Histological Study of Arterial and Venous Grafts Before their Use in Aortocoronary Bypass Surgery

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Introduction: In this study we investigated the morphology of grafts from the internal thoracic artery and the great saphenous vein, before their use in aortocoronary bypass surgery, in order to draw conclusions concerning their suitability and viability.

Material and Methods: Sections of grafts from the great saphenous vein and left internal thoracic artery obtained for use in bypass surgery were examined using light microscopy and transmission and scanning electron microscopy.

Results: The histological changes in the walls of the vessels were classified as acute or chronic. The acute lesions concerned the endothelium and the subendothelial layer. There was extensive necrosis of endothelial cells, resulting in the basement membrane being left uncovered and becoming the target of blood cells. The endothelial necrosis was accompanied by subendothelial oedema and focal destruction of the inner elastic lamina of the internal thoracic artery. The chronic lesions affected mostly the venous grafts and included the presence of distinct atheromatous plaques or thickening of the intima and media.

Conclusions: The combination of ischaemic and chronic atheromatous lesions in bypass grafts may contribute to a decrease in their viability, especially in the case of venous grafts.

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sis rate of foam cells originating from monocytes.\textsuperscript{9,10} This combination is an index of sensitivity to atheromatous plaque.\textsuperscript{7}

Histological examination of all occluded grafts has shown a large degree of intimal thickening. The main cause of intimal hyperplasia in venous grafts is considered to be the combination of the insertion of the venous graft into the arterial circulation and endothelial dysfunction as a consequence of surgical stress.\textsuperscript{12} In contrast, arterial grafts appear to be more resistant to the action of atherogenic factors and incur only minor traumatic and ischaemic lesions, since they are not removed from the blood circulation but are prepared locally, with few ligations and preservation of blood flow.\textsuperscript{13} Large studies have shown that 7-13\% of grafts from the great saphenous vein used for aortocoronary bypass become obstructed during the first postoperative month, while 5-14\% of venous grafts develop >50\% luminal stenosis. Complete occlusion is seen in 15-26\% of grafts after 6-8 months with 5-10\% having >50\% luminal stenosis. The most significant finding, however, is that 20\% of venous grafts show complete occlusion during the first year after bypass and 10 years later 50\% of venous grafts have become completely occluded, while the remaining 50\% have significant atheromatous lesions.\textsuperscript{14-16} Arterial grafts seem to have better survival, since 90\% of them remain functional for the next 12 years, at least in the case of arteries that are not removed from the circulation for anastomosis.\textsuperscript{17,18}

The question that arises is, which factors are implicated in the early obstruction of grafts, especially venous grafts? Those factors act during both the preoperative and the postoperative period.\textsuperscript{19,20} For this reason it is considered vital to carry out a preoperative quality assessment of grafts, which can provide important information for predicting a graft's viability. Angioscopy and intravascular ultrasound have been proposed as the most reliable methods for the preoperative screening of grafts.\textsuperscript{21-24} A combination of both methods allows the detection of small lesions in the vessel wall, including intimal hyperplasia, and this makes the quality assessment of grafts even more useful. It should be noted, though, that ultrasound alone cannot detect small or incipient vascular wall lesions, which can easily be recognised using angioscopy or histological examination.\textsuperscript{24}

For these reasons we used light and electron microscopy to examine biopsies of grafts taken from the great saphenous vein and the left internal thoracic artery, remnants from surgical coronary artery bypass procedures, with a view to drawing conclusions concerning the preoperative morphology of the grafts and correlating this with their future viability.

### Material and methods

Sixty biopsies from the great saphenous vein and 10 from the internal thoracic artery were examined using a light microscope and a transmission and scanning electron microscope. The mean age of the male patients from whom the grafts were taken was 61 ± 8 years and that of the female patients was 65 ± 5 years.

The great saphenous veins were obtained from the upper third of the lower limb. Immediately after removal, the great saphenous vein was catheterised and washed with heparinised normal saline (pH 7.2). Care was taken that the pressure within the vein should not exceed 40-60 mmHg. The venous grafts were then stored in a normal saline solution containing 2000 units of heparin /100 ml at room temperature while the surgical field was prepared. The time the grafts remained in the heparinised solution ranged from 15 to 45 minutes. Special care was taken to avoid injury to the great saphenous vein during preparation. Most of the venous graft was used to bypass an occluded coronary artery. The remnants were flushed with 10\% neutral formalin or 3\% glutaraldehyde in a PBS 0.1M pH 7.2 buffer solution, for observation using a light or electron microscope, respectively. The grafts in the buffer solution were immediately sent to the Histology Department for further examination.

For light microscopy, the grafts were left in the 10\% neutral formalin solution for 7 days, then dehydrated in ascending alcohol concentrations and embedded in paraffin. Sections 4 µm in thickness were stained with eosin-haematoxylin, using the Weigert-Van Gieson method, and examined with an Axionlab light microscope.

For transmission electron microscopy the two end sections of the graft were removed and the remainder was cut into smaller pieces (1x1x1 mm) which were left in a new 3\% glutaraldehyde solution for 2 hours. The sections of the internal thoracic artery, because of their small size, were cut immediately into smaller pieces in the same fixing agent without undergoing the flushing procedure. All tissue samples were post-fixed in 2\% osmium tetroxide solution, dehydrated in ascending alcohol concentrations for 60 minutes and embedded in EPON 812 resin. Thin, 80 nm sections were examined with a Jeol 200CX transmission electron microscope.
The samples destined for scanning electron microscopic examination were initially cut transversely into pieces 5 mm in length and then each piece was split longitudinally into two tissue samples of equal thickness. In this way the lumen of the graft was revealed for easier observation. The tissue samples were then fixed in a 3% glutaraldehyde solution for 4 hours, postfixed in 2% osmium tetroxide solution, dehydrated in ascending alcohol concentrations for 1.5 hours and dried in a critical point freeze-drying device (Balzers). The samples were covered with a gold-palladium mixture and then with carbon in a BALZERS SCD 004 Sputter Coater and were examined with a Jeol 6400 scanning electron microscope.

Results

Histological findings from the venous grafts

The histological lesions from the venous grafts were classified as acute or chronic. Acute lesions were seen mainly in the endothelium and involved the loss of endothelial cells. The endothelium was missing from extensive regions of the intima and blood cells could be seen adhering to the uncovered basement membrane (Figure 1). In three venous grafts there was thrombus formation and deposition on the endothelium, with the presence of fibrin or large accumulations of trapped red cells (Figure 2).

The chronic lesions included thickening of the intima and media (Figure 3) and the presence of more organised atheromatous lesions (Figures 4-6). The thickness of the intima in the venous grafts ranged from 16 µm to 2 mm and it was within normal limits in only 10 of the 60 venous grafts. The intimal thickening was focal (Figure 5) or concentric (Figures 3, 4, 6) and caused a significant degree of luminal stenosis, though not >50%.

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Figure 1. Microphotograph from transmission electron microscope, showing an endothelial cell (C) that has become detached from the basement membrane (BM) and is free within the lumen (L) of the venous graft. There is a macrophage (M) between the detached endothelial cell and the basement membrane, which can be seen to be thickened. Smooth muscle cell (MC). x 10,000.

Figure 2. Microphotograph from scanning electron microscope showing a venous graft with thrombus on the endothelium. E - endothelium, F - fibril, RC - red cell. x 2,000.

Figure 3. Photograph from light microscopy of the great saphenous vein, showing thickening of the intima and media. I - intima, LM - longitudinal layer of the media, CM - circular layer of the media, FC - foam cells, CF - collagen fibres. Weigert-Van Gieson stain. x 100.
The wall of a normal great saphenous vein consists of the intima, which contains the endothelium and a thin subendothelial layer, the media, which is comprised of the inner longitudinal and outer circular layers of smooth muscle fibres, and the adventitia (Figure 7).

Intimal thickening is due to the accumulation of smooth muscle fibres, fibroblasts, collagen fibres, foam cells (Figure 3), neovasculature and the presence of an extracellular matrix (Figure 4), as well as to thickening of the basement membrane (Figure 8). The neovascularature includes the presence of arterioles of various sizes, venules and capillary vessels in the intima and media. Haemorrhagic irrigation was seen between the bundles of smooth muscle fibres in the longitudinal and circular layers of the media. The neovascularature of the intima is made up of smaller vessels than that of the media.

The most organised atheromatous lesions included type Vc (Figure 5) and type IV (Figure 6) atheromata,
Figure 8. Microphotograph from transmission electron microscopy, showing a many-layered, thick basement membrane, indicated by the black arrows. Apart from general thickening, the basement membrane shows additional, focal thickening, indicated by the line. The white arrow shows limited intracellular oedema and detachment of the cell membrane on the side of the lumen. x 15,000.

Figure 9. Microphotograph from transmission electron microscopy, showing a smooth muscle cell in apoptosis. The black arrows show the basement membrane at its periphery, which is preserved in spite of the muscle cell apoptosis, and the white arrow shows an apoptotic body. x 10,000.

Figure 10. Microphotograph from transmission electron microscopy, showing a necrotic muscle cell in the subendothelial layer. The necrotic region of the cytoplasm and its conversion to myeloid (M) bodies can be clearly seen. x 8,000.

according to the American Heart Association’s classification. The atheromatous lesion in figure 5 is compact and contains mainly fibres and fewer foam cells, which are located close to the surface of the plaque that is exposed to the lumen. At one point there was rupture and detachment of a region of the plaque, but with no thrombus development. The atheromatous plaque in figure 6 is classified as stage VI, according to the American Heart Association’s classification, and is characterised mainly by the presence of cholesterol crystals, which extend throughout the entire depth of the plaque as far as the intima. This plaque also shows the presence of calcium salts, infiltration with inflammatory cells and rupture, which resulted in the release of material into the lumen.

The thickness of the basement membrane in the venous grafts ranged from 100 nm to 1.5 mm. Thickening of the basement membrane was continuous or focal. The basement membrane was ordered into more layers, or appeared completely disorganised, converted into fine, unstructured, granular material that alternated with fine fibrils woven into nets (Figure 8). The degeneration of the basement membrane was accompanied by subendothelial oedema. The thickness of the basement membrane at its thickest point (Figure 8) was 1.5 mm.

In 81% of the venous grafts there was thickening of the longitudinal and circular layers of the muscularis, in which four types of muscle cells were identified according to their functional roles: apoptotic, necrotic, secretory and contractile. The necrotic muscle cells clearly outnumbered the apoptotic. The apoptotic muscle cells were characterised by shrinkage and by the intracellular presence of apoptotic bodies (Figure 9), and the necrotic cells by rupture of the cell membrane and the release of organelles in the extracellular region (Figure 10). The secretory muscle cells were increased in size and had an abundance of secretory granules near to the cell membrane (Figure 11), while the contractile muscle cells contained a large quantity of actin fibrils. Features of the interior of the contractile muscle cells included

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lipid droplets in contact with the nuclear membrane, extreme dilatation of the endoplasmatic reticulum, giving the appearance of large vacuoles, and swelling and loss of matrix in many mitochondria (Figure 12).

**Histological findings in arterial grafts**

On the wall of the arterial grafts there were mainly acute lesions, similar to those in the venous grafts. The intimal thickness of arterial grafts was clearly smaller and ranged from 8 to 20 µm. In two cases there was dissection of the intima in its external third, in a region that contained alternating layers of elastin laminas (Figure 13).

The loss of endothelium was extensive and was accompanied by intense subendothelial oedema, while in places there was destruction of the inner elastic lamina and ordering of the basement membrane into multiple parallel layers. The subendothelial oedema also extended below the inner elastic lamina in regions where the latter appeared to have been destroyed (Figure 14). The inner elastic lamina appeared normal in some re-
gions, while in others it was replaced by fine-grained, unstructured material, with only a small number of elastic fibres being preserved (Figure 14). The basement membrane had the form of continuous, multiple layers, or granular material, or filaments of varying length aligned in various directions (Figure 14). Their thickness ranged from 60 to 600 nm.

Discussion

The results of this study using light and electron microscopy showed that before their implantation 90% of vascular grafts exhibit histological lesions of small or large degree on the vessel wall. Venous grafts appear to show the most severe lesions.

The vascular wall lesions were classified as acute or chronic. For the total grafts studied the most commonly encountered acute lesions were extensive destruction of the endothelium, oedema of the subendothelial layer, thrombus adhesion in the vessel lumen and necrosis of a significant number of muscle cells in the uppermost subendothelial layer.

The most frequently encountered type of chronic lesion, mainly in the venous grafts, was local thickening of the vessel wall, although this did not cause >50% luminal stenosis in any vessel. The stenosis was due mainly to hyperplasia of the wall and more rarely to real atheromata, alternating in the same vessel with regions where the vessel diameter was within normal limits. Chronic lesions were much more rare in the arterial grafts.

Of course, the findings of this study refer to sections of arterial and venous grafts that were not used in bypass surgery and they could thus be considered not to reflect the real histological picture of the grafts that were implanted. In our view, however, the histological picture of the great saphenous vein and the internal thoracic artery in regions closely bordering on the implanted sections provides useful and reliable information about the condition of the grafts prior to implantation. Furthermore, given that the vascular grafts undergo manipulations at the surgical stage that prolong the time of hypoxia and increase the chance of injury, one might suppose that the lesions in implanted grafts are likely to be even more severe.

The findings of the present study are also confirmed by other experimental studies reported in the international literature. In particular, 10 minutes after experimental arteriovenous anastomosis in healthy rats a small degree of endothelial destruction has been demonstrated in the grafts, which does not cause cell death but over the next few days leads to the formation and deposition of thrombus, infiltration by neutrophils and a small degree of intimal thickening. The same experimental study reported that one week after anastomosis a large number of smooth muscle fibres showed necrosis, apoptosis, as well as mitotic activity that led finally to intimal thickening in the graft.

Studies of patients with aortocoronary bypass report intimal thickening in venous grafts during the first postoperative month. The thickening is located mainly in the region of the anastomosis where manipulation of surgical instruments can cause injury to the graft. Westerband et al maintained that exposure of the endothelial cells of venous grafts to mechanical stress, because of the increased pressure they are subjected to in the arterial circulation, can cause an increase in the genetic expression of adhesion molecules, growth factors and the production of proteins in the extracellular region, which leads finally to thickening of the vascular intima.

A number of cardioplegic solutions have been implicated in acute endothelial lesions that lead to cell deficiency and damage to the cytoskeleton. According to reports in the literature, intimal thickening in the great saphenous vein is a hallmark of smoking, while in the media it is a sign of varicosity. The wall of the upper third of the great saphenous vein is thinner and more elastic than the remainder and for this reason it is often used as a graft. Our observations from a parallel study of occluded venous grafts that were removed during reoperation for the restoration of arterial circulation (data not given) showed that the main cause of obstruction of venous grafts was the great degree of thickening of the vessel’s intima and media, which was due either to the proliferation of muscle fibres, or to the formation and incorporation of thrombus from previous rupture of atheromatous plaque. Thrombus formation and organisation caused the greatest obstruction and perhaps complete occlusion. These observations of ours also agree with an earlier report by Waller et al who studied the histological picture of 400 atheromatous coronary artery lesions and concluded that the formation of atheromatous plaque and thickening of the intima after atherectomy were the main causes of obstruction.

Studies have reported that as luminal stenosis develops in venous grafts there is a progressive change...
in the phenotype of secretory and contractile muscle cells in the media, with a tendency for the contractile type to be converted into the secretory type. The new type of secretory muscle cells shows a higher index of cell proliferation and migration towards the intima. In their new location these cells produce and deposit elements of connective tissue as well as extra-cellular material, resulting in the thickening and progressive obstruction of the lumen of the graft.

Most vascular grafts are influenced by risk factors such as hypertension, hyperlipidaemia, diabetes mellitus, age, sex and smoking. Hypertension, because of the mechanical stress it exerts on endothelial cells, activates mitogenic factors, such as the α-receptor of platelet growth factor, PDGF receptor α, which in cell cultures cause proliferation of muscle cells. It has been proved, however, that hypertension alone does not cause thickening of the vascular intima, whereas in combination with hypercholesterolaemia or diabetes mellitus it can cause significant thickening of the intima and media. It seems, then, that the most important risk factor leading to hyperplasia of muscle cells in the vascular intima and media, as well as to vasomotor disturbances, is hypercholesterolaemia, either alone or in combination with diabetes mellitus or hypertension. The above findings were evident in venous grafts that had been implanted in the arterial circulation of Apo-E deficient mice. Although diabetes mellitus causes changes in the endothelium, muscle cells and basement membrane, it cannot on its own be considered as a sign of high risk for luminal stenosis in a graft. The arterial graft in figure 13, in which scission was seen within the media, came from a patient who had three risk factors, hyperlipidaemia, hypertension and smoking. The grafts in figures 3 and 4, which showed a great degree of wall thickening and neovascularisation in the media and intima, respectively, came from a patient who suffered from type 1 diabetes mellitus.

In the present study the lesions in the wall of venous grafts were clearly more severe than those in the wall of arterial grafts. The reason is that the arterial grafts undergo more gentle manipulations during their placement and that the latter does not require their removal from the blood circulation. The flushing of venous grafts under pressure, their storage in heparinised normal saline at 4°C and their placing within a higher pressure system are all factors that have a deleterious effect on the venous graft’s vessel wall. These peri-operative factors lead to necrosis, mainly of the endothelial and muscle cells of the intima and the upper layer of the media. Necrosis of endothelial cells over a great area leaves the basement membrane uncovered and allows contact between structured blood elements, mainly platelets and monocytes, and the subendothelial layer. This contact represents the beginning of the process through which a new atheromatous lesion is created.

Venous grafts are used widely by surgeons. However, the atheromatosis to which they are constantly subjected reduces their lifespan and means that there is an urgent need to investigate this predisposition and to find ways of combating it effectively.

Today, genes have been identified for vascular smooth muscle fibres that are overexpressed in atheromatosis and in-stent stenosis. Disturbance of the balance between cell proliferation and apoptosis of muscle and foam cells leads to the destabilisation of atheromatous plaque and finally to occlusion of the graft.

Until the discovery of new therapeutic methods for combating already existing as well as progressive atheromatous lesions in grafts, there is a need for better handling of grafts during the period of the operation and an improvement in the diagnostic methods of choice. A large study of venous grafts, based solely on histological examination, found that 91% of grafts showed varying degrees of histological lesions, whereas an ultrasound study of the same grafts found that 90% had a physiological picture. Angioscopy has been proposed as the best method for preoperative evaluation of the suitability of venous grafts and can detect incipient wall lesions or those of small degree, as has been confirmed later by histological examination. The creation of a bank of venous grafts from selected donors, which have previously been screened histologically, could prevent the implantation of grafts with pre-existing histological lesions.

References

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