Immunocytochemical Study on Endothelial Integrity of Saphenous Vein Grafts Harvested by Minimally Invasive Surgery with the Use of Vascular Mayo Stripers. A Randomized Controlled Trial


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Objectives. The aim of this study is to compare the endothelial integrity of saphenous vein grafts harvested by minimally invasive surgery and veins harvested conventionally for coronary artery bypass surgery in 200 participants who were assigned to interventions by using random allocation.

Design. Randomized controlled trial.

Methods. Immunocytochemistry with anti-CD 31 antibodies and anti-nitric oxide synthase (NOS) antibodies were employed to identify the endothelial integrity.

Results. The CD 31 immunostaining showed that the endothelial cell integrity of the minimally invasive harvested veins was preserved in 82 ± 13% of the circumference of luminal endothelium, while in conventionally harvested grafts it was reduced to 64 ± 15% (p = 0.05). This was associated with the lack of CD 31 expression in vasa vasorum (10 and 18%) in both groups, respectively, (p = 0.02). The NOS immunostaining revealed that the endothelial integrity of the minimally invasive harvested grafts was preserved in 96 ± 4% of the luminal endothelium circumference as compared to 74 ± 10% in conventionally harvested grafts (p = 0.05). The percentage of cases with the lack of NOS expression in all vasa vasorum was 12 and 21%, in G1 and G2, respectively, (p = 0.02).

Conclusion. The endothelial integrity of saphenous vein grafts harvested by minimally invasive surgery is better preserved than with the grafts obtained by the conventional manner. This could play an important role in improving vein graft patency rates.

Key Words: Saphenous vein harvesting; Minimally invasive surgery; Endothelial integrity; CD 31 antigen; Nitric oxide synthase.

Introduction

The autologous saphenous vein is the most commonly used conduit for coronary artery bypass surgery (CABG).1,2 Occlusion rates for saphenous vein grafts used in CABG are relatively high: up to 15–30% during the first year and approximately 50% within 10 years.3,4

The simplest method of saphenous vein harvesting includes a continuous skin incision over the entire length of the vein. In this conventional technique, the perivascular tissue is stripped from the vein and venospasm observed during vein harvest is overcome by vein distention. This may result in the significant denudation of the endothelium.5 Recently, less invasive approaches have been reported in order to minimize surgical trauma and decrease the number of postoperative complications.6–9 One of them includes saphenous vein harvesting with the use of vascular Mayo strippers.6 The rationale for this technique is that it provides a cushion of surrounding tissue and reduces venospasm, so that it eliminates the need of distention.

CD 31, expressed on all continuous endothelia, is a single chain type-1 transmembrane protein belonging to the immunoglobulin superfamily.10 Nitric oxide, which mediates vasodilatation and plays an important role in the maintenance of vascular tone, is synthesized from L-arginine in the vascular endothelium by the endothelial nitric oxide synthase (eNOS).11 Endothelial injury results in impaired nitric oxide synthase activity.
release by endothelial cells. Thus, the preservation of endothelial integrity in vein grafts may improve graft patency rates by preserving nitric oxide activity. The aim of this study was to compare, both histologically and immunocytochemically, the endothelial integrity of saphenous vein grafts harvested by minimally invasive surgery with the use of vascular Mayo strippers to those obtained by the conventional technique in a randomized controlled trial.

Patients and Methods

The study group comprised 200 patients whose autogenous saphenous veins were used for CABG in the Department of Cardiac Surgery, University of Medical Sciences in Poznań, Poland, between 2000 and 2002. All patients who were between 45 and 70 years of age were candidates for inclusion in the study. They were not admitted to the study if any of the following criteria were present: (1) age > 70 year, (2) body weight > 95 kg, or (3) the need of bilateral vein harvesting. Patients were simply randomized, according to a random coin-throw, to harvest a saphenous vein either by means of the Mayo technique (group 1) or the conventional technique (group 2). An adequate allocation concealment was performed to protect the sequence after allocation. Local ethics committee approval and informed consent from patients was obtained.

In group 1 (G1, \( n = 100 \)) the skin was incised over a length of 1–2 cm at the site of branches traced during USG-Doppler examinations. The vein between skin incisions was dissected free with the use of vascular Mayo strippers. When the distal end of the vein was ligated, it was cut and introduced into the stripper’s eye. Vascular stripper movements along the vein’s course caused dissection of adjacent soft tissues. The mean time taken to harvest the saphenous vein in this method was 63 ± 16 min. The mean length of harvested graft was 34 ± 9 cm.

In group 2 (G2, \( n = 100 \)) the conventional technique was employed. The mean time taken to harvest a vein for CABG using this technique was significantly longer (77 ± 19 min, \( p < 0.05 \)). The mean length of a vein graft, as compared to G1, did not differ significantly (35 ± 7 cm). Also, patient age, height, weight and rate of diseases potentially influencing wound healing did not differ between groups (Table 1).

The distal part of harvested veins (about 1 cm long) were taken with their surrounding tissue for immunohistochemical analysis just prior to grafting. All tissue samples were fixed in a 10% formalin solution, embedded in paraffin and cut into 5–6 μm thin sections.

CD 31 antigen and eNOS detection involved the use of the immunocytochemical procedure with mouse monoclonal antibodies against human CD 31 (Dako, M 0823) and against human endothelial eNOS (Sigma, N 9532) consequently, followed by the StreptABCComplex/HRP method modified by biotinylated tyramine (Dako Catalysed Signal Amplification System, Peroxidase, K 1500). The endogenous activity of peroxidase was blocked by a 10 min pre-incubation in 10% hydrogen peroxide. The sections were incubated with anti-CD 31 antibodies diluted 1:500–1:1000 and anti-eNOS antibodies diluted 1:3000 for 18 h at +4 °C. Incubation with the second antibody (biotinylated goat anti-mouse, Dako E 0433, diluted 1:300) was performed in room temperature for 60 min. This was followed by incubation with diaminobenzidine (Dako S 3000). Both analyses were performed blind on coded samples and complied with the principles of positive and negative controls.

Quantitative assessment of CD 31 stainings of saphenous vein sections were carried out using Olympus MicroImage imaging software. This was performed on vessels from all 200 patients. The amount of positive luminal immunostaining was measured with a Polaroid CCD camera attached to a Nikon Eclipse E 600 microscope at ×4 magnification. The perimeter of the lumen was measured and endothelial integrity, defined by CD 31 and eNOS stainings, was expressed as a percentage of the lumen length (Table 2).

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<th>Table 1. Preoperative patient data</th>
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<td>G1 (( n = 100 ))</td>
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<tr>
<td>Age (year)</td>
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<td>Height (cm)</td>
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<td>Length of vein graft (cm)</td>
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<td>Time of vein harvesting (min)</td>
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<td>Diabetes mellitus (number of cases)</td>
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<td>Obliterative atheromatosis (number of cases)</td>
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To have an 85% chance of detecting a significant difference in endothelial integrity between the two groups, with an assumed standard deviation of 20.0, 100 patients (200 in total) in each group were required. The results were reviewed every six months to enable the study to be terminated early if clear results emerged. According to this, three interim analyses were performed during the trial. The results, however, were not statistically significant until the final analysis.

Data analysis was performed by means of paired and unpaired t-student and chi-squared tests with continuous Yates’ correction. $P < 0.05$ was considered as statistically significant.

**Results**

*Diagrammatic representation of the sample size is presented in Fig. 1*

The CD 31 immunostaining showed that the endothelial cell integrity of the minimally invasive harvested veins was preserved in $82 \pm 13\%$ of the luminal endothelium circumference, while in conventionally harvested grafts it was reduced to $64 \pm 14\%$ ($p = 0.05$). In the former, (Fig. 2(a)), positive CD 31 immunostaining was often evident as folds within the vessel lumen, whereas staining in traditionally obtained vessels (Fig. 2(b)) was patchy and less folded due to the saline distension. In the minimally invasive harvested grafts, immunostaining was also present in some vasa vasorum (Fig. 2(c) and (d)). The percentage of cases with the lack of CD 31 expression in vasa vasorum was 10 and 18%, in G1 and G2, respectively, ($p = 0.02$).

NOS was identified both in the luminal and microvascular endothelium. In G1, the presence of NOS was almost continuous with the perimeter of the lumen in all vessels harvested by means of the minimally invasive technique (Fig. 2(e)) while in the traditionally harvested grafts it was reduced (Fig. 2(f)). This immunostaining showed that the endothelial integrity of the minimally invasive harvested grafts was preserved in $96 \pm 4\%$ of the circumference of the luminal endothelium as compared to $74 \pm 10\%$ in the conventionally harvested grafts ($p = 0.05$). The expression of NOS in vasa vasorum was present in perivascular tissues (G1) (Fig. 2(g)) and in the media (G1 and G2) (Fig. 2(h)) of...
saphenous grafts. The percentage of cases with a lack of NOS expression in all vasa vasorum was 12 and 21%, in G1 and G2, respectively, (p = 0.02).

No marked surgical complications were observed in G1 during the postoperative period. In G2, one patient required revision due to wound bleeding, and in four cases wound infection followed by delayed healing were noted.

Discussion

Damage to the venous wall as a consequence of incautious dissecting is a significant factor influencing the patency of aorto-coronary grafts during the long-term follow-up. It facilitates thrombus formation within its lumen, and eventually may lead to occlusion of a part of venous grafts even one year after the operation. Results of post-mortem examinations conducted by other authors demonstrate that even more that 70% of occluded graft cases are preceded by parietal thrombi covering the damaged endothelial surface. On the other hand, one of the major factors associated with vein graft failure is thought to result from the adaptation of the vein to arterial conditions (increased pressure, pulsative motion). The reconstruction of the venous wall can take place if the endothelial integrity is well preserved. Nitric oxide plays an important role by influencing vascular smooth muscle cell proliferation and migration. Removal of the adventitia, which usually occurs in conventional vein harvesting, reduces the tissue mass of the graft vein, making it more prone to distention, endothelial damage and subsequent graft failure. There is also evidence to show that adventitial injury is involved in vessel occlusion, via activation of adventitial fibroblasts and local tissue ischaemia as a result of damage to adventitial microvessels. Preservation of the adventitia has been suggested to improve vein graft patency.

The introduction of the new minimally invasive technique for vein harvesting could result in more expressed saphenous vein damage than when using the conventional technique. The first prospective study to assess the functional quality of a saphenous vein harvested with the use of a vascular Mayo stripper was undertaken between 1979 and 1984. Both light electron microscopy and scanning electron microscopy demonstrated that the harvested vein was of good quality and that the intima had been well preserved. Postoperative graft angiography and exercise testing confirmed high patency rates for the vein graft (93%). This method, however, has not been universally accepted because of a perceived increase in injury to the venous conduit. For this reason, in 1997 and 1998 studies were assessed to check the vascular reactivity and endothelial integrity of veins harvested with the use of Mayo stripper. To compare contractile function, a saphenous vein was harvested both by Mayo stripper and in the traditional ‘open’ technique in the same patient. The study revealed that there was no significant difference in response to both constrictors and dilators, in a medium containing 5-hydroxytryptamine or noradrenaline, between vein grafts taken using both methods. The endothelial release of vasoactive substances after endoscopic harvesting was also similar to that after the traditional, extended incision technique. Histological examination by light and electron microscopy was unable to show any significant damage to the vessel wall. However, these studies enrolled relatively small numbers of patients and employed varied designs.

In our study, the endothelial integrity of saphenous vein grafts was assessed by means of the endothelium-specific antibody anti-CD 31 and anti-eNOS. Before this analysis was performed, the authors expected that endothelial integrity in minimally invasive harvested grafts would not be worse than that in traditionally obtained veins. The presented randomized controlled trial has revealed that endothelial integrity is even better preserved than that in conventionally harvested grafts. This integrity could also be observed in vasa vasorum.

Although the CD 31 antigen is a useful marker for

Fig. 2. (a) The CD 31 immunostaining of luminal endothelium (arrows) of saphenous vein graft harvested by means of minimally invasive surgery. It is evident as folds within the vessel lumen ×100. (b) The CD 31 immunostaining of luminal endothelium (arrows) of saphenous vein graft obtained in traditional manner. The staining is patchy and less folded due to the saline distention ×100. (c) The CD 31 immunopositive microvascular endothelium (arrows) in media of saphenous vein section presented on Fig. 1. Luminal endothelium with positive expression of CD 31 (stars) is also seen ×250. (d) Immunocytochemical staining of CD 31 in vasa vasorum (arrows) of saphenous vein graft from G2 group ×250. (e) The immunocytochemical presence of NOS in the luminal epithelium of saphenous vein graft harvested by means of minimally invasive surgery (stars). The expression of NOS is also present in microvascular endothelium of media and adventitia (arrows) ×100. (f) Extremely reduced NOS immunostaining of luminal endothelium of saphenous vein graft obtained in traditional manner ×100. (g) The immunocytochemical expression of NOS in microvascular endothelium in media (arrows) of saphenous vein graft from G1 ×250. (h) The immunocytochemical expression of NOS in microvascular endothelium in media (arrows) of saphenous vein graft from G2 ×250.
the presence or absence of endothelium, its presence does not exclude the possibility that intimal hyperplasia may develop—the pathologic lesion responsible for the majority of early vein graft failures.  

On the other hand, the results of NOS immunodetection suggest that the enzyme expression is also better preserved on the luminal endothelium of grafts harvested by the minimally invasive method as compared to the traditional technique. Apart from being located on the luminal endothelium, NOS was also expressed in the microvascular endothelium within the media (G1 and G2) and adventitia (G1), which may result in a better supplementation of NO to the media. Vessels harvested by means of minimally invasive surgery might maintain their NO-producing capacity in vivo, which could reduce the incidence of vessel occlusion by promoting vasodilatation and reducing platelet aggregation and thrombus formation.

The available literature repeatedly signifies that different minimally invasive surgery techniques limit surgical complications and postoperative pain. Only individual reports demonstrate that minimally invasive techniques enable improved preservation of the endothelial integrity in lumen of harvested grafts and in vasa vasorum with the use of the immunocytochemical technique and electron microscopy study. These methods also result in a lower occlusion rate in a prospective study of coronary artery bypass surgery.

Despite the size and duration of this trial, it is important for clinicians to be able to estimate the occlusion rate for saphenous vein grafts harvested by the Mayo technique. Therefore, the observation of the study group (up to 10 years) must be fully completed. Consequently, the results of this study do not address the occlusion rate in saphenous vein grafts. Moreover, the present results, for patients who did not meet eligible criteria, cannot be simply extrapolated to all patients seen in general clinical practice.

Conclusion

The endothelial integrity of saphenous vein grafts harvested by minimally invasive surgery is better preserved as compared to grafts obtained by the conventional manner. The preservation of endothelium in minimally invasive techniques could play an important role in improving long term vein graft patency rates of vein grafts.

References


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