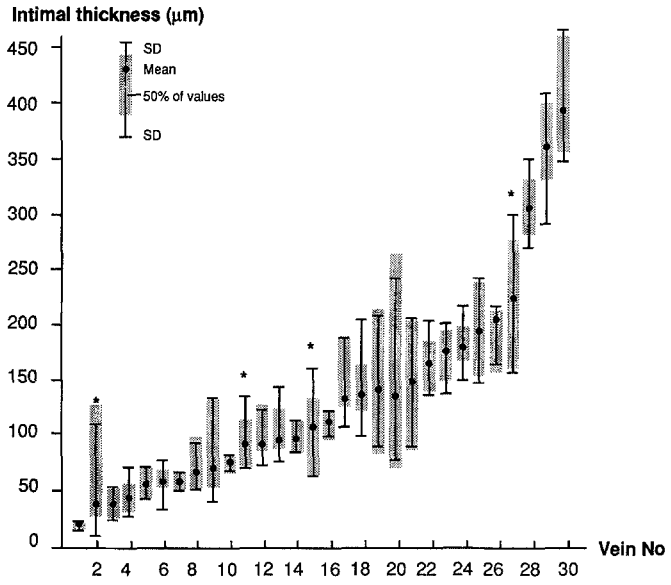

Fig. 5. Values of the same 30 veins as in Fig. 5 by means of planimetry. *, Veins with 25 or more specimens investigated

The values of the two most severe phlebosclerotic veins (corresponding to the last two values in each figure) and those with only minor phlobosclerosis (in the first 10 respectively 16 veins) do not overlap. Very minor intimal fibrosis (thickness up to 25 µm by estimate and 50 µm by planimetry) in one ring is found only in veins with very little intimal fibrosis. Very thick pads (with a mean intimal thickness of >0.3 mm) occur in veins with severe phlebosclerosis; we did not observe a range of very little to severe intimal thicknesses in any one vein.

Discussion

In adults, a smooth intima in the saphenous vein, with a narrow intima, is rarely found. The internal elastic lamina of the saphenous vein is smaller and more irregular than that of an artery of muscular type. Phlebosclerosis of the saphenous vein is prevalent in about 83% of veins at all ages (Vogt 1978), but the extent of the changes varies markedly (Leu 1980). Intimal thickening may be regionally pronounced, resembling a pad (Thiene et al. 1980), the main part of which consists of collagen with varying numbers of slender elastic fibres and fusiform cells possibly of smooth muscle origin occurring as solitary cells or as small groups of cells. The pads are often separated from the internal elastic lamina at their base and the lamina is full of gaps is split or is not clearly identifiable at this site.

Occasionally, the main part of the pad is situated between two layers of the internal elastic lamina. The increase in collagen is most often found in the medial component and a separation outwards by the internal elastic lamina is missing. Smooth muscle cells run longitudinally with a circular arrangement of muscle fibres in the outer part of the media. Our measurements in-

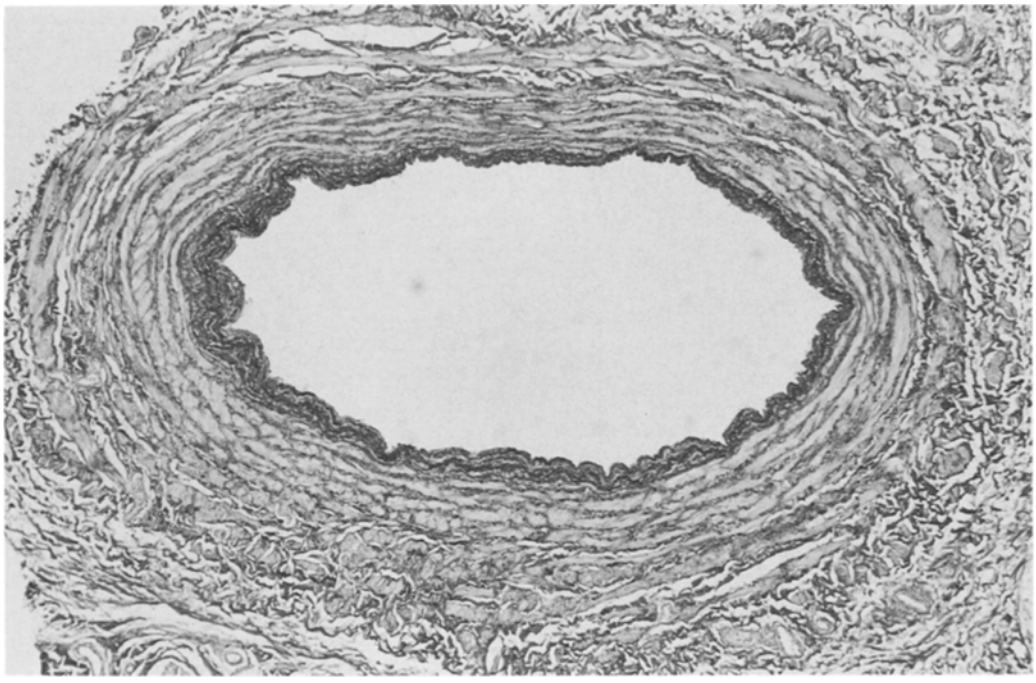

Fig. 6. Saphenous vein with minimal thickening and peg-shaped folding of the intima. Elastic van Gieson, ×10

clude the fibrous thickening of the media, because fibrosis had destroyed the clear separation of intima and media. In these cases the term "fibrous thickening of the intimal layer" is preferred to "intimal fibrosis". To simplify matters, the fibrous thickening of the inner intima plus inner media is called intimal fibrosis or phlebosclerosis. Very rarely, in other vascular regions, phlebosclerotic plaques may contain lipid deposits or sclerosis, but we did not observe these severe changes in this investigation or in numerous intraoperative specimens.

The origin of phlebosclerosis is undefined. Correlation of morphological changes with age is not as strong as it is in atherosclerosis but an increasing prevalence is seen in the elderly (Leu 1991). With age the thickness of the entire venous wall and the intimal layer increases; the amount of collagen is uniform in all layers but increase of longitudinal muscle layers and decrease of circular muscle layers occurs. Post-mortem folding with a peg-shaped configuration of the intimal layer (Fig. 6) is due to contraction of the circular muscular layer; the folding declines after the 19th h post mortem (Neumann 1937) and the lumen increases with time. Taking this into account the specimens for our investigation were prepared between the 18th and 36th h post mortem. We did not fulfil the request of Fischer (1967) that morphological or planimetric investigations should only be performed on inflated veins to reflect the wall/lumen ratio more realistically. In our study we investigated the veins as intra-operative specimens: that is to say the veins were collapsed and the diameters were therefore smaller than in situ.

The fact that phlebosclerosis is a common phenomenon, especially in the saphenous vein, can be explained in part by the high hydrostatic pressure. If the hydrostatic pressure increases most of the wall stress is said to act on the circular muscular layer (Fischer 1967) which is not well-defined in the saphenous vein.

To quantitate regionally pronounced fibrous thickening of the intima (phlebosclerosis), one can measure the most thickened area or try to establish the mean of the entire ring. We preferred the latter method because it reveals a better view of the mean changes in the entire vein. Nevertheless, all methods have disadvantages. Planimetry does not take into consideration the fact that the "intimal radius" is not the radius of a circle as the ideal condition with circular configuration rarely exists. Approximately oval-shaped venous rings predominate but this exerts little influence on the planimetric calculations. An exact mean intimal thickness can be determined only by means of extensive measurements, but this is not practical. A mean from a limited number of rings can be determined by the four-point method but this method is not much better than that produced by estimation. The estimate is based upon the eye's capacity for integration and works with only one diameter (the mean estimated diameter). For practical purposes – provided the investigator is experienced – this value is sufficiently precise.

The intimal thickness shows marked deviations not only in the different veins but also within single veins. These deviations reduce the representative value of a single ring but the variability is limited and minor and severe changes are not seen in a single vein. A ring with a very thin intima always belongs to a vein with little intimal fibrosis; very thick intimal layers are always found in veins with severe phlebosclerosis. However most of the rings with slight, moderate or severe intimal thickening allow only limited extrapolation to the entire saphenous vein.

From our data, extrapolation can be made as follows:

if the intima in the small operative specimen is very smooth, one can assume that the entire vein will present slight intimal thickening. If there is severe intimal thickening, the bypass vein will probably be severely phlebosclerotic. A specimen with an intimal thickness of less than 100 μm normally derives from a vein with little or moderate intimal thickening but an intimal thickness between 100 and 250 μm derives from a vessel with moderate or severe intimal fibrosis. If the thickness is more than 300 μm the vein has very severe phlebosclerosis. The broad range in between does not allow us to evaluate the saphenous vein accurately. Extreme values can clearly be related to the entire bypass vein.

References

Cheanvechai C, Effler DB, Hooper JR, Eschenbruch EM, Sheldon WC, Sones FM Jr, Levin HS, Hawk WA (1975) The structural study of the saphenous vein. Ann Thorac Surg 20:636–645

Eschenbruch EM, Cheanvechai C, Levin HS, Hawk BH, Spillner G, Schlosser V (1974) Histologische und radiologische Untersuchungen zur Frage des Frühverschlusses des aorto-koronaren Venenbypass. Thoraxchirurgie 22:601–605

Fischer H (1967) Der lichtmikroskopische Bau der Venenwand mit besonderer Berücksichtigung der Einwirkung des hydrostatischen Druckes. Zentralbl Phlebol 6:209–220

Langes K, Körfer R, Bircks W, Hort W (1984) No effect of saphenous intimal thickness on patency of aorto-coronary bypass grafts. J Thorac Cardiovasc Surg 88:304–306

Leu HJ (1980) Aspects morphologiques des altérations de la paroi veineuse. Phlebologie 33:223–232

Leu HJ, Brunner U (1973) Zur Pathogenese der degenerativen Veränderungen an autologen Venentransplantaten. Dtsch Med Wochenschr 98:2433–2436

Leu HJ, Rüttner JR, Schneider J (1971) Zur Frage der Beziehungen zwischen Arteriosklerose und Phlebosklerose der Beingefäße. Schweiz Med Wochenschr 101:1323–1326

Leu HJ, Vogt M, Prunder H, Odermatt BF (1991) Phlebosclerosis: disorder or disease? Vasa 20:230–236

Milroy CM, Scott DJ, Beard JD, Horrocks M, Bradfield JW (1989) Histological appearances of the long saphenous vein. J Pathol 159:311–316

Neumann R (1937) Histologie der Vena saphena magna unter dem Gesichtswinkel der Architekturpathologie. Virchows Arch [A] 299:479–512

Seydewitz V, Staubesand J (1990) Calciumbestimmungen, enzymbiochemische und elektronenmikroskopische Untersuchungen an Saphenavenen von Patienten mit Koronarsklerose oder Varikosis. Z Gesamte Inn Med 45:96–100

Stolte M (1977) Die aorto-koronare Bypass-Vene. Klinikarzt 6:211–218

Szilagyi DE (1979) Autogenous vein grafting in femoropopliteal atherosclerosis: the limits of its effectiveness. Surgery 86:836–851

Thiene G, Miazzi P, Valsecchi M, Valente M, Bortolotti U, Casarotto D, Galucci V (1980) Histological survey of the saphenous vein before its use as autologous aortocoronary bypass graft. Thorax 35:519–522

Vogt M (1978) Altersabhängige morphologische Veränderungen der Vena saphena magna. Vasa 7:54–60

Waller BF, Roberts WC (1985) Remnant saphenous veins after aortocoronary bypass grafting: analysis of 3,394 centimeters of unused vein from 402 patients. Am J Cardiol 55:65–71