Original Article

Novel No-touch Technique of Harvesting the Saphenous Vein for Coronary Artery Bypass Grafting

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Summary

Background: **Optimization of the saphenous vein for myocardial revascularization.**

Objective: **To present the no-touch technique of the saphenous vein preparation. This technique consists of harvesting the vein with a pedicle of surrounding tissue, which protects the vein from spasms, obviating the need for distension.**

Methods: **A prospective, randomized study with 156 patients who underwent artery bypass grafting was performed comparing three saphenous vein harvesting techniques: conventional, intermediate, and no-touch. A morphological study of the endothelium was carried out using scanning microscopy. An angiographic assessment of the vein graft patency was performed at a mean follow-up time of 18 months. Also, an immunohistochemical assessment was carried out to identify the endothelial enzyme nitric oxide synthase (eNOS) in the vein wall.**

Results: **The preservation of the endothelial cell integrity was greater in the no-touch technique than in the other procedures. At angiographic follow-up, the patency for the no-touch group was 95.4%, 88.9% for the grafts of the conventional technique group, and 86.2% for the grafts performed in the intermediate technique group. The immunohistochemical assessment revealed eNOS in all three layers of the vein wall in the no-touch group and reduction of this enzyme in the conventional group.**

Conclusion: **The endothelial integrity and eNOS activity were better preserved when using the no-touch technique for vein graft harvesting. The mechanical protection provided by the cushion of surrounding tissue in the no-touch group, the vasorelaxation and thromboresistant activities of nitric oxide may be responsible for the reduction of vasospasms and improved patency rate. (Arq Bras Cardiol 2008; 90(6): 356-362)**

Key words: **Saphenous vein; endothelium, vascular; myocardial revascularization.**

Introduction

Atherosclerotic coronary disease is the major cause of morbidity and mortality in industrialized countries. Over a million people die every year in the world due to atherosclerotic coronary disease.[1] Myocardial revascularization using saphenous vein grafts, developed in the 1960s, deeply affected the outcomes and natural history of ischemic coronary disease and improved patient quality of life. However, graft failure may lead to recurrent angina[2], one of the several causes of redo surgeries. About 30% of saphenous vein grafts occlude in the first year after revascularization[3], and this rate reaches over 50% at 10 years.

The recent use of arterial grafts has yielded better long-term results, but saphenous vein grafts are still widely used in coronary artery bypass grafting (CABG)[4,5]. Several procedures have been adopted to increase the long-term patency of saphenous vein (SV) grafts. The preparation of the SV plays a very important role in outcomes because inadequate manipulation of the SV may cause morphological and functional damage to the graft[4].

This study evaluated a no-touch technique to dissect the SV with surrounding tissue, which ensures very little manipulation and avoids graft distention[6], and analyzed the clinical importance of this technique in the preparation of the saphenous vein.

Methods

Patient selection **-** After approval by the Ethics Committee and written informed consent from participants, 156 patients underwent CABG in the Cardiothoracic Surgery Division of the University Hospital of Örebro, Sweden. Patients were randomly assigned to three groups according to the techniques used for saphenous vein preparation. Inclusion criteria ensured that patients would be available for long-term follow-up. Surgeries were performed from June 1993 to April 1997.

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Manuscript received February 02, 2007; revised manuscript received September 05, 2007; accepted October 16, 2007.

Saphenous vein preparation techniques.

Conventional technique - C group **-** The saphenous vein was harvested through a longitudinal incision in the leg. The adventitial layer was removed, and tributaries were ligated with 3-0 cotton thread suture. The vein was removed immediately after dissection, distended with saline solution injected with a syringe at a constant pressure of 300 mmHg for one minute, and then stored in saline solution at room temperature.

Intermediate technique - I group **-**The saphenous vein was dissected as in the C group, but was not immediately distended. It was left in situ and covered with a compress soaked in saline solution containing papaverine (1 mg/ml), and perfusion was maintained. After removal, the SV was stored in blood obtained from the cannula introduced in the aorta. Although papaverine was used, all veins had to be distended because of spasm.

No-touch technique - NT group **-** A continuous incision in the leg exposed the saphenous vein. All visible tributaries were ligated at about 0.5 cm from the SV wall with 3-0 cotton thread suture. The SV and a surrounding tissue pedicle were isolated and left in situ; perfusion was maintained, and the vein was covered with a compress soaked in pure saline solution. After removal from the leg, the SV was stored in blood obtained from the arterial cannula. (Fig. 1)

In summary, the SV was stripped and distended in the conventional technique; in the intermediate technique, it was stripped but not distended; and in the no-touch technique, it was neither stripped nor distended.

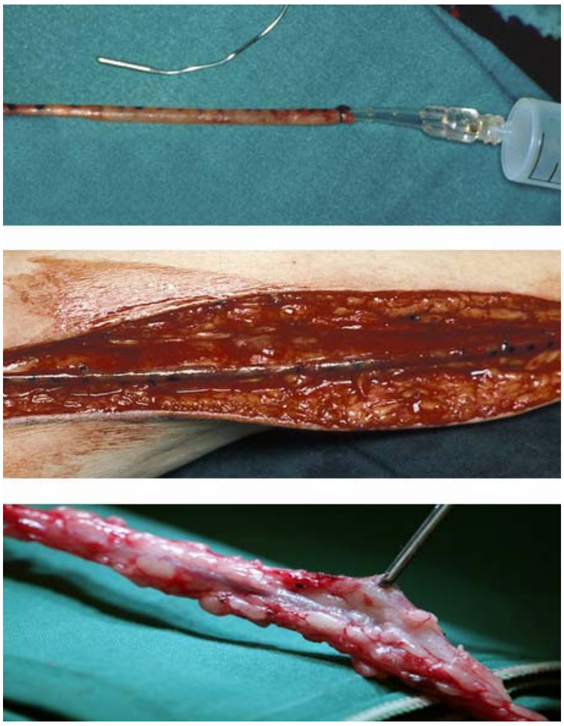

Figure 1 - *Saphenous vein grafts dissected and treated with conventional, intermediate or no-touch technique.*

Surgical aspects - Cardiopulmonary bypass (CPB) and cardioplegia at 4º C were routinely used, and the diameter of coronary arteries was measured with a probe.

In all groups, distal anastomosis was the first to be made. The proximal portion of the SV was rapidly connected to the arterial cannula of the CPB unit to check possible bleeding of anastomosis or tributaries in the NT and I groups. In the C group, SV was manually tested with a syringe containing saline solution connected to a pressure transducer. All internal thoracic arteries (ITA) were anastomosed to the anterior descending artery (ADA). The proximal anastomosis was made after aortic declamping. The SV characteristics, as well as its quality and origin (proximal, medial or distal) were recorded. Blood flow through the grafts was measured using ultrasound (Flow Measure Probe, Transonic System Inc., US).

Morphological evaluation - Endothelial morphology was evaluated using scanning electron microscopy (SEM) and light microscopy (LM) of SV segments obtained from ten consecutive patients in each group. Control segments were obtained from the distal portion of the vein immediately after removal. The control sample, also called the primary sample, was immediately fixed, and was not distended or stored. After proximal anastomosis was completed, another vein sample, named secondary, was obtained and immediately fixed.

Samples were fixed by immersion in 2% paraformaldehyde and 2.5% glutaraldehyde in Millonig phosphate buffer.

Immunohistochemical evaluation **-** In ten other patients, the proximal portion of the SV was prepared according to the NT technique, whereas the distal portion was treated using the conventional technique. Both segments were stored in heparinized blood up to the moment of distal anastomosis. Samples of SV excess were obtained after proximal anastomosis and the control sample was obtained from the stripped vein that was not distended.

Conventional immunohistochemical methods were used to detect endothelium and collagen. The SV samples were fixed in acetone, and serum activity of endothelial enzymes was inactivated to block possible changes in color. Samples were incubated with anti-CD31 antibodies to detect endothelial cells, and with rat monoclonal antibodies to detect collagen. A 3.3'-diaminovenxidine tetrahydrochloride solution was used as substrate; the sections were stored in Mayer hematoxylin and prepared for microscopy. Analysis with NADPH-diaphoresis (Sigma- Aldrich, Poole, Dorset, UK) was used to detect endothelial nitric oxide synthetase (eNOS), which is a precursor of nitric oxide.

In-vitro autoradiography and nitroarginine (NOARG) marked with a radioactive isotope were used to detect eNOS in endothelium. Slides were pre-incubated in buffered HCl solution and later incubated in a solution of 10 nM radioactive NOARG. After incubation, they were wiped and let to dry in open air. The slides were post-fixed in paraformaldehyde and kept in nuclear emulsion overnight. They were then stored in light-tight slide boxes and stored at 4º C for 12 weeks. After that, tissues were stained with

Mayer hematoxylin-eosin and examined using bright-field microscopy under black light.

This procedure was conducted as described above to rule out suspicions that the better veins were used for the NT technique. Our purpose was to investigate immunohistochemical effects on two segments of the same SV treated according to two different techniques.

Angiographic evaluation - Angiographic control using the Judkins technique was performed about 18 months after surgery[7]. Visipaque, a low osmolarity medium, was used as contrast (Nycomed Amersham AB, Stockholm). Angiographic evaluations were randomly performed by two radiologists. The grafts were visualized in two planes, and patency ratios, as well as the degree of localized and diffuse lesions, were determined. Occlusion was identified by locating the remaining graft neck after selective contrast injection in the proximal anastomosis and by the type of graft opacification after injection of 50 ml of contrast in the ascending aorta.

Statistical analysis - The unpaired Kruskal-Wallis test was used for the retrospective comparison of endothelial histological findings between the three groups, as described by Siegel & Castellan[8].

Logistic regression was used to evaluate graft patency. The main explanatory factor was the three different methods of vein preparation: conventional, intermediate and no touch. Additional factors were evaluated individually. Factors that were statistically significant and irregularly distributed between the three different techniques were selected and used in the final model.

Results

Patient characteristics **-** No significant patient differences were found between groups (Table 1). Patients treated because of high cholesterol levels or whose cholesterol values were above normal (2.6 – 5.0 μmol/l) were classified as patients with dyslipidemia. Hypertension was defined when diastolic pressure was greater than 90 mmHg and/or systolic pressure was greater than 140 mmHG. Ventricular function was determined according to ejection fraction.

The use of aspirin was initiated on the first postoperative day at a dose of 160 mg per day. At the time of angiographic examinations, 42 patients in the C group, 35 in the I group, and 35 in the NT group were receiving 160 mg/day; 3 patients in the C group, 4 in the I group, and 7 in the NT group, were receiving 75 mg/day; and one patient in the C group, 2 in the I group, and 3 in the NT group were not taking any antiplatelet drugs.

Morphological findings - Secondary samples from the NT group showed that cell integrity was similar to that found in the control group. Almost half of the endothelial surface of samples in the C group had no endothelial cells, and the endothelial integrity of samples in the I group varied between that of the C and of the NT group.

The comparison of samples of the three groups showed a significant difference in endothelial integrity, and p values for

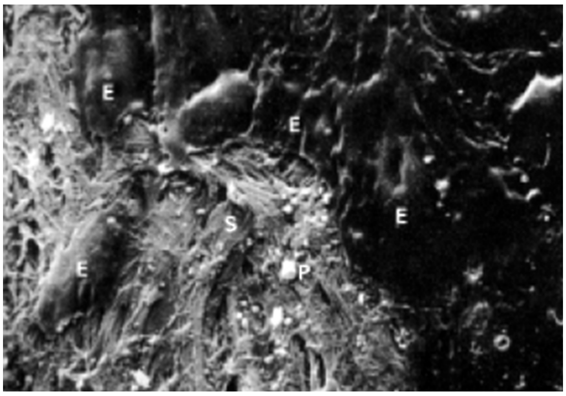

Figure 2 - *Electron microscopy of vein prepared with conventional technique. Discontinuity of endothelial cell layer (E); exposure of subendothelial connective tissue (S); adhesion and platelet activation (P). bar = 50μm.*

Table 1 - Sample characteristics

Patient characteristics	Conventional	Intermediate	No Touch
Number of patients (n)	46	41	45
Sex – Female/Male	6/40	8/33	7/38
Mean age at surgery (range)	58 (45-70)	60 (41-71)	58 (43-67)
Preoperative AMI n(%)	29 (63%)	19 (39%)	25 (56%)
Mean preoperative EF % (range)	63 (41-81)	67 (39-87)	67 (40-86)
Hyperlipidemia n(%)			
Preoperative	29 (63%)	19 (46%)	29 (64%)
18 months postoperative	22 (48%)	20 (49%)	27 (60%)
Smoking (%)			
Preoperative	9 (20%)	4 (10%)	9 (20%)
18 months postoperative	9 (20%)	1 (2%)	6 (13%)

p > 0.05. AMI - acute myocardial infarction.

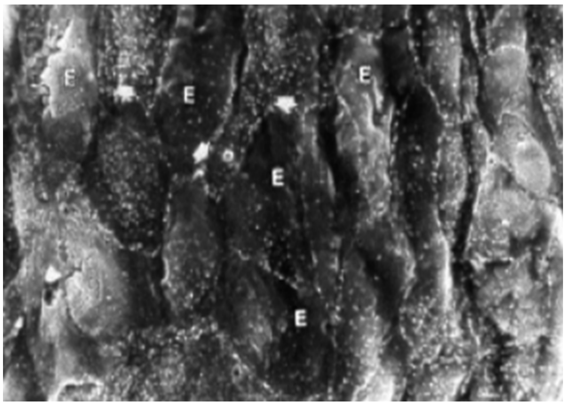

Figure 3 - *Electron microscopy view of vein prepared with no-touch technique. Endothelium is intact, and endothelial cells (E) have prominent borders and microvilli, which indicate that metabolic activity was preserved at time of fixation. bar = 50µm.*

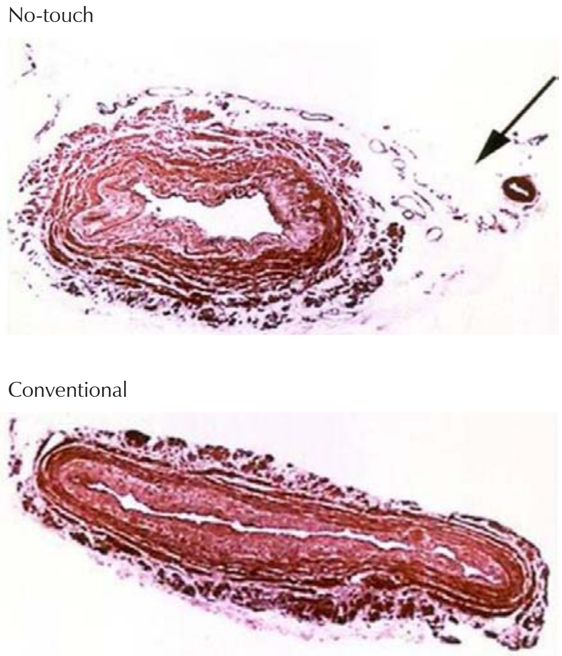

Figure 4 - *Preparation of samples using rat monoclonal antibody and anti-collagen IV antibody. Marked staining of adventitial layer of vein in NT group (not seen in vein of C group) and many microvessels. bar = 50µm.*

the comparisons were: control *versus* conventional samples - $p<0.01$; control *versus* intermediate samples - $p<0.05$; and control *versus* NT samples - $p<0.05$. Figures 2 and 3 show the degree of endothelial damage in samples of the NT and C groups.

Immunohistochemical findings - Immunohistochemistry to identify collagen fibers revealed an intact adventitial layer with microvessels in the NT group. No similar findings were seen in SV treated with the conventional technique (Fig. 4).

Endothelial nitric oxide synthase (eNOS) was identified by autoradiography of tissues incubated in a solution containing the NOARG marker. There was a direct association between NOARG binding to the lumen of vessels and area of intact endothelium, and the veins in the NT group had their morphological structure preserved. Such binding was reduced in the grafts of the C group (Fig. 5).

The analysis with NADPH-diaphoresis revealed a reduction of 19.5% in eNOS concentration in the samples of the I group (control), that is, the veins were stripped, but not distended, when compared with the sample of the NT group. The reduction was over 35% greater in the veins in the C group.

Surgical findings **-** Mean duration of aortic clamping and CPB was 121 (59 to 187) minutes and 64 (34 to 95) minutes for the C group; 126 (90 to 180) and 69 (41 to 117) for the I group, and 139 (88 to 195) and 72 (41 to 113) for the NT group. Patients in the three groups received saphenous vein and left internal thoracic artery (ITA) grafts.

A total of 127 SV grafts and 41 ITA grafts were implanted in the C group, 116 SV and 35 ITA grafts in the I group, and 124 SV and 42 ITA grafts in the NT group. In the C group, 107 free, 16 double sequential and 4 triple sequential grafts were used; In the I group, 100 simple, 14 double sequential and 2 triple sequential grafts were used; and in the NT groups, 109 simple and 15 double sequential grafts were implanted. The ITA was not considered a good graft in 5 patients of the C group, 5 of the I group, and in 3 of the NT group. Thromboendarterectomy (TEA) was performed in two posterior descending (PD) coronary arteries in the C group, and in one in the I group, but was not necessary in the NT group.

Mortality rate was 0% in the 3 groups. Perioperative acute myocardial infarction (AMI) occurred in two patients in the C group. One patient in the NT group was re-operated due to bleeding in the vascular bed of ITA. Minor complications were associated with leg wound, such as cellulites and superficial infection, and affected about 10% of the patients in each group.

Graft patency **-** One hundred thirty-two patients underwent angiographic control, 46 from the C group, 41 from the I group, and 45 from the NT group. Six patients in the C group, 11 in the I group, and 7 in the NT group refused the examination. Mean time of angiographic follow-up was 17.2 $\pm$ 4.4 months for the C group, 16 $\pm$ 2.6 months for the I group, and 16.3 $\pm$ 2.7 for the NT group.

SV graft patency in the NT, C and I groups was 118/124 (95.4%), 113/127 (88.9%) and 100/116 (86.2%). ITA patency was 108/118 (91.5%).

Grafts with low blood flow and anastomosed to small caliber veins had a high incidence of occlusion in the three groups (Table 2). The quality of coronary arteries did not affect graft patency in any of the groups. The two grafts in the C group that were anastomosed to PD after TEA worked well. Occlusion rates of low-quality veins were 4/10 (44.4%) in the C group, 6/19 (31.6%) in the I group, and 3/29 (10.3%) in the NT group.

All sequential grafts in the NT and I groups worked

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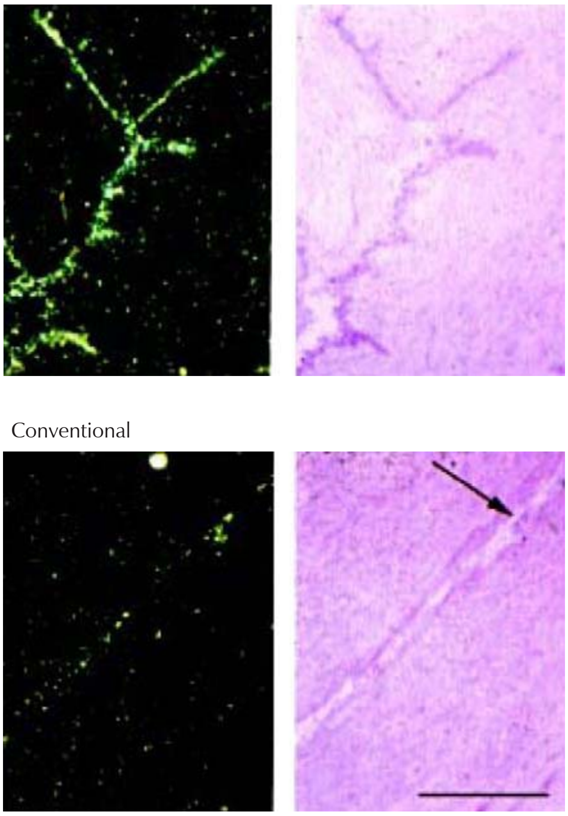

Figure 5 - Autoradiograph with labeled nitroarginine shows greater concentration of eNOS in endothelial layer of vein in NT group compared with sample prepared using conventional technique.

adequately. In the C group, however, two sequential grafts were totally occluded, and two were partially occluded. Therefore, 47/51 (92.2%) of all grafts were fully patent.

Logistic regression analysis of factors that substantially affected results of graft patency rates showed a better statistical difference in the NT group than in the C group (p=0.025). The poorest results were found in the I group (Table 2).

No correlation was found between graft occlusion and classical clinical factors that may affect results, such as age, preoperative AMI, cholesterol levels, smoking and hypertension.

Figure 6 shows a NT graft that was anastomosed to a 1 mm coronary artery. When the vein is protected by a fat pedicle, kinking does not occur (Fig. 7).

Discussion

For several reasons, SV and ITA are still widely used in CABG. Better SV graft patency is the greatest advance in cardiovascular surgery. The indiscriminate use of ITA grafts together with other grafts does not seem to be fully justified by scientific findings of controlled studies[5].

Structural differences between arteries and veins may explain the better results of arterial grafts[9]. At the same time, the damage to the SV wall, which occurs during graft preparation, may contribute to the poor results obtained with venous grafts. The ITA endothelium is protected during all the operation. The SV endothelium, however, suffers severe lesions during the procedure, and becomes thrombogenic[10] The proliferation of smooth muscle of the medial layer is similar in the uninjured vein and in the ITA[11]. Proliferation is greater in the veins that suffer endothelial lesion during operation. Therefore, the major cause of intimal layer hyperplasia, detected in the SV, may be a result not of intrinsic differences in the smooth muscles between arteries and veins, but of the greater sensitivity of veins to trauma.

Several strategies have been used to avoid vein occlusion. Many advances have come from medical therapy, pharmacological agents[12], and genetic therapy[13], and have undergone constant improvement. Surgical techniques to obtain the SV without important damage to the venous wall during the operation may represent an important improvement.

Removal of the SV with a pedicle of surrounding tissue protects the vein from spasms and, consequently, avoids the need for distension. Moreover, the pedicle can be useful for the careful manipulation of the vein from its removal to the time of implantation.

The no-touch technique results in excellent preservation of endothelial integrity, which is confirmed by electron microscopy[14,15]. The fat pedicle contains numerous collagen fibers that may provide external protection to the vein wall against the deleterious effects of aortic pressure. Changes in the distribution of eNOS in the SV prepared using the NT technique were compared with those of veins treated with the conventional technique[16]. We found that eNOS was preserved in the endothelium, both in the medial and adventitial layers, in the veins of the NT group. The amount of eNOS was reduced when stripping and distention were used, which suggests that these procedures contribute to a reduction of eNOS in the veins prepared with the conventional technique. Distention plays an important role in the reduction of eNOS concentration in the endothelium and in the medial layer; and a large amount of eNOS is removed from the adventitia when the vein is stripped. The presence of eNOS indicates that available nitric oxide, usually reduced in the SV treated with the conventional technique[17], may be found in high concentrations in the veins treated with the NT technique. This may be one of the many explanations for the better results of the NT technique.

Initial patency of SV treated with the NT technique was very high (95.4%) even in grafts with low blood flow[18]. To investigate whether the fat pedicle had a role in the improved patency of venous grafts, the angiographic results were compared between grafts treated with the NT technique and those treated with the I and C techniques. Results showed that patency was better when the NT technique was used (p=0.02).

When the vein is stripped of surrounding tissue, graft size has to be carefully adjusted to avoid the risk of kinking and later functional impairment. Several techniques have been

Table 2 - **Analysis of simple graft patency of the three groups according to logistic regression model, additional explanatory factors and vein preparation technique.**

	No. of grafts - Patent / Total (%)	Odds	95% CI	P value
Technique				
Conventional (reference)	95/107 (88.7)	1.0	-	-
Intermediate	84/100 (84)	0.9	0.3-2.2	0.745
No-touch	103/109 (94.5)	3.9	1.2-12.6	0.025
Diameter of coronary artery (mm)				
1.0 (reference)	21/31 (67.7)	1.0	-	-
1.5	162/181 (89.5)	3.2	1.2-8.3	0.016
≥ 2.0	99/104 (95.2)	4.7	1.4-15.4	0.0011
Vein quality				
Good (reference)	236/257 (91.8)	1.0	-	-
Poor	46/59 (77.9)	0.2	0.1-0.2	0.001
Vein segment				
Distal (reference)	92/96 (96.8)	1.0	-	-
Medial	103/123 (83.7)	0.2	0.1-0.8	0.019
Proximal	87/97 (89.6)	0.2	0.1-0.9	0.04
Graft flow (ml/min)				
≤ 20 (reference)	8/64 (75)	1.0	-	-
21-40	87/98 (88.7)	1.6	0.6-3.8	0.336
≥ 41	147/154 (95.4)	4.9	1.8-13.4	0.002

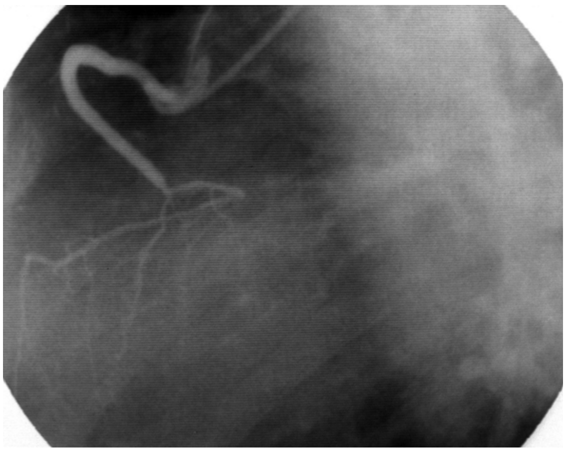

Figure 6 - Saphenous vein graft of NT group anastomosed to coronary artery with a 1 mm diameter.

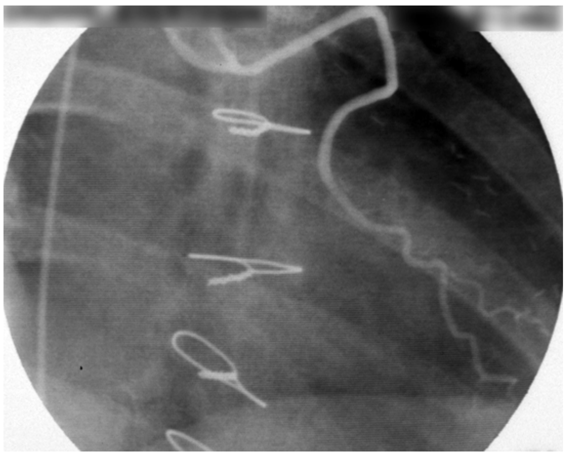

Figure 7 - Long saphenous vein graft of NT group without kinking.

recommended to avoid kinking[19], but our study shows that it may be simply avoided if the SV is removed with a fat tissue pedicle.

Conclusions

Endothelial integrity was better when the no-touch technique was used. The adventitial layer and other structures in the fat tissue have mechanical and functional properties that protect the SV from spasms and ischemia. The activity of a nitric oxide precursor (eNOS) in the endothelium and also in the microvessels of the adventitial layer, suggests that the availability of nitric oxide is preserved in the grafts treated with the NT technique. Consequently, nitric oxide may be one of the factors responsible for the increase in initial patency of the SV grafts in the NT group. In addition, surrounding tissue protects excessively long grafts against kinking.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This article is part of the thesis of Doctoral submitted by Domingos Sávio Ramos de Souza, from Acta Universitatis Upsaliensis – UPPSALA – Faculty of Medicine.

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