Clinical Research

The Role of Apoptosis in the Failure of Vascular Grafts

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Key words:
Vascular grafts, apoptosis, prognosis, viability.

Indroduction: Apoptosis expresses programmed cell death and is an important function of the organism that ensures the proliferation and renewal of tissues. Atherogenic factors, such as diabetes, smoking, high blood pressure and dyslipidaemias, operate by activating the apoptosis mechanism, so their contribution to vascular occlusion is significant. Vascular grafts, arterial or venous, are used widely for myocardial reperfusion after occlusion, and arteriovenous anastomoses provide a model of atheromatosis in a vein that has been placed within the arterial circulation, in a higher-pressure system than it was destined for.

Methods: We studied samples of grafts from the great saphenous vein and the left internal thoracic artery that were used for aortocoronary bypass and samples of occluded arteriovenous anastomoses of the radial artery in patients undergoing haemodialysis. Biopsies from the above grafts were examined with a light microscope and a scanning and transmission electron microscope. The avidin-biotin-peroxidase immunocytochemical method was employed during light microscopy, using the caspase-3 antibody and the TUNEL method (fragment end labelling of DNA).

Results: Extensive apoptosis of endothelial and muscle cells was seen in the intima and media of both arterial and venous grafts. The arterial grafts showed extensive apoptosis of the fibrocytes of the adventitia. The wall of the arteriovenous anastomoses showed a significant degree of thickening of the intima and media because of hyperplasia of the smooth muscle fibres, which were in a universal state of apoptosis. A significant number of endothelial cells and smooth muscle fibres in the grafts showed signs of necrosis. In conclusion, apoptosis takes place in a significant number of cells of vascular grafts and anastomoses.

Conclusions: Before arterial and venous grafts are implanted to bypass occluded coronary arteries they already show extensive apoptotic and necrotic lesions in the endothelium and in myocytes of the intima and media. In view of this, an assessment of the degree of apoptosis and vascular grafts could be used as a prognostic index of their viability.

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Apoptosis is a discrete expression of programmed cell death and contributes to the proliferation and homeostasis of tissues. Pathological regulation of the phenomenon of apoptosis leads to a variety of diseases, including cancer, neurological and cardiovascular disorders.[1-3] Many studies have shown that injury to the endothelium triggers a healing process, which is realised through the migration of myocytes from the media and their establishment in the intima, cell proliferation and the production of extracellular material at the new site.[4-6] This process leads to an increase in the thickness of the intima, which is a standard finding from all venous grafts during the first postoperative month. The focal thickening of the intima is often accompanied by a loss of endothelium in the underlying region.[7] Intimal hyperplasia in animals is not seen when a venous graft replaces a section of vein or when an arterial graft is placed within the arterial

circulation in apoE-null mice, even 8 weeks after the graft is implanted.[8] Since the total number of cells in the vascular wall depends on the relation between cell death and proliferation, cellular apoptosis is considered to be a causative factor for intimal hyperplasia of vascular grafts after their implantation in an occluded region.[7-10]

It is generally accepted today that venous grafts show much earlier signs of obstruction than do arterial grafts, which seem to be clearly more resistant. Thus, during the first postoperative year after aortocoronary bypass, 20% of venous grafts show complete occlusion, in contrast to arterial grafts, 90% of which remain functional for the following 12 years.[11,15] The latter percentage refers to arteries that have not been cut off from blood circulation.

Many factors have been implicated in disease of venous grafts. A primary cause is considered to be the mechanical stress that the endothelial cells of venous grafts undergo when they are placed in a system with ten times greater pressure, such as the arterial circulation. This effect leads to changes in the genetic expression of endothelial and smooth muscle cells, which are manifested by apoptosis, proliferation and a concentration of intracellular lipids in the early stages following surgery.[16,17] An acute rise in blood pressure is known to cause an increase in both apoptosis and proliferation of myocytes, through the activation of metabolic pathways of the PDGF receptor, which acts via the MAP kinase.[18,19] The role of apoptosis in the survival of venous grafts is thought to be a significant one.[20-23] The extent and the rate of apoptosis in atheromatous plaque is an index of the plaque's stability.[24] The expression of apoptosis-related proteins in smooth muscle fibres in the intima and media is an early sign of disease in venous grafts.[25]

In this study we investigated the extent of the apoptosis phenomenon in grafts from the great saphenous vein and the internal thoracic artery before their implantation in aortocoronary bypass surgery. We also examined occluded anastomoses of the radial artery to the cephalic vein with a view to drawing conclusions regarding the contribution of apoptosis to the early occlusion of venous grafts.

Material and Methods

Light microscopy employing immunocytochemical and molecular biological methods was used to examine biopsies of samples from 60 great saphenous veins, 10 internal thoracic arteries and 5 occluded arteriovenous anastomoses of the radial artery to the cephalic vein in chronically haemodialysed patients. The mean ages of the male and female patients who received vascular grafts was 60 ± 9 years and 65 ± 4 years, respectively. 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. 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. All the pieces of the vessels that were not used for the grafting procedure were sent to the Histology Department for histological and ultrastructural examination. Segments from the arterial and venous grafts were processed appropriately for examination by a light and an electron microscope. Biopsies were also taken from both the arterial and venous parts of the occluded anastomoses. For light microscopy, biopsies from the grafts and anastomoses were fixed in 10% neutral formalin and embedded in paraffin. Sections 4 μm in thickness were stained with eosin-haematoxylin, using the Weigert-Van Gieson method, the avidin-biotin immunoperoxidase method, and the TUNEL method. For the immunocytochemistry the caspase-3 monoclonal antibody (Serotec) was used in a dilution of 1/100. The FragEL DNA Fragmentation Detection Kit In situ apoptosis assay (Oncogene) was used for the TUNEL. For the TUNEL method, following paraffin removal the sections were incubated with proteinase K for 20 minutes, rinsed with large quantities of buffer solution, incubated with 3% hydrogen peroxide for 5 minutes, and were then flushed and incubated with a mixture containing terminal deoxynucleotidyl transferase (TdT) enzyme, biotinylated and non-biotinylated deoxynucleotides. The sections were kept in an autoclave at 37˚ C for 1.5 hours, after which the reaction was interrupted by flushing and the sections were incubated with avidin-peroxidase for 30 minutes. Sections were stained with diaminobenzidine in the presence of hydrogen peroxide. The sections were rinsed, dehydrated in ascending alcohol concentrations, covered with Histomount and examined with a light microscope.

For the transmission electron microscope examination the lumen of the great saphenous vein was flushed with 3% glutaraldehyde solution in a PBS 0.1M pH 7.2 buffer solution. Special care was taken

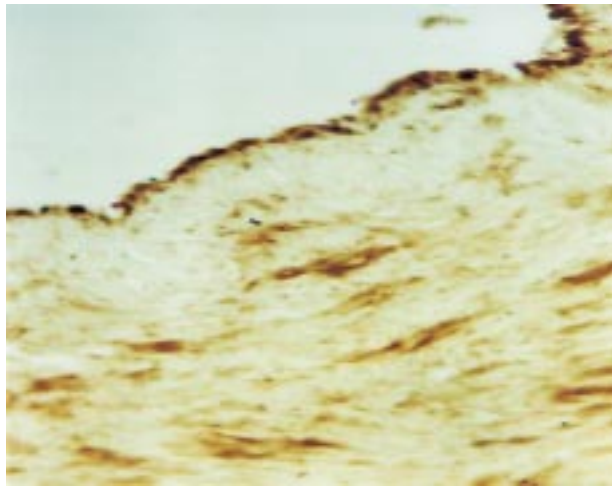


Figure 1. Detection of caspase-3 in the endothelium and in some myocytes of the intima and media of the internal thoracic artery (x 400).

Figure 2. Detection of caspase-3 in the inner layer of the media of the internal thoracic artery (x 400).

that the pressure within the lumen should not exceed 40-60 mmHg. The graft was then cut into smaller pieces and was left in the same fixing solution for 2 hours. The sections of the internal thoracic artery and the arteriovenous anastomoses, because of their limited size, were cut immediately into smaller pieces in the same fixing agent without undergoing the flushing procedure. The above biopsies were post-fixed in 2% osmium tetroxide solution, dehydrated in ascending alcohol concentrations and embedded in EPON 812 resin. Thin, 80 nm sections were examined with a Jeol 200CX electron microscope.

Fifty-one patients returned for re-examination 3 months and 1 year after operation. The re-examination included a cardiological examination and in certain cases angiography. The clinical examination focused on the detection of a new infarction, chronic heart failure and arrhythmias. The case of sudden death was also investigated.

Results

Study of the apoptosis phenomenon

In the arterial grafts from the internal thoracic artery positive detection of caspase-3 was seen in extensive regions of the endothelium and in a small number of myocytes in the intima (Figure 1). The external longitudinal layer of the media had a significantly greater number of positive myocytes than the internal orbicularis (Figure 2). The nuclei of the myocytes of the internal layer of the intima, according to the TUNEL method, showed a weak reaction compared to the nuclei of both the myocytes of the external layer and those of the adventitia, which showed the strongest reaction (Figure 3). The TUNEL method showed that the nuclei of the endothelial cells in serial sections were strongly positive in the same regions where caspase-3 was detected.

The venous grafts showed a strongly positive reaction to caspase-3 in an extensive and continuous

Figure 3. Detection of apoptotic cells by the TUNEL method in the intima (arrow) and media, especially in the outer layer of the media (white arrow), and the adventitia of the internal thoracic artery (x 100).

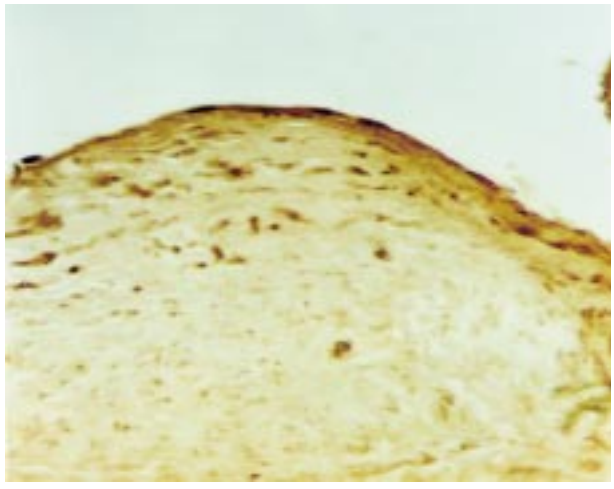


Figure 4. Detection of caspase-3 in the endothelium and subendothelial layer of the great saphenous vein (x 400).

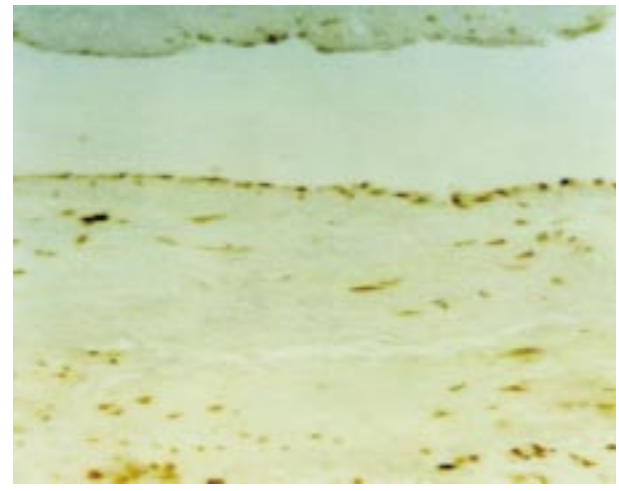


Figure 5. Apoptotic cells detected by the TUNEL method in a large area of the endothelium and in a small number of myocytes of the intima and media of the great saphenous vein (x100).

region of the endothelium and in the myocytes of the underlying thickened subendothelial layer (Figure 4). The TUNEL method revealed that for serial sections the nuclei of the endothelial cells were strongly positive in regions where caspase-3 activity was detected, while a smaller number of myocytes in the thickened subendothelial layer and the media showed positive (Figure 5).

The arteriovenous anastomoses showed a very great increase in the thickness of the intima and media. The thickening of the intima was due to an increase in the number of myocytes and to the assimilation and organisation of thrombus. The development of neovasculature in the thickened intima is characteristic (data not shown). The thickening of the media was due to the proliferation of myocytes.

The venous section of the arteriovenous anastomoses had extensive regions of endothelium, subendothelial layer and media that showed a strongly positive reaction to caspase-3 antibody, while there were also regions with sparse positive cells (Figure 6). The TUNEL method revealed a large number of cells in all wall layers with a strong positive reaction. A very strong positive reaction was also seen in the endothelial cells of the neovasculature, from the assimilation of thrombus into the intima (Figure 7).

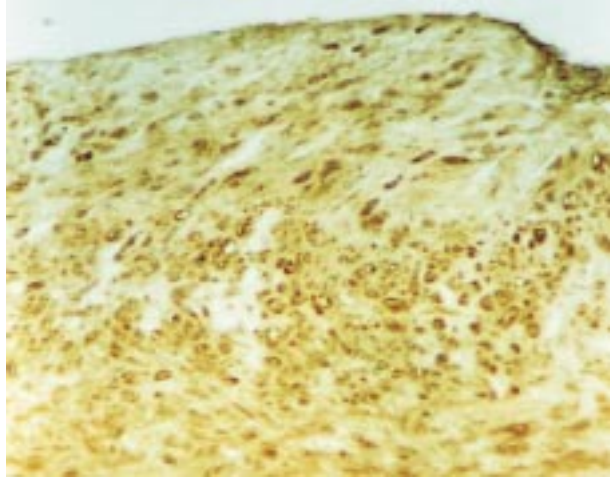


Figure 6. Detection of caspase-3 in the endothelium and in a large number of cells of the thickened endothelial layer of the cephalic vein at the point of anastomosis (x 400).

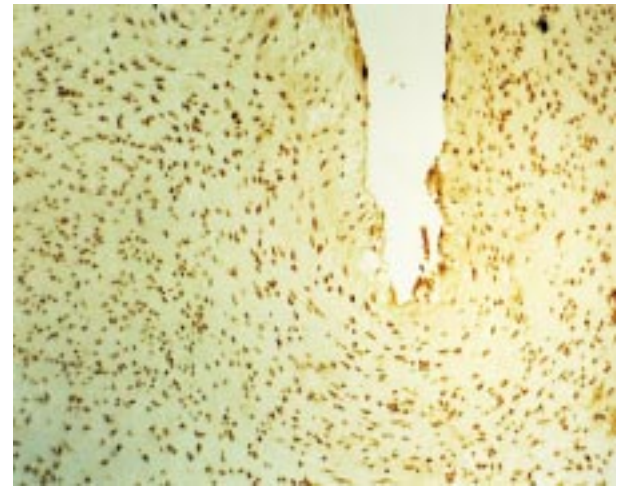


Figure 7. Apoptotic cells detected by the TUNEL method in the endothelium and in the thrombus-thickened subendothelial layer of the cephalic vein at the point where its anastomosis to the radial artery is occluded. The endothelial cells of the neovasculature are also apoptotic (x 100).

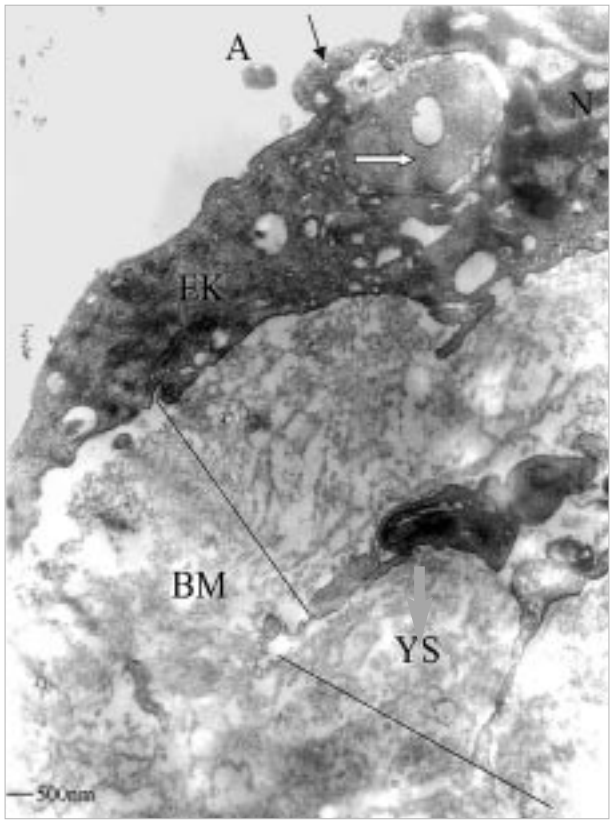
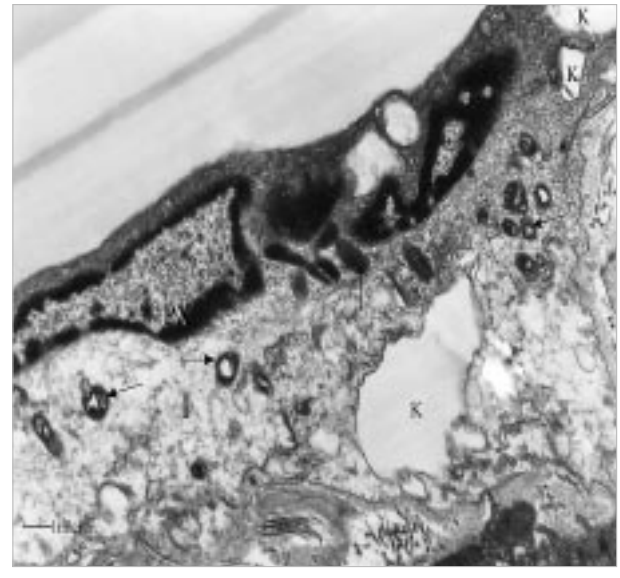


Figure 8. Electron microscope image showing a section of an endothelial cell (EK) and a section of the subendothelial layer (YS) of the internal thoracic artery. The endothelial cell is apoptotic, as can be seen from the multiple infoldings of the nuclear membrane (N), the condensation of chromatin, the ejection of a small apoptotic body into the vessel lumen (A), the creation of an autophagosome (white arrow) and the start of the ejection of a second apoptotic body (arrow). The basal membrane (BM) appears thickened (thickness >1000 nm) and is distended into multiple non-contiguous layers interrupted by oedema, or converted into masses of formless granular material. The elastic fibres of the inner elastic lamina in the subendothelial space (YS) appear to have been completely destroyed.

Figure 9. Electron microscope image showing a section of endothelial cell whose cytoplasm contains many Weibel-Palade bodies (arrows), vacuoles (K) of various sizes and an increase in cytoskeletal fibrils (I). The nucleus shows infoldings and condensation of peripheral heterochromatin (PX).



The arterial section of the anastomoses had a clearly smaller number of cells with a positive reaction to caspase-3 antibody and to the TUNEL method. The cells positive for caspase-3 were located mainly in the endothelium and to a lesser extent in the subendothelial layer. Cells positive in the TUNEL method were mainly located in the endothelium and the external layer of the media. The intima contained a smaller number of cells that showed a weaker positive reaction. The occluded arteriovenous anastomoses, and mainly the venous section, contained a significantly greater number of cells in the stage of apoptosis, compared with the arterial and venous grafts used for aortocoronary bypass. The arterial sections of the anastomoses had a smaller number of apoptotic cells than the venous sections, which were universally apoptotic.

Morphological findings of apoptosis using the electron microscope

The electron microscope was used to examine vessels that showed indications of atheromatosis on light microscopy and to look for signs of cellular apoptosis. Morphological wall lesions of varying degrees were found in 90% of the grafts and in the most severe of these the detection of apoptotic bodies was easier. A significant number of endothelial cells showed deep evaginations and long protrusions of nuclear membrane, as well as strongly dark-coloured or condensed nuclei. The cellular membrane on the side of the lumen showed spherical protrusions in places, a precursor to the creation of apoptotic bodies. Endoplasmic reticulum dilatation, large vacuoles and many intracellular Weibel-Palade bodies were observed in many endothelial cells (Figures 8, 9).

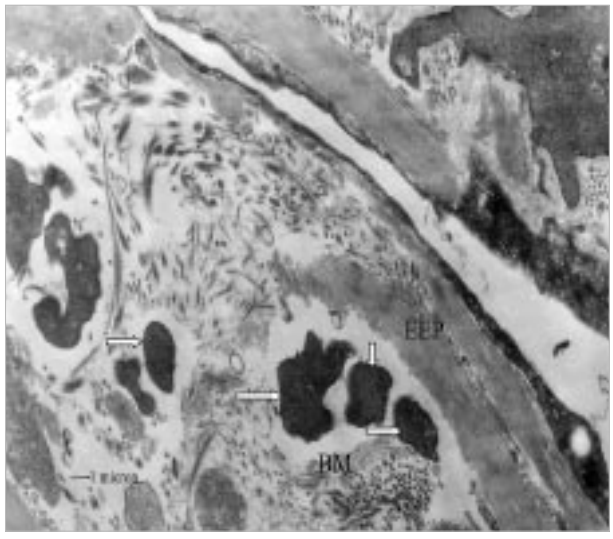


Figure 10. Electron microscope image showing apoptotic bodies in a muscle cell (white arrows) in the media of the internal thoracic artery. Preservation of elements of the basal membrane (BM) can be seen, as well as the presence of the inner elastic lamina (EEP).

Figure 11. Electron microscope image showing loss of endothelium, great development of fibrils (I) in the subendothelial space (YX) and necrosis of muscle cells (MK). The muscle cells show intracellular oedema (O), cell membrane rupture (PM) and a release of organelles (YO), myofibrils (MI) or medullary bodies (PS).

A large number of smooth muscle cells in the intima and media showed signs of necrosis and apoptosis. The apoptotic cells where characterised by shrinkage of the cell and the presence of apoptotic bodies, while necrosis was indicated by cell membrane rupture and the release of organelles into the surrounding area. Identification of these cells was based on the presence of remains of basement membrane on their periphery (Figures 10, 11).

When the patients were re-examined at the first postoperative month they were all alive and taking appropriate medication. Eight patients with venous grafts had angina that was not controlled by medication. One year later a further 5 patients had angina, 2 an infarction and 2 chronic heart failure. Angiography in the patients with infarction and angina showed obstruction of the venous grafts, while grafts from the internal thoracic artery were functioning normally. The histological findings from the 8 patients who developed early cardiological problems showed signs of apoptosis in extensive regions of the vascular wall of the grafts.

Discussion

In this study we investigated the extent of apoptosis in arterial and venous grafts prior to their implantation for aortocoronary bypass and the relation between the degree of apoptosis and the viability of the grafts. We also investigated apoptosis in the arterial and venous segments of occluded arteriovenous anastomoses, looking for a possible relation between the extent of apoptosis and the obstruction of the anastomosis. The originality of this study lies in the fact that there have been no previous reports in the international literature concerning the extent of apoptosis in vascular grafts before their implantation for coronary bypass and the possible relation between the degree of apoptosis and the length of time the grafts remain in good functional condition. In contrast, there have been many studies of the extent and rate of apoptosis and the distribution of apoptotic cells in the atheromatous plaque of occluded venous grafts after their implantation in bypass operations involving the arterial circulation.[8,21,25-30] During their preparation, these grafts are subjected to the effects of hypoxia, which leads to cell death according to the length of time involved.

As an index for the recognition of apoptotic cells we used the activation of the caspase system, in combination with the presence of fragments of DNA in the nucleus. The activation index chosen from the caspase system was caspase-3, which belongs to the cysteine proteases, is activated during the middle stage of the apoptosis phenomenon and causes extensive disorganisation of the nuclear shell and the

cytoskeleton. One result of the destruction of lamines is the entry into the nucleus of plasmins, which cause extensive proteolysis of nucleases related to DNA repair and the Rb protein, which is an important regulator of the cellular cycle.[31] The activation of the caspase path starts 30 minutes after ischaemia, while the DNA fragmentation starts after 80 minutes. The free segments of DNA can be detected by the presence of free groups of 3′ HO, which have arisen from the action of apoptotic endonucleases during the final stage of the apoptosis phenomenon.

The activation of the caspase system is not the only route that leads to cell death, since there have been reports of the presence of ubiquitine inclusions in the cytoplasm, which are an indication of autophagic cell death, irrespective of the activation of the caspase path.[32] Autolysis of the cytoplasm with a physiological picture of the nucleus has been reported as cell death of cardiac muscle fibres in chronic heart failure with an increased stroke volume.[32] The terms DNA fragmentation, apoptosis, positive staining with the TUNEL method, activation of the caspase path and autolysis of the cytoplasm are an expression of a one-way street leading to cell death. Detection of DNA fragmentation in the nucleus of a cell does not by itself amount to a sign of programmed cell death.[21,25] Insufficient and delayed fixation, along with increased RNA synthesis, a sign of intensive functional cell activity, can lead to false positive results. In both cases the TUNEL method detects segments of DNA that result either from post-mortal changes in the cell or from transient DNA splitting during transcription. For this reason, this study of apoptosis in grafts was based on the detection of the phenomenon using morphological and functional criteria, using two indices that are activated at two stages, one earlier in the caspase path and one that follows DNA fragmentation.

The endothelial cells in vascular grafts sustain large necrotic or apoptotic lesions, which can be easily discerned by morphological methods but cannot be distinguished through the detection of activation of the caspase system or DNA fragmentation. A number of myocytes in the subendothelium and media are also in apoptosis and in fact this number increases at the sites of formation of atheromatous plaque in the subendothelial space. Many myocytes of the endothelium and media are seen to be in a phase of necrosis or apoptosis. It has been reported that the apoptosis of the cell population occurs more in thin, symptomatic atheromatous plaques than in asymptomatic ones,[9] although necrosis and apoptosis occur in non-atheromatous stenotic regions of grafts from the great saphenous artery that are used in aortocoronary bypass procedures.[27] The necrotic cells outnumber the apoptotic. A characteristic of the necrotic cells is the presence of a thickened basal membrane, which surrounds the vesicles that result from the destruction of the cytoplasm. The vesicles have the appearance of medullary bodies, rich in phospholipids. Analysis of the contents of the vesicles with X-rays has shown the presence of calcium and phosphorus.[33,34] The calcified vesicles in atheromatous plaques are also known to contain cholesterol.[34]

During cell apoptosis phosphatidyloserine appears in the cell membrane.[35] Phosphatidyloserine is a hydrophobic lipid that is normally turned towards the inner section of the double layer and is not detected on intact cell membrane. During cell apoptosis, because of disruption of the double layer, the phosphatidyloserine changes its orientation and can be detected on the outer surface of the cell membrane. Phosphatidyloserine and other phospholipids have a strong cohesion with calcium, especially in the presence of phosphorous, and cause a strong influx of calcium to the cytoplasm of smooth muscle cells.[35] The intracellular increase of calcium concentration is the start of the process of cell apoptosis.[36-38] The presence of vesicles surrounded by basal membrane is a manifestation of increased cell death in smooth muscle fibres, reduced phagocytosis and instability of atheromatous plaque. In symptomatic atheromatous plaques the apoptosis rate of smooth muscle cells in the region underlying the atheromatous lesion is significantly increased and is considered to be a destabilising factor for atheromatous plaque.[24]

The presence of macrophages in apoptosis has also been reported in atheromatous regions.[21] In spite of that, apoptosis of macrophages and foam cells arising from macrophages is not common. The concentration of macrophages in the subendothelium and atheromatous plaque aggravates the development of atheromatous plaque, given that they secrete metalloproteases that cause the destruction of collagen fibres and plaque destabilisation, or they secrete cytotoxic factors that cause necrosis of adjacent muscle cells.[20,39,40] A third reason for cell death of smooth muscle fibres is the presence in atheromatous plaque of T-lymphocytes that secrete interferon-γ, which is known to inhibit the expression of α-

actin, to inhibit the proliferation of smooth muscle cells and to stimulate macrophages.[41] It is a fact that the presence of macrophages is accompanied by increased cell apoptosis in the region.[42]

Apoptosis was also seen in the external layer of the media and in the adventitia, especially in arterial grafts. The dissection of the perforating branches for the removal of the internal thoracic artery is likely to be responsible for the apoptotic lesions, given that they are nutritive vessels for the adventitia and also for the external layer of the media. Apoptosis and mitotic activity of the myofibroblasts of the adventitia have been reported at sites of intra-arterial removal of atheromata and indeed movement of these cells has been considered responsible for hyperplasia of the intima and media. The proliferation and local persistence of these cells in the adventitia is also thought to be responsible for the overall increase in the arterial wall thickness following endarterectomy, as well as of the constriction of the wall that results in a reduction of the arterial lumen.[43]

Smooth muscle cells coming from atheromatous plaque and not from a physiological media are unable to proliferate in cell cultures, since they secrete the pro-apoptotic BAX protein of the BCL-2 family, which accelerates cellular apoptosis.[44] The expression of the BAX protein in smooth muscle cells activates cellular apoptosis, either via the caspase system or not. The contractile type of smooth muscle cells, which show lipids in their cytoplasm in contact with the nuclear membrane, express BAX protein and are converted to foam cells.[45] It is thought likely that intracellular lipid concentrations are related with the expression of BAX protein, while lipid oxidation may activate the caspase path and the reduction in BCL-2, which leads to cell apoptosis.[46] The foam cells in the intima and media come either from smooth muscle cells or from macrophages migrating into the atheromatous region. And while foam cells that come from smooth muscle cells are susceptible to apoptosis, those coming from macrophages are able to proliferate and to produce factors that accelerate cellular apoptosis in smooth muscle cells.[7]

In the venous section of arteriovenous anastomoses the myocytes that register as positive in the TUNEL method predominate. Caspase-3 is detected in limited sections of the wall of arteriovenous anastomoses, showing that the caspase system has already been exhausted and the cells are in the stage of DNA fragmentation. These regions show that the failure of the anastomosis is progressive and when the apoptosis spreads to a large number of cells the anastomosis is already incapable of functioning. The intima and media of the venous sections of anastomoses are in universal apoptosis, which is due to stenosis of the lumen and inadequate perfusion. The corresponding arterial section of the anastomoses contains cells that register as positive with the TUNEL method in the adventitia and the outer layer of the media, with only a few in the subendothelial layer. In contrast, caspase-3 is detected in a small number of endothelial cells. From these findings one can concluded that the venous section is responsible for the failure of arteriovenous anastomoses. In contrast, in grafts it appears that caspase-3 is the main index of apoptosis and is detected mainly in the endothelium, and indeed over a great area. Prolonged apoptosis, which is more conspicuous in arteriovenous anastomoses in combination with increased proliferation of smooth muscle fibres, is an important factor that contributes to the process of graft failure.

Before their implantation in a bypass procedure, the grafts already contain a number of apoptotic cells that depends on their degree of atheromatosis. Factors associated with the procedure, such as hypoxia, storage and injury, exacerbate the pre-existing state of the vessels and in this way the degree of damage to the grafts continues to increase, resulting finally in occlusion, especially in the case of venous grafts. Apoptosis of endothelial cells over a wide area leaves the basal membrane uncovered and allows communication between blood cells, such as platelets, and the subendothelial layer. This contact is the start of the process of atheromatosis at the site in question. Activation of the caspase system starts at least 30 minutes after the action of cytotoxic factors, while DNA fragmentation starts after 80 minutes. Caspase-3 and fragmented sections of DNA were detected in all grafts. That means that the grafts had been under conditions of intense stress for at least 80 minutes.

The literature contains reports of cases where the inhibition of caspase activity was sufficient to prevent cell death,[47,48] but also cases where this inhibition was unable to prevent apoptosis.[49] The subsequent histological evaluation of the grafts in patients who showed early, severe postoperative cardiological problems showed extensive apoptosis of the endothelium, necrosis of a large number of smooth muscle cells in the subendothelial layer, and the presence of many and variously-sized medullary bodies. The question is whether apoptosis is beneficial or detrimental to the grafts. It would be beneficial if it occurred only in the

macrophages of atheromatous lesions and if the apoptotic bodies moved quickly away from the region. Since apoptosis of macrophages always coexists with apoptosis of smooth muscle cells, which is also more intense, the atheromatous plaque is weakened to the point of rupture, with all the complications that ensue. A reduction of blood lipids has been reported to be accompanied by a reduction in the rate of apoptosis in smooth muscle cells.[22]

Venous grafts are widely used by surgeons today. However, the atheromatosis to which they are always subject and which begins immediately after their implantation to the arterial circulation, reduces their lifespan and raises an urgent need for the investigation and effective management of this predisposition. Nowadays, we are aware of genes in the vascular smooth muscle fibres that are overexpressed in atheromatosis and in post-stent stenosis.[50,51] The overexpression of DAP kinase at the mRNA level and of protein that has been recognised in atheromatous lesions may in the future be the beginning of a new pharmaceutical treatment of atheromatosis.[51] Successful reduction in coronary artery wall thickness and significant widening of the lumen have also been reported after intra-arterial irradiation with β- or γ-rays in animals that had undergone angioplasty.[52]

To sum up, prior to their implantation in coronary artery bypass surgery, grafts from the great saphenous vein and the internal thoracic artery already exhibit a significant degree of apoptotic and necrotic damage to the endothelium and to smooth muscle fibres in the intima and media. Such damage represents the start of new atheromatous lesions, or the deterioration of existing ones, which are considered responsible for a reduction in lifespan, especially in venous grafts. Apoptosis is the main phenomenon that predominates in occluded arteriovenous anastomoses and raises the question whether extensive apoptosis is the cause of the occlusion, or the occlusion the cause of the apoptosis.

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