Endoscopic versus conventional radial artery harvest for coronary artery bypass grafting: Functional and histologic assessment of the conduit

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Background: The radial artery's propensity for vasospasm and vulnerability to surgical trauma are well known. A less invasive endoscopic method to harvest the radial artery was recently introduced, but its effect on radial artery integrity is unknown.

Methods: To compare the effects of harvest method on radial artery function, we prospectively randomized 54 patients undergoing coronary artery bypass grafting with the radial artery into 3 groups on the basis of harvest techniques: endoscopic, conventional with cautery, and conventional with harmonic scalpel. We assessed endothelium-dependent and endothelium-independent relaxation of radial artery segments to sequential doses of acetylcholine and nitroglycerin, respectively, using standard organ-chamber methodology. Vasospasm was assessed as the vasoconstrictor response to the thromboxane analog U46619. We assessed endothelial integrity using light and electron microscopy and by rating intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and P-selectin expression by means of immunohistochemistry on a semiquantitative 0- to 3-point scale. Harvest procedures were performed by a single surgeon, and data analyses were blinded to the harvesting method.

Results: Maximal relaxation-contraction responses to acetylcholine, nitroglycerin, and U46619 and effective drug concentration yielding 50% response were similar in the 3 groups. Adhesion molecule expression and histologic changes, as assessed by means of light and electron microscopy, were similar in the 3 groups.

Conclusions: Endoscopic harvest does not alter radial artery vasoreactivity or endothelial integrity compared with conventional harvest techniques. Because the endoscopic technique is less invasive, it might prove to be the technique of choice to harvest the radial artery.

The radial artery has become the second arterial graft of choice after the internal thoracic artery in patients undergoing coronary artery bypass grafting.¹ Improved patency rates of the radial artery observed in recent years were attributed to improved harvesting technique and routine use of antispasmodic agents, such as calcium-channel blockers or nitrates.^{2,3}

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Abbreviations and Acronyms

ANOVA	= analysis of variance
EC ₅₀	= drug concentration producing 50% of maximal relaxation
ICAM-1	= intercellular adhesion molecule 1
NO	= nitric oxide
PBS	= phosphate-buffered saline
VCAM-1	= vascular cell adhesion molecule 1

The overall morbidity of conventional radial artery harvesting is not insignificant and includes bleeding, hematomas, and wound infection, as well as motor and sensory nerve deficits.¹⁻⁵ The cosmetic results are also of major concern for many patients because the resulting scar is plainly visible. In an effort to reduce the morbidity of radial artery harvesting, a less invasive endoscopic method to harvest this artery was recently described.⁶⁻⁸ Reported early clinical outcomes of endoscopic radial artery harvesting are excellent.⁶⁻⁸ However, the endoscopic technique involves manipulation of the conduit and dissection with harmonic shears in a narrow tunnel, potentially injuring the conduit and inducing vasospasm.⁶⁻⁸

The purpose of this study was to assess the effect of the endoscopic harvesting technique on the vasoreactivity and structural integrity of the radial artery compared with 2 conventional harvesting techniques in a prospective randomized fashion.

Patients and Methods

Patients

Fifty-four patients undergoing first-time isolated coronary artery bypass grafting with the use of the radial artery were enrolled in the study. The Boston University Medical Center Institutional Review Board approved the study protocol, and informed consent was obtained from each patient. By using a computer-generated random list, patients were prospectively randomized into 3 groups on the basis of radial artery harvest technique: endoscopic (Endo group), conventional with cautery (ConC group), and conventional with harmonic shears (ConH group).

Radial Artery Harvesting

The radial artery was usually harvested from the nondominant hand. A modified Allen test was performed preoperatively to screen patients for radial artery harvesting. It was repeated intraoperatively with the use of a pulse oximeter placed on the thumb to ascertain an intact palmar arch. Harvest procedures were performed by a single surgeon (OMS).

Conventional radial artery harvesting was performed according to the method described by Reyes and colleagues² through a full-length forearm incision along the course of the radial artery. Key points include harvesting the radial artery as a pedicle with the accompanying veins and fat, minimal manipulation of the graft ("no-touch" technique), and avoiding probing or hydrostatic dila-

tion of the conduit. In the ConC group dissection of the pedicle was performed by using low-intensity electrocautery, and control of the branches was achieved with stainless-steel vascular clips. In the ConH group dissection of the pedicle and control of the branches were performed with Harmonic shears (UltraCision Harmonic Scalpel, Ethicon Endo-surgery Inc) without clipping.

The endoscopic radial artery technique was performed as described by Connolly and associates.⁶ Briefly, the patient was placed supine with the arm abducted and fixed on a board and the wrist in hyperextension. A 3-cm longitudinal skin incision starting 1 cm proximal to the radial styloid was performed. The radial artery was dissected as a pedicle with its accompanying veins and fat. The Ultra-Retractor (CardioVations) was then introduced, and dissection of the radial artery pedicle was performed with video assistance with a 30° scope by using the harmonic shears (UltraCision Harmonic Scalpel). The dissection extended proximally to just distal to the brachial artery bifurcation. Proximal control of the radial artery was achieved with an Endo-loop ligature (Ethicon). The artery was divided with Endo-scissors (Ethicon), gently flushed with heparinized Plasma-Lyte solution (Baxter Healthcare Corp), and stored in a papaverine basin (King Pharmaceuticals Inc).

Intravenous nitroglycerin was commenced after induction of anesthesia and continued for 24 hours after the operation, switching to oral nitrates for 6 to 12 months postoperatively, as previously described.³

Materials

Kreb's buffer contained the following: NaCl, 118.3 mmol/L; KCl, 4.7 mmol/L; CaCl₂, 2.5 mmol/L; MgSO₄, 1.2 mmol/L; KH₂PO₄, 1.2 mmol/L; NaHCO₃, 25.0 mmol/L; glucose, 11.1 mmol/L; and Na₂EDTA, 0.026 mmol/L. U46619 was obtained from Cayman Chemicals, acetylcholine from Sigma Aldrich, and nitroglycerin from Baxter. Primary anti-P-selectin, vascular cell adhesion molecule 1 (VCAM-1), and intracellular adhesion molecule 1 (ICAM-1) antibodies for immunohistochemistry were obtained from BD PharMingen, DakoCytomation, and Santa Cruz Biotechnology, respectively.

Organ-chamber Methodology

The time interval between radial artery harvesting and grafting ranged between 30 and 45 minutes. On grafting, the exact vessel length needed was determined, and segments of the radial artery that would otherwise be discarded were placed in ice-cold Kreb's buffer and immediately transferred to the laboratory for studies of vascular function. Segments were consistently taken from the proximal end of the vessel in all 3 groups to truly evaluate the effect of the endoscopic technique. The vessels were carefully dissected from their surrounding fat tissue and cut into 2 to 3 segments measuring 3 to 4 mm in length. Vessel segments were placed in organ chambers (37°C) containing 25 mL of Kreb's buffer, suspended between 2 tungsten stirrups for measurement of isometric tension (as previously described),⁹ and constantly aerated with 95% O₂/5% CO₂. Each vessel was then progressively stretched in 1-g increments to its optimal resting tension that produced a maximal response of 80 mmol/L KCl. Vessels were then allowed to equilibrate for 1 hour before introduction of vasoactive drugs, as previously described.⁹ Relaxation studies

were performed after vessels were precontracted with 0.1 to 1 $\mu\text{mol/L}$ of the thromboxane analog U46619 so that contraction was 60% to 70% of maximal KCl-induced contraction. The contractile response to U46619 was assessed to evaluate radial artery vasospasm. All experiments were done in the presence of 10 $\mu\text{mol/L}$ indomethacin (INN: indometacin) to inhibit prostanoid synthesis.

Light and Electron Microscopy

Tissue samples from randomly selected patients ($n = 8$; ConC group, $n = 2$; ConH group, $n = 2$; and Endo group, $n = 3$) were processed for light microscopy to assess vascular morphology and endothelial integrity. Segments of the radial artery were fixed with a solution of 10% formalin in phosphate-buffered saline (PBS), pH 7.4, for 20 minutes. Segments were then cleaned and washed in cacodylate-sucrose. Samples were then dehydrated, embedded in paraffin, and sectioned (5 $\mu\text{mol/L}$). Two sections from each vessel were stained with hematoxylin and eosin and evaluated by a pathologist (LJ) blinded to the harvest technique for neutrophil, eosinophil, and lymphocyte infiltration and evidence of endothelitis and vasculitis.

For electron microscopy, segments of radial arteries were fixed overnight in 2.5% glutaraldehyde in 0.2 mol/L sodium cacodylate buffer at 4°C. They were washed in buffer and postfixed in 1% aqueous osmium tetroxide for 1 hour. They were dehydrated in acetone and embedded in Epon Araldite. Ultrathin cross-sections of endothelium were cut and stained with uranyl acetate and lead citrate. Sections were examined in a transmission electron microscope at a magnification of 7000 $\times$. Random micrographs of endothelial cells were obtained and studied for signs of injury by a pathologist (TGC) blinded to the harvesting method.

Immunohistochemistry

Midsegments of radial artery specimens obtained from 45 patients were immediately washed with cacodylate-sucrose buffer for 5 minutes and then fixed with 3% glutaraldehyde in 0.1 mol/L solution of cacodylitic acid for 24 hours to assess the expression of leukocyte adhesion molecules. Specimens were then immersed in OCT compound (Sakura Tissue-Tek), snap-frozen in liquid nitrogen, and stored at -80°C . Sections (9 μm) were cut from the middle of the block, placed on precleaned microscope slides (Fisher), immersed in acetone, dried, and then rehydrated in PBS. The slides were then placed in a Biogenex I-6000 machine for incubation with the primary anti-human P-selectin, VCAM-1, and ICAM-1 antibodies. Primary antibody was diluted in Biogenex antibody diluent at concentrations of 1:200, 1:200, and 1:50, respectively. P-selectin and VCAM-1 slides were left to incubate at room temperature with the primary antibody for 30 minutes. ICAM-1 slides were left at 4°C overnight and warmed up for 20 minutes in the morning before proceeding with the protocol.

For secondary antibody staining, slides were washed in PBS and treated with StrAviGen Multilink Biotinylated Kit (Biogenex). Slides were treated with biotinylated secondary antibody (20-minute incubation) and streptavidin (20 minutes), respectively, at room temperature. Next, the Biogenex DAB kit was applied for 5 to 10 minutes for chromagen visualization. After DAB treatment, the slides were washed in distilled water and counterstained with hematoxylin (Shandon-Lipshaw) for 30 seconds. Intensity of stain-

ing was assessed by an average score of 3 observers blinded to the harvest technique using a semiquantitative 0- to 3-point scale (0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining).

Data Analysis

Unless otherwise specified, data are expressed as means $\pm$ standard deviation or as absolute values with percentages. Vessel relaxation is expressed as percentage reduction in tension induced by U46619. EC_{50} represents the drug concentration producing 50% of maximal relaxation determined by means of sigmoidal curve fitting with the use of commercially available software (Origin, Microcal Inc). Data analyses were blinded to the harvesting method. The clinical characteristics for the 3 groups were compared by using 1-way analysis of variance (ANOVA) for continuous variables and the χ^2 test for categorical variables.

The peak relaxation response to acetylcholine in a prior study⁹ was $92\% \pm 16\%$. The sample size of 18 subjects per group (total of 54 patients) was calculated to provide 80% power ($\alpha = .05$) to demonstrate a 17% difference between groups in the maximal relaxation response to acetylcholine. The dose-dependent relaxation-contraction responses to acetylcholine, nitroglycerin, and U46619 were compared for the 3 groups by using 2-way ANOVA (with group and dose as the 2 factors). The Kruskal-Wallis 1-way ANOVA on rank test was used to compare histologic changes and adhesion molecule expression between the groups. Statistical analyses were performed with SigmaStat for Windows software (version 3.0.1, 1992-2003; SPSS, Inc).

Results

Patients

Baseline patient profile of the 3 study groups is depicted in Table 1. As a result of the randomization, baseline patient profile was similar among the groups. There were no statistically significant differences among the groups with respect to risk factors known to affect endothelial function.

Clinical Outcomes

Harvest time was longer with the endoscopic technique (Endo group, 61 ± 24 minutes, ConC group, 45 ± 19 minutes; ConH group, 41 ± 10 minutes; $P = .0005$). Clinical outcomes were similar among the groups, with no mortality, postoperative myocardial infarction, or stroke in any group. Radial artery harvest-related outcomes were also similar, with no incidence of hand ischemia, motor deficits, hematoma, or wound infection. There was a significant difference with respect to sensory deficits. Patients undergoing conventional harvest had sensory symptoms (paresthesias and numbness) in the distribution of both the lateral antebrachial cutaneous nerve and the superficial radial nerve. In contrast, patients undergoing endoscopic harvest had sensory symptoms limited to the superficial radial nerve only. Cosmetic results were clearly superior after endoscopic harvest. Endoscopic harvest was more expensive. (An endoscopic harvest kit, including the disposable harmonic shears, costs \$550.)

TABLE 1. Baseline profile of the study groups

	ConC (n = 18)	ConH (n = 18)	Endo (n = 18)	P value
Age (y)	58 ± 12	62 ± 12	60 ± 10	.577
Sex (male/female)	14/4	16/2	12/6	.276
CCS angina class	3.2 ± 0.7	3.1 ± 0.8	2.9 ± 1.0	.466
LVEF (%)	51 ± 14	54 ± 11	53 ± 13	.773
Hypertension	15 (83%)	14 (78%)	13 (72%)	.730
Diabetes mellitus	11 (61%)	4 (22%)	8 (44%)	.059
Dyslipidemia	15 (83%)	17 (94%)	17 (94%)	.812
Smoking	14 (78%)	12 (67%)	12 (67%)	.706
Renal failure	1 (6%)	0 (0%)	0 (0%)	.362
Prior CVA	2 (11%)	2 (11%)	2 (11%)	1.000
Peripheral vascular disease	6 (33%)	2 (11%)	4 (22%)	.276
Medications				
ASA	17 (94%)	16 (89%)	16 (89%)	.805
β-Blockers	16 (89%)	16 (89%)	15 (83%)	.849
Calcium-channel blockers	0 (0%)	4 (22%)	2 (11%)	.104
ACE inhibitors	11 (61%)	8 (44%)	8 (44%)	.518
Statins	12 (67%)	16 (89%)	13 (72%)	.267
Lipid profile				
Total cholesterol (mg/dL)	181 ± 58*	163 ± 38†	163 ± 42‡	.451
Triglycerides (mg/dL)	170 ± 114†	176 ± 88†	193 ± 158‡	.873
HDL (mg/dL)	40 ± 10*	35 ± 8†	43 ± 11‡	.110
LDL (mg/dL)	108 ± 50‡	94 ± 39‡	102 ± 27‡	.669

ConC, Conventional with cautery; ConH, conventional with harmonic shears; Endo, endoscopic; CCS, Canadian Cardiovascular Society; LVEF, left ventricular ejection fraction; CVA, cerebrovascular accident; ASA, acetylsalicylic acid; ACE, angiotensin-converting enzyme; HDL, high-density lipoprotein; LDL, low-density lipoprotein. *n = 16. †n = 15. ‡n = 14.

Vasoreactivity of the Radial Artery

To examine endothelium-dependent and endothelium-independent vascular relaxation, we exposed segments of U46619-precontracted radial arteries to increasing doses of acetylcholine and nitroglycerin, respectively. Radial artery vasoreactivity remained intact after endoscopic harvest (Figures 1 and 2 and Table 2). Maximal relaxation-contraction response and the sensitivity of the

conduits as reflected by EC₅₀ were similar among the groups.

To examine vasospasm, we exposed radial artery segments to increasing doses of the thromboxane analog U46619. U46619-induced maximal relaxation-contraction response and EC₅₀ values were similar among the groups (Figure 3 and Table 2).

Figure 1. Assessment of endothelial-dependent, NO-mediated radial artery relaxation by dose-response curves to acetylcholine (ACh; P = .639).

Figure 2. Assessment of endothelial-independent radial artery relaxation by dose-response curves to nitroglycerin (Ntg; P = .365).

TABLE 2. Radial artery vasoreactivity in the study groups

	ConC (n = 18)	ConH (n = 18)	Endo (n = 18)	P value
Acetylcholine E _{max} (%)	96 ± 9	94 ± 13	95 ± 11	.865
Acetylcholine EC ₅₀ (Log M)	−8.32 ± 0.72	−8.05 ± 0.79	−8.21 ± 0.77	.969
Nitroglycerin E _{max} (%)	99 ± 2	92 ± 15	100 ± 1	.116
Nitroglycerin EC ₅₀ (Log M)	−9.48 ± 0.61	−8.90 ± 1.42	−9.14 ± 0.81	.231
U46619 E _{max} (%)	100 ± 0	100 ± 1	100 ± 2	1.000
U46619 EC ₅₀ (Log M)	−8.16 ± 0.31	−8.19 ± 0.18	−8.20 ± 0.36	.913

ConC, conventional with cautery; ConH, conventional with harmonic shears; Endo, endoscopic; E_{max}, maximal relaxation-contraction response; EC₅₀, drug concentration producing 50% of maximal relaxation.

Light and Electron Microscopy

To assess the effect of harvest technique on the integrity of the radial arteries, we examined histologic sections of the conduits using hematoxylin and eosin staining in randomly selected patients (n = 8). There was minimal leukocyte infiltration, with no evidence of endothelitis or vasculitis and no apparent difference among the groups (data not shown). Representative hematoxylin and eosin-stained sections from each group are depicted in Figure 4. Assessment of the endothelial layer by means of electron microscopy in randomly selected patients showed minimal structural damage, with no difference among the groups. Representative electron micrographs of endothelial cells obtained from each group are depicted in Figure 5.

Immunohistochemistry

To focus on the effect of harvest technique specifically on the endothelial cell layer, we examined the expression of 3 leukocyte adhesion molecules by means of immunohistochemistry using a semiquantitative scale. We observed minimal expression of ICAM-1, moderate expression of VCAM-1, and strong expression of P-selectin in all 3 groups (Figure 6 and Table 3). There were no differences in adhesion molecule expression among the groups (Figure 6 and Table 3).

Discussion

The less invasive endoscopic radial artery harvest technique was recently introduced to reduce the morbidity associated with conventional radial artery harvest and to improve cosmesis.⁶⁻⁸ Reported short-term clinical outcomes of the endoscopic technique are excellent. However, the technique involves manipulation of the conduit in a limited space and dissection of the pedicle with control of side branches by using harmonic shears in close proximity to the conduit.⁶⁻⁸ Thus there is a potential for mechanical or thermal injury to the conduit, particularly the endothelium.

Normal structure and function of the vascular endothelium is essential for maintaining normal vascular tone, leukocyte and platelet interaction with the vessel wall, and vascular smooth muscle cell proliferation and migration.¹⁰

Figure 3. Radial artery contractile response to U46619 (P = .679).

Figure 4. Representative low-power (A, C, and E) and high-power (B, D, and F) magnification hematoxylin and eosin-stained slides from the 3 study groups: ConC, conventional with cautery; ConH, conventional with harmonic shears; Endo, endoscopic.

Figure 5. Representative electron micrographs (original magnification 7000 $\times$) of endothelial cells from the 3 study groups: *ConC*, conventional with cautery; *ConH*, conventional with harmonic shears; *Endo*, endoscopic.

Endothelial nitric oxide (NO) has a pivotal role among the substances produced by the endothelium.^{10,11} NO acts as a strong vasodilator, and it inhibits platelet aggregation, leukocyte adhesion, and vascular smooth muscle proliferation and migration.^{10,11} As such, NO is a strong antithrombotic and antiatherosclerotic agent.¹¹ Endothelial dysfunction has

Figure 6. Leukocyte adhesion molecule expression in the 3 study groups: *ConC*, conventional with cautery; *ConH*, conventional with harmonic shears; *Endo*, endoscopic.

TABLE 3. Leukocyte adhesion molecule expression

	ConC (n = 18)	ConH (n = 18)	Endo (n = 18)	P value
ICAM-1	0.8 ± 0.7	0.6 ± 0.9	0.7 ± 1.0	.823
VCAM-1	1.3 ± 0.7	1.2 ± 0.7	1.3 ± 0.8	.912
P-selectin	2.0 ± 0.7	2.2 ± 1.0	2.2 ± 0.6	.725

ConC, Conventional with cautery; *ConH*, conventional with harmonic shears; *Endo*, endoscopic; *ICAM-1*, intracellular adhesion molecule 1; *VCAM-1*, vascular cell adhesion molecule 1.

been correlated with the development of atherosclerosis,¹⁰ and enhanced NO production has been hypothesized to be a major factor explaining the superior patency rates of arterial coronary bypass conduits compared with saphenous vein grafts.^{9,12} Mechanical trauma to the conduit during harvest has been identified as a major factor responsible for endothelial dysfunction.¹³⁻¹⁶

In the present study we have demonstrated that structural integrity and the vasoreactivity of radial arteries harvested endoscopically remained intact. Using light and electron microscopy, we observed no significant structural injury to the conduit with any of the 3 techniques. Using organ-chamber methodology, we showed that endothelial-dependent, NO-mediated vascular relaxation and endothelial-independent vascular relaxation were normal and similar to that seen in radial arteries harvested with either electrocautery or harmonic scalpel through a full-length incision. Also, the contractile response to U46619 was similar among the groups.

The harmonic shears are an essential component of the endoscopic radial artery harvest technique. The harmonic shears are used both to dissect the pedicle and divide the branches in a very limited space, working in close proximity to the radial artery. The harmonic scalpel was introduced for radial artery harvest in the late 1990s.¹⁷⁻²⁰ The principle of harmonic scalpel technology is conversion of electrical energy to an ultrasonic energy within the blade of the instrument. The harmonic scalpel blade vibrates at a frequency of 55.5 kHz, resulting in mechanical breakage of tertiary hydrogen bonds and protein denaturation.^{21,22} Although there is heat generation, the harmonic scalpel works at a temperature range of 50°C to 100°C, which is substantially lower than conventional electrocautery (operating temperature range of 150°C to 450°C).^{21,22} Moreover, heat transmission through the tissue is significantly greater with electrocautery compared with the harmonic scalpel.^{21,22} Thus it is much safer to use the harmonic scalpel at close proximity to the vessel. Studies assessing the use of the harmonic scalpel for radial artery harvest through a conventional full-length incision showed that this technology is safe and fast and minimizes the use of metal clips.¹⁷⁻²⁰ Although one study documented reduced radial artery vasospasm with the harmonic scalpel,¹⁸ another study did not show any difference in vasoreactivity of the radial artery when tested in organ

chambers.²⁰ Finally, ultrasonic energy produced by the harmonic scalpel at levels used for conduit harvest was found to induce internal thoracic artery vasorelaxation through a time-dependent endothelial NO and prostacyclin release.²³ A direct effect on the vascular smooth muscle was also observed. Both effects appeared to be unrelated to tissue heating (tissue temperature increase of only $0.3^{\circ}\text{C} \pm 0.03^{\circ}\text{C}$) or to damage to endothelium structural integrity, as assessed by means of electron microscopy.²³

Under normal conditions, only a few leukocyte adhesion molecules are expressed by the vascular endothelium.^{14,24} Upregulation of adhesion molecules in response to a variety of stimuli, such as mechanical trauma, shear stress, and cytokine stimulation, is an essential first step in the interaction between the endothelium and the leukocytes, which in turn is one of the fundamental mechanisms of atherosclerosis. Thus upregulation of adhesion molecules is considered a marker of endothelial injury.^{14,24-26} Using immunohistology, we observed differential expression of the 3 adhesion molecules tested. We observed minimal expression of ICAM-1, intermediate expression of VCAM-1, and strong expression of P-selectin. Thus mechanical or other types of trauma clearly resulted in some endothelial cell injury, as assessed by using this very sensitive measure. However, there were no statistical differences among the harvest techniques, and in the present study it did not affect vascular reactivity. Similar to our observation in the radial artery, Chello and colleagues¹⁴ described differential expression of adhesion molecules in saphenous grafts exposed to pressure distention. This phenomenon can be, at least in part, explained by the fact that expression of ICAM-1 and VCAM-1 involves DNA transcription and therefore takes several hours to reach a peak. In contrast, P-selectin is constitutively present in the Weibel-Palade bodies within the endothelial cells, and its expression on the membrane involves translocation to the cell surface, a much faster process.¹⁴ The clinical implication of upregulation of some of the adhesion molecules needs to be further investigated.

In summary, endoscopic harvest with harmonic shears does not alter radial artery vasoreactivity or endothelial integrity compared with conventional harvest techniques. Because the endoscopic technique is less invasive, it might prove to be the technique of choice to harvest the radial artery.

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