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Combined Distal Venous Arterialization and Free Flap for Patients with Extensive Tissue Loss

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Abstract: Aim: We evaluated the mid-term outcome of distal venous arterialization (DVA), and the role of a combined free flap as a bridgehead for blood supply.
Methods: In the past 6 years, 9 patients with extensive tissue loss and lucking graftable distal arteries underwent DVA. These consisted of 4 primary DVAs, 3 combined DVA and free flap procedures, and 2 adjuvant DVAs for hemodynamically failed distal bypasses. After 9 primary DVAs, 3 redo DVAs were performed for early failure. Etiologies were 4 Buerger's disease and 5 arteriosclerosis obliterans, including 3 dialysis patients.
Results: Among the 9 DVA cases, there were 5 primary failures, consisting of 2 who underwent amputation, 2 had successful redo DVA, and the remaining one did not require redo-DVA. Primary patency, secondary patency, and limb salvage rates were 44.4%, 55.6%, and 77.8%. The postoperative period was 1-36 months (median 12 months). Angiography demonstrated DVA was effective in the early period, and development of collaterals or a capillary network from the free flap replaced the DVA function in the intermediate period.
Conclusion: DVA can be effective as a procedure for limb salvage in patients without graftable distal arteries, and a combined free flap is effective and functions as a bridgehead for blood supply to the ischemic zone.
Key words: Distal venous arterialization, Free flap, Critical limb ischemia, Tissue loss, Limb salvage

Combined distal venous arterialization and free flap for patients with extensive tissue loss

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ABSTRACT

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INTRODUCTION

In limb salvage, there have been three steps in the development of surgical management. The first step was paramalleolar arterial bypass, which is the choice of operation for foot salvage in patients with Buerger's disease (thromboangiitis obliterans, TAO) as well as diabetic ischemic gangrene, and this aggressive bypass strategy has been justified by a significant decrease in major amputation rates.[1,2] The second evolution was application of free tissue transfer combined with distal arterial bypass[3], which has also contributed to limb salvage in patients with extensive tissue loss. The latest step is distal venous arterialization (DVA); in which the procedure is truly indicated, although candidates are limited. When angiography demonstrates the absence of graftable distal arteries, DVA may be possible as a last resort for limb salvage. Taylor et al. improved the techniques and reported in 1999 an immediate high success rate of limb salvage[4]; however, according to a recent meta-analysis of DVA reports,[4-7] secondary patency and foot preservation rates at one year were only 46% and 71%, respectively,[8] indicating that the intermediate-term outcome is not acceptable.

Against this evolutionary background of limb salvage treatment, if a patient with extensive tissue loss has no graftable distal arteries, the patient is a suitable candidate for combined DVA and free flap; however, because of the limited number of DVA candidates, neither the combined procedure nor the functional role of the free flap has been reported. In the present study, we report the outcome of DVA with or without a free flap for patients with extensive tissue loss without graftable distal arteries, and discuss the mid-term biological fate of DVA and the role of the free flap as a nutrient for limb salvage.

PATIENTS AND METHODS

Patients: Between September 2003 and August 2007, we employed DVA for limb salvage as a final resort. DVA candidates were definitively selected and there were 9 patients who required DVA because of no graftable distal arteries (Fig. 1A, Table 1). The 9 included 4 TAO and 5 ASO, including 3 on dialysis, and they complained of intractable rest pain, which was mainly controlled by continuous epidural analgesics. They had progressive extensive tissue loss with exposed bone, and 6 had virulent organism infection such as methicillin-resistant staphylococcus aureus (MRSA), escherichia coli, and/or pseudomonas aeruginosa. The indications of DVA were progressive gangrene due to disease progression in all TAO patients (Fig. 2A, B), as well as in 3 ASO patients with dialysis, and extensive thrombosis of paramalleolar and foot arteries after percutaneous angioplasty (Fig. 1A) or blue toe syndrome (Fig. 3A) associated with ASO. The indications were evaluated by preoperative angiography, but 4 ASO patients required intraoperative angiography from the below-knee popliteal artery for final decision making. All 5 ASO patients had no history of arterial bypasses, whereas all TAO patients had a long clinical history, with single or multiple redo bypasses or lumbar sympathectomy.

DVA procedures: Among the 9 patients, there were 4 primary solitary DVAs, 3 combined DVA and free flap procedures, and 2 adjuvant DVAs performed in dialysis patients at the time of an attempted bypass because of low graft flow or hemodynamically failed conventional arterial bypass (Table 2). After these 9 primary DVAs, in one simultaneous DVA and free flap case, an additional DVA was performed to create an arterial access for the free flap, and 3 redo DVAs were performed for DVA failure.

Operative technique *(Fig. 4)****:*** The target vein was selected based on the location of gangrene with infection, and the anatomical advantage for a free flap. In 6 of 9, we primarily used plantar veins; in another we selected the dorsal pedal vein because of the advantage for a later free flap, but thrombotic occlusion occurred within 24 hours, so the plantar vein was used in redo DVA. In the remaining 2, neither veins were available, and the superficial dorsal arch vein or the anterior tibial vein was used.

After intravenous administration of heparin, the common plantar vein was ligated 2 cm proximal to the bifurcation, and longitudinally opened distal to the ligation. Proximally located valves were bluntly destroyed by means of a surgical probe (Fig. 4A), and then fine catheters, such as a trial production balloon catheter with an outer diameter of 0.75 mm (Nipuro Co., Osaka, Japan), a 2 Fr Fogarty balloon catheter (Edwards Lifesciences, Irvine, CA, USA), and/or a Parsonnet probe (CR Bard Co., Murray Hill, NJ, USA) with an outer diameter of 1 mm, were passed up and placed immediately proximal to the distal bifurcation of the medial plantar vein or to deep plantar arch vein of the lateral plantar vein for distal valve destruction (Fig. 4B). The distal fine valves were easily destroyed by gently passing a catheter through without the firm push required for proximal valves, and weak resistance could be felt through the catheter at the site of destruction. In 3 cases, we used a microangiofiberscope with an outer diameter of 0.75 mm (Microendoscope®, Fibertech, Rochester, NY, USA) to confirm the valve destruction. After completion of the valve destruction, retrograde venography was performed from the venotomy to confirm visualization of the plantar metatarsal veins or the dorsal metatarsal veins connecting to the plantar or the dorsal pedal veins (Fig. 1B).

Vein grafts were used exclusively: 4 in-situ saphenous vein grafts, and 8 reversed vein grafts, including 3 spliced vein grafts, were employed. In one TAO patient, an expanded polytetrafluoroethylene graft was used for redo DVA. For proximal anastomosis of DVA grafts, we selected the common femoral artery, superficial femoral artery, below-knee popliteal artery, or hemodynamically failed femoro-anterior tibial artery or plantar artery bypass graft. Anastomotic techniques were the same as for a conventional arterial bypass: the length of the anastomosis was 8-10 mm, and all anastomoses were performed with 8-0 polypropylene continuous sutures using a 3x magnification loupe.

There are many connections between superficial veins and plantar veins which decrease the pressure of the DVA system, whereas complete ligation may cause graft failure due to extremely low graft flow. Several branches originating from the proximal segment of the plantar veins become AVFs, but small branches originating from the distal segment of plantar veins contribute to the maintenance of adequate graft flow. In order to obtain the optimal flow volume (20-40 ml/min), the proximally located branches were ligated individually under monitored graft flow (ultrasound transit time flow meter; Medi-stim Vutterfly®, Oslo). When the graft flow decreased below 20 ml/min after ligation, the last branch was left unligated (Fig. 4C), and the graft flow after completion of ligation was ultimately controlled at 15 to 50 ml/min (median: 40 ml/min).

A free flap was indicated for extensive tissue loss with exposed bone. In 3 patients receiving combined DVA and free flap, one TAO patient underwent a simultaneous operation, and 2 others received vacuum-assisted closure management for infection control after DVA, and then underwent free flap as a second stage operation, with intervals of 3 weeks to 4 months. The type of free flap was selected based on the size of tissue loss, thickness of the flap, whether or not the area to be repaired was weight bearing, and/or locational advantage in harvesting, and the rectus abdominis flap, the scapular fasciocutaneous flap, or latissimus dorsi muscle flap was used. A free flap was harvested immediately before transplantation, and the free flap artery was anastomosed to the distal site of the DVA graft in an end-to-side manner. The vein of the free flap was usually anastomosed to the proximal segment of the vein used for DVA, such as the terminal posterior tibial vein or the dorsal pedal vein.

Postoperative management: Neither the skin perfusion pressure nor the toe pressure measurement was applicable because of extensive gangrene. Therefore, effective retrograde perfusion of the DVA was evaluated by the change of skin color to a characteristic darkish pink. Newly-opened AVFs were the most important factor for reaggravation of ischemia. When significant bruits developed and manual AVF occlusion improved the skin color to pink, ligation of the AVF was performed. Postoperative anticoagulants were not given, but statins or low dose aspirin, or both, were administered.

Serial angiographies were performed to evaluate changes of blood supply patterns and the DVA outcome. In 7 cases with successful limb salvage (2 DVAs alone, 3 DVAs with free flap, and 2 adjunctive DVAs for hemodynamically failed distal arterial bypass), 3 cases with primary patent DVA, 1 with failed DVA and functioning free flap, and 2 redo functioning DVAs were available for evaluation. The remaining 1 case was excluded because of the short observation period after surgery. During observation periods ranging from 4 -36 months (median 12 months), serial angiographies were performed 2 – 6 times /case (total 19 times) at 1-30 months after DVA.

RESULTS

Patients who underwent primary solitary or adjuvant DVA: Of 4 patients who underwent primary solitary DVA, 2 had early DVA graft failure due to graft shortness or acute irreversible ischemic change, resulting in amputation. In remaining 2 (who had successful DVA or redo DVA performed for early thrombosis) we succeeded in limb salvage. The foot in which DVA was performed regained a characteristically pink skin (Fig. 1C). Recurrence of ischemia occurred in one patient 2 weeks after DVA because of a delayed open AVF; however, the skin color immediately returned to pink after ligation for the AVF. The deep wound was gradually covered by granulation tissue, and pedolplasty with surviving excess tissue was performed after 4 months (Fig. 1D). In the meantime, sufficient collateral arteries developed while the DVA lapsed into dysfunction, and the vein graft hemodynamically failed without any symptoms after 6 months.

Of 2 adjuvant DVAs for an arterial bypass, one failed after 5 months and the subsequent redo DVA also failed after 3 months; however, a wound with extensive tissue loss completely healed 7 months after DVA, and we succeeded limb salvage. In the other patient, the DVA continued functioning, and the distal half of the foot with extensive tissue loss was exclusively nourished by the DVA, but he died of myocardial infarction 2 months after surgery.

Patients who underwent combined DVA and free flap: Of the 3 patients who underwent combined DVA and free flap (Figs. 2, 3), 2 with TAO demonstrated characteristic darkish pink skin, and rest pain was relieved after 2 to 4 weeks. One patient with a spliced vein graft had graft stenosis 18 months after surgery. Because no veins were available, a 10-cm-long right gastroepiploic artery was used for replacement of the graft segment. The remaining patient, who had ASO, had operative wound dehiscence due to MRSA infection after DVA; however, angiography after 8 weeks demonstrated a distal venous system with retrograde perfusion. After control of the infection, adjunctive DVA bypass for free flap was placed from the initial DVA graft to the dorsal pedal vein, and a free flap was performed 3 months after the initial DVA (Fig. 3B, C).

Changes in blood supply patterns to the ischemic zone: In the 7 patients with definitely successful procedures and limb salvage, the DVA was obviously functioning and effective in the early postoperative period; however, serial angiographies in the intermediate period in 5 patients demonstrated 3 different changes of blood supply modality, which were summarized as follows: 1) DVA and free flap both functioning well without development of collaterals in one patient (Fig. 5A); 2) failed DVA without development of collaterals and functioning free flap with supplementary vascular ingrowth to surrounding ischemic zone in 2 patients (Fig. 5B); 3) failing or failed DVA with development of vigorous collaterals in 2 patients (Fig. 5C). In 1), there was narrowing of the vein graft and DVA venous system, probably due to arterialization, but both the DVA and the free flap nourished their respective areas in the foot. In 2), the DVA venous system atrophied and failed, but the free flap connected to the DVA graft continued to function, and the free flap formed a blood supply network to the ischemic zone, with an increase of caliber in the free flap artery. In 3), complete recovery from critical limb ischemia was attained, while the DVA became meager, changed to a simple AVF, and ultimately failed.

DVA patency rate and Limb salvage: In 9 patients, the lengths of hospital stay were 1 week to 3 months (median 3 weeks), and they underwent 1 to 3 procedures (median 2). Of 9 various types of DVAs, there were 5 primary failures, of which 2 resulted in amputation, but 2 underwent successful revision or redo surgery, and the remaining patient, who had a free flap, did not require redo DVA because of improvement of ischemia by microangiogenesis from the free flap. Seven patients with successful limb salvage started rehabilitation 3 to 20 weeks after DVA or pedoplasty. Of 7, 5 had passed 12 months after DVA, and regained a satisfactory gait function without any prosthesis for short walks after 5 to 8 months (median 3 months). The primary and secondary patency rates of DVA were 44.4% (4/9) and 55.6% (5/9), and limb salvage was attained in 7 of 9 patients (77.8%) during the follow-up period of 2-36 months (median 12 months).

DISCUSSION

In critical limb ischemia, 30% of patients who underwent amputation died within 2 years and another 30% required re-amputation or contralateral limb amputation,[9] while several studies have proved that successful revascularization improves quality of life.[10,11] Against this background, we set out to clarify efficacy of DVA and combined free flap grafting as a final resort for limb salvage, and selected the 9 patients as a definitive DVA candidate, which were 3.9% in patients underwent bypasses for critical limb ischemia in the same period of time. All of the patients positively accepted a long-term treatment plan with multiple procedures which might enable limb salvage.

To evaluate efficacy in limb salvage, it is necessary to differentiate the effects of intervention from those developing from natural improvement. Ischemia may induce microangiogenesis, and 25% of critical limb ischemia naturally improves[9]; however, if gangrene is progressive, it is necessary to provide a definitive treatment for stopping the progression. In the present candidates, natural improvement was not expected because gangrene was clearly progressive, and the progression stopped after DVA in either DVA alone or DVA with free flap. Taylor speculated that neovascularization takes place in response to AVF with the development of new arteries, and due to development of collaterals limb salvage can be attained even after graft failure,[5] while Root reported that peripheral AVFs may be a potent stimulus to the growth of arterial and venous collateralization.[12] Nevertheless, if DVA is actually effective, retrograde blood flow must reach a significantly distal level of the microcirculation, and it appears that limb salvage is accomplished by different mechanisms in the acute and the intermediate phases.

In the acute phase, nourishment by diffusion is speculated to be the main mechanism: as many previous reports regarding diffusion have suggested[13], arterial walls thinner than 0.5 mm must be nourished entirely by diffusion from the lumen or adventitial vessels, and diffusion efficiency is enhanced by arterialization of the venous system with thinner walls. In DVA, therefore, an ischemic area beyond 500 μm must be nourished by diffusion alone, even though oxygenized blood does not reach capillaries.

In the intermediate phase, the patency rates of DVA bypasses are poor, and the mechanism of DVA dysfunction still remains unclear. In order to clarify the reasons for DVA failure and the mechanism of clinical improvements in each successful case, we performed repeated angiography, which clearly demonstrated changes in the perfusion zone of the DVA and dominant perfusion vessels. We found three angiographic patterns, and concluded that DVA may continue to work as long as development of significant collaterals does not occur, while the DVA venous system gradually diminishes and lapses into dysfunction in association with vascular ingrowth from the free flap or development of native collaterals. Furthermore, the mechanisms leading to limb salvage are considered to differ between cases of DVA alone and those of DVA with a free flap: in DVA alone, development of collaterals gradually progresses for several months, and then antegrade flow becomes dominant, while retrograde flow via the DVA gradually decreases and ultimately changes to a simple AVF. On the other hand, in DVA with a free flap, a capillary network between microvessels of the free flap and ischemic tissue is established within about 3 weeks.[14] Thereafter antegrade flow from the free flap gradually becomes dominant in the same way as DVA alone.

In the DVA procedure, adequate valve destruction in the target vein and its distal venous system is important, and metallic olives[5], Parsonnet probe[4], Fogarty catheter, and /or guide wires[15] have been used. The proximally located valves can be surgically destroyed, but incompetence of microvalves is induced only by high venous wall tension generated by arterial pressurization. Major AVFs not only steal blood flow from the distal venous system, but also reduce vessel wall tension, suppressing induction of distal valve incompetence. As seen in the present cases, delayed opening of a significant AVF markedly aggravated ischemia. Historically, Szilagyi attempted DVA of the superficial femoral vein in 9 cases, but no responders were found.[16] Use of the superficial pedal venous arch of the distal saphenous vein,[15,17,18] or crural saphenous vein[19] have been reported. Since small superficial veins from toes feed into the dorsal venous arch which continues to the saphenous veins, DVA using the superficial veins is theoretically feasible; however, it is not easy to destroy valves of the dorsal venous arch, and the dorsal arch has many communicating branches, leading to major AVFs. Thus, we conclude that the venae comittantes of the medial and/or lateral plantar arteries are the veins of choice for DVA as far as these areas are not encroached upon by gangrene or infection. Proximal branches of these veins close to the anastomosis lead to major AVFs and commonly require ligation, but ligation for distal branches requires graft flow monitoring. Because of a contradiction between sufficient graft flow produced by AVFs and high pressure in the DVA venous system, we routinely perform AVF ligations while measuring graft flow, controlled at about 40 ml/min as the adequate value securing vein graft patency. Reducing the flow means not only reduction of excessive AVF flow, but maintenance of higher venous wall tension.

In order to assure limb salvage for a long-term period, a free flap as an alternative blood supply resource after DVA failure is necessary. Angiography in the present study demonstrated that a free flap combined with DVA not only covers a wound that has exposed bone, but also plays the role of a bridgehead for blood supply to the ischemic zone. Mimoun, et al. reported the concept of the nutrient flap,[14] and stressed the following three functions of the free flap: 1) it provides supplementary blood flow to ischemic zones, 2) it assists venous drainage in regions of venous insufficiency, and 3) it induces the formation of a capillary network. In critical limb ischemia, immediate effective arterial blood supply is necessary to prevent impending necrosis. Sunar, et al. reported a free flap technique using an arteriovenous shunt for limb salvage;[20] however, since a free flap requires 3 weeks to establish capillary ingrowth to surrounding tissue, it will not be effective to stop progression of gangrene; in addition, blood flow steal through an arteriovenous shunt may aggravate the ischemia. Therefore, even in combined free flap cases, DVA, not an arteriovenous shunt, is essential until the formation of vascular connections with surrounding tissue.

CONCLUSIONS

In patients without graftable distal arteries, DVA may be the procedure of choice for limb salvage; however, because long-term durability is limited, an adjunctive blood supply resource is necessary for the foreseeable dysfunction. When such patients have extensive tissue loss, a combined free flap with DVA not only is effective for wound coverage, but also retains vein graft patency and continues function as a nutrient flap, resulting in a more satisfactory outcome in limb salvage when compared with DVA alone.

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Table 1. Patient characteristics

Patients				DVA			
#	**Age**	**Sex**	**Disease**	**Indications**	**Op. Date**	**ABI**	**Tissue loss**
Combined DVA and free flap:							
1.	64	M,.	TAO	Disease progression	Jan. ‘03	0.1	Lateral half of foot
2.	58	M	TAO	Disease progression	Sept. ‘04	0	Distal to Lisfranc joint
3.	60	M	ASO/DM	Microatheroembolism	July ‘06	0.3	1st-2nd metatarsal bone
Primary solitary DVA:							
4.	74	F	ASO	Thrombosis	Jun. ‘06	0.4	Distal to Chopart joint
5.	51	M	TAO	Disease progression	Mar. ‘06	0	Forefoot to Chopart joint
6.	53	M	TAO	Disease progression	May ‘07	0.4	2nd toe
7.	58	F	DM/dialysis	Disease progression /thrombosis	Apr. ‘07	inc.	Entire sole
Adjuvant DVA for hemodynamically failing distal arterial bypass:							
8.	48	M	DM/dialysis	Disease progression	Aug. ‘06	inc.	Lateral half of heel
9.	72	M	DM/dialysis	Disease progression	July ‘07	inc.	2-4 toes-metatarsal area

DVA, distal venous arterialization; TAO, thromboangiitis obliterans; ASO, arteriosclerosis obliterans; DM, diabetes mellitus; Op. date, date of DVA operation; ABI, ankle-brachial index; inc., incompressible artery

Table 2. Results of DVA with or without free flap

DVA **Free flap**

 # Procedure Graft type Graft type Outcome/status

Function (follow-up) Function (follow-up)

Combined DVA and free flap:

1. Graft-MPV RVG (arm vein) RcAb

F (1 mo) P/Fu (26 mo) healed/died 26 mo

2. FA-MPV RVG (spliced vein) RcAb

P/Fu (36 mo) P/Fu (34 mo) healed/ambulation

3. BKP A-MPV ISVG

P /Fu (14 mo) -

Adj-DVA graft-DPV RVG Scpl

P/Fu (10 mo) P/Fu (10 mo) healed/ambulation

Primary solitary DVA:

4. BKPA-LPV RVG

P/meager (12 mo) - healed/died

5. FA-ATV RVG (spliced vein graft)

F (3 days) -

Redo FA-CPV ePTFE/6mm

F (1 day) - amputation

6. BKPA-DPV ISVG

F (1 day) -

Graft revision RVG (spliced vein graft)

P/Fu (4 mo) - healed/rehabilitation

7. BKPA–DVA ISVG-RVG

F (1 day) - amputation

Adjuvant DVA for hemodynamically failing distal arterial bypass:

8. adj Graft-CPV Direct anastomosis

F (5mo) -

Redo adj Graft-DPV RVG arm vein

F (3mo) - healed/rehabilitation

9. adj Graft-LPV ISVG-direct anastomosis

P/Fu/ (2 mo) - healing/rehabilitation/died

DVA, distal venous arterialization; Graft, distal arterial bypass graft; MPV, medial plantar vein; FA, femoral artery; BKPA, below knee popliteal artery; adj, adjuvant DVA; DPV, dorsal pedal vein; LPV, lateral plantar vein; ATV, anterior tibial vein; CPV, common plantar vein; DVA, dorsal venous arch; RVG, reversed vein graft; ISVG, in situ vein graft; ePTFE, expanded polytetrafluoroethylene; F, failed; P/Fu, patent/functioning; P, patent; RcAb, rectus abdominus; Scpl, scapular fasciocutaneous

LEGENDS

Fig. 1. A 74-yr-old female ASO, candidate for DVA. A, intraoperative angiography from below the knee popliteal artery demonstrated entire paramalleolar and foot artery occlusion; B, venography after complete valve destruction, visualizing the plantar arch and metatarsal veins (arrows); C, 7 days after DVA, showing a characteristic darkish pink skin; D, 4 months after pedoplasty.

Fig. 2. A 56-year-old TAO patient who underwent simultaneous operation of combined DVA and free flap for progressive gangrene: A, 1 month before DVA; B, preoperative finding of extensive tissue loss; C, 6 months after surgery.

Fig. 3. A 60-year-old diabetic ASO patient who underwent a 2-stage operation of combined DVA and free flap. A, before DVA; B, free flap performed 3 months after DVA; C, 5 months after free flap.

Fig. 4. Technique of valve destruction. A, The common plantar vein is dissected, and ligated 2 cm proximal to the bifurcation, and longitudinally opened distal to the ligation. Proximal valves of the medial and/or lateral plantar veins are destroyed by a surgical probe; B, Fine catheters are inserted distally to the plantar vein for destruction of fine valves; C Visualization of plantar metatarsal veins is confirmed

by venography, and then anastomosis between vein graft and common plantar vein is performed.

Fig. 5. Blood supply modalities in the foot in the intermediate period after DVA with or without free flap: A, well-functioning DVA (arrows G1,G2: vein grafts of primary and secondary DVA) and free flap seen in the case of Fig. 3 (10 months after DVA); B, failed DVA, but patent vein graft (arrow G) with functioning free flap (arrow F: free flap artery) with supplementary vascular ingrowth and blood supply to the surrounding ischemic zone seen in the case of Fig. 2 (30 months after DVA); C, failing DVA graft (arrow) and collateral development from native proximal arteries seen in the case of Fig. 1 (5 months after DVA);.

Cover Letter

May 2, 2008

Ramon Berguer, MD
Editor-in-Chief
Annals of Vascular Surgery

Dear Dr. Berguer:

Enclosed please find our paper entitled "Combined distal venous arterialization and free flap for patients with extensive tissue loss," by Tadahiro Sasajima et al., which we are submitting for your consideration for publication in the Annals of Vascular Surgery.

(1) Category of manuscript: Clinical research (Report of clinical series)
(2) The material has not been previously published or submitted elsewhere for publication and will not be sent to another journal until a decision is made concerning publication by Annals of Vascular Surgery
(3) Any personal conflicts of interest of any of the authors: Nil
(4) Sources of outside support for research, including funding, equipment, and drugs: Nil
(5) Any financial support whatsoever from industry: Nil

Your kind consideration of this manuscript would be appreciated.

Yours sincerely,

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Nobuyuki Akasaka, MD

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Figure 1A
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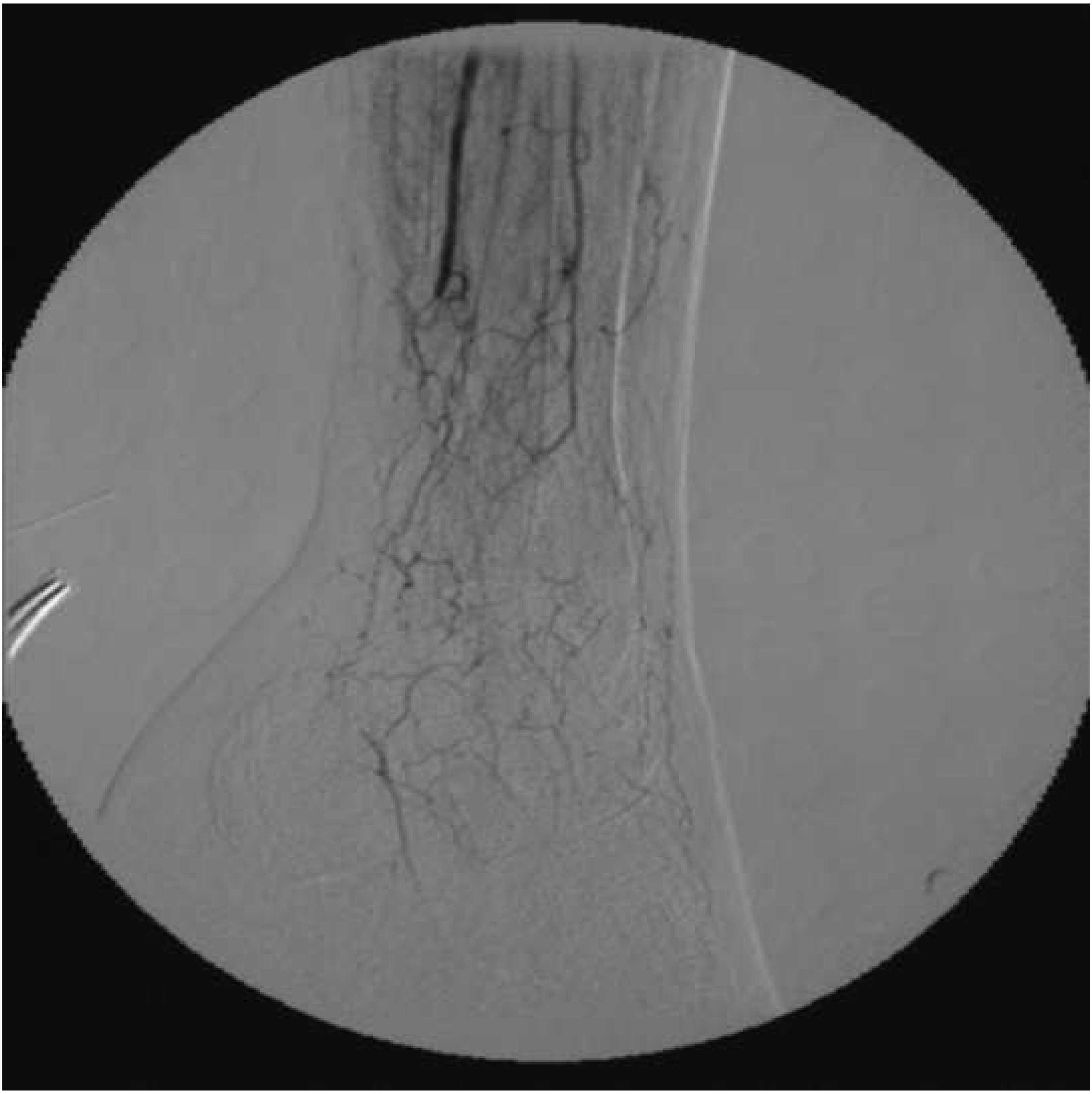

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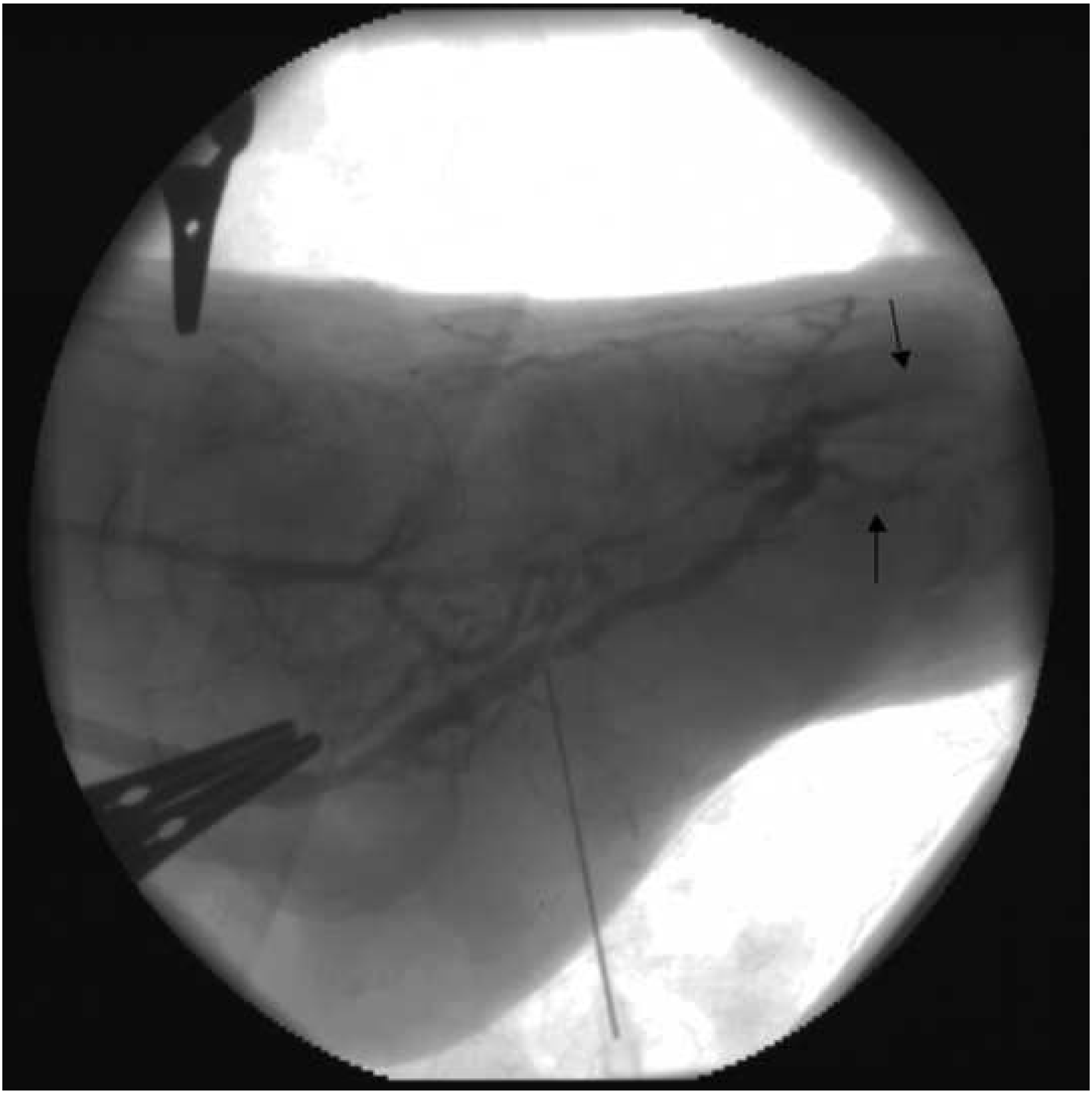

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Figure 3B

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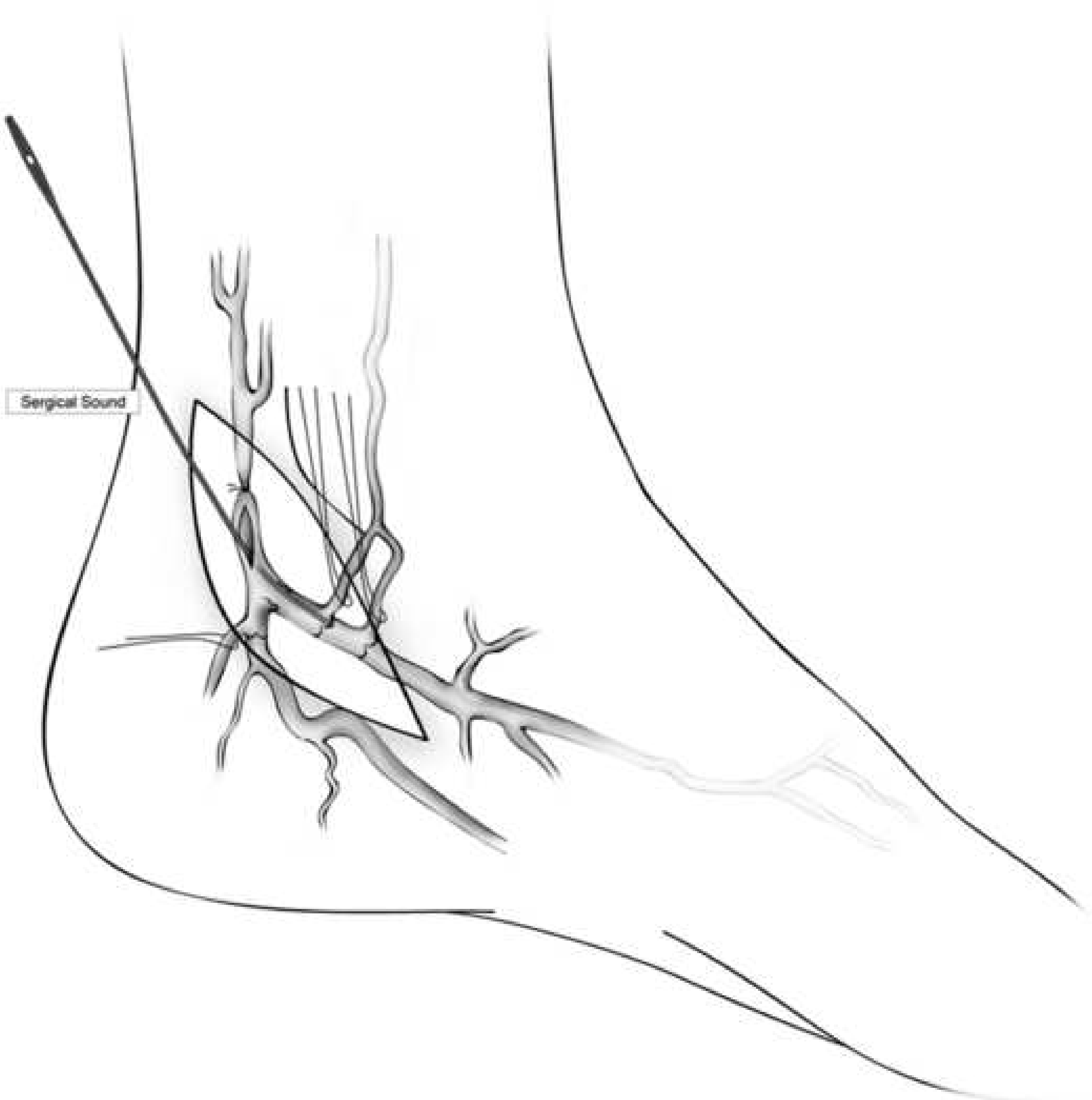

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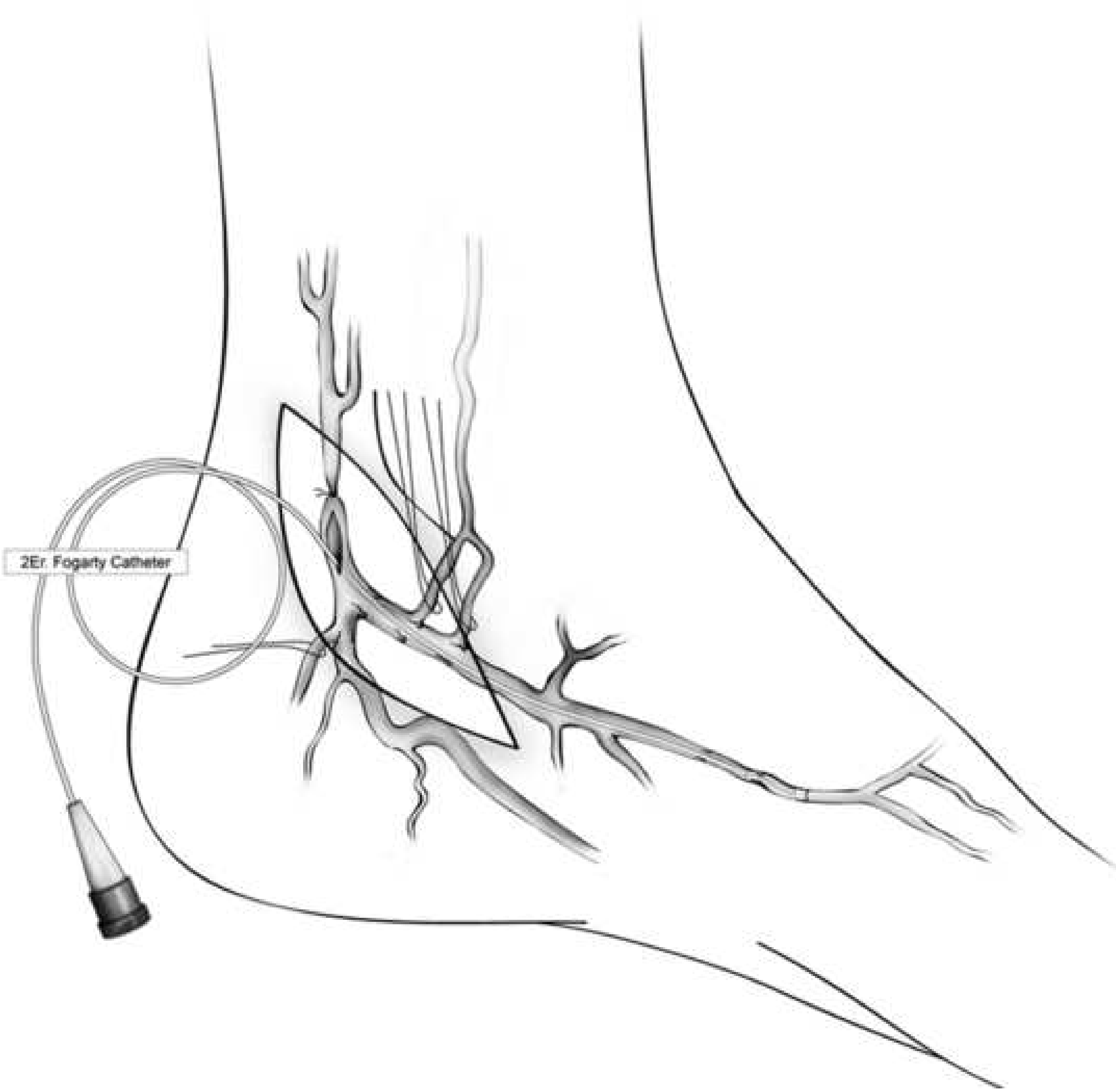

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Figure 5A

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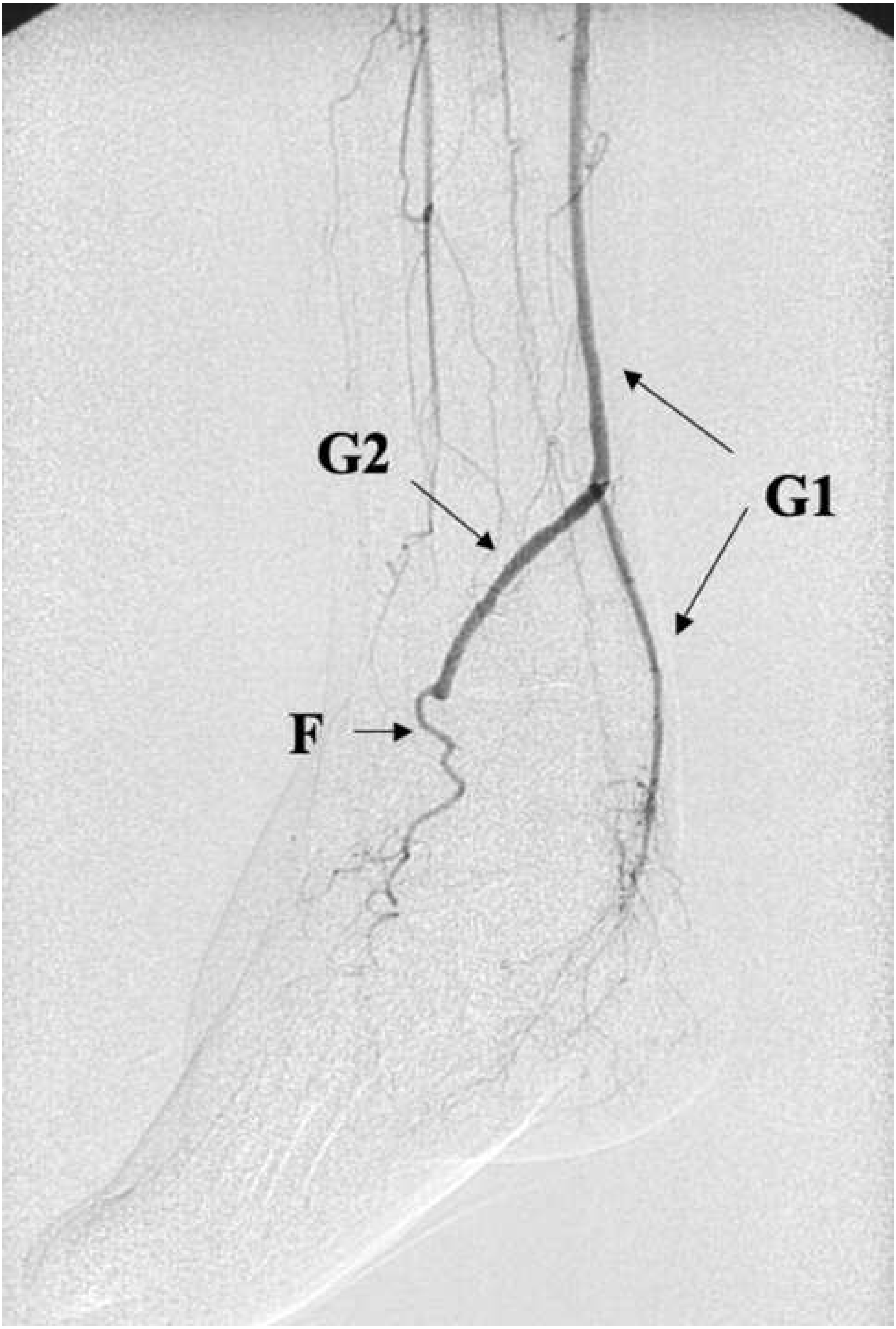

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