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Investigation of Venous Vascular Occlusion Mechanisms in the Great Saphenous Vein Treated with Radiofrequency Endovenous Ablation for Varicose Veins in Clinical Cases

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Abstract

Objective: We assessed the mechanism of venous vascular occlusion in the great saphenous vein (GSV) following radiofrequency venous ablation (RFA) in clinical cases of varicose veins. **Materials and Methods:** RFA was performed on 35 limbs from 25 patients presenting with symptomatic varicose veins and GSV incompetence. Immediately following RFA, a portion of the highly ligated GSV was pathologically examined. The venous segments were stained and examined microscopically using hematoxylin (eosin) and Masson's trichrome staining. Post-procedure, the condition of the ablated GSV, including vascular properties, thrombus presence, and blood flow status, was analyzed using duplex scanning of the lower extremities. **Results:** Major complications such as thrombophlebitis or skin burns were not observed during RFA. Histological damage beyond the endothelial layer of the vessel wall was observed in all treated varicose veins. In the veins treated with RFA, intima edema was noted in 85.7% of the cases, and microthrombus in 88.6%. Postoperative duplex ultrasound was performed in all cases between days 7 and 59 (median, 21.2 ± 19 days). Intravascular thrombi were present in all cases, with intimal hyperplasia in 80.0%. Complete occlusion of the entire GSV was not observed. Postoperative duplex scanning revealed blood flow through the perforating branches of the varicose veins in 14 limbs (40.0%); when scanning lower extremities, it showed intravascular thrombi in all cases and vessel intima thickening in 80.0% of cases. **Conclusion:** Although changes were identified in the vascular tissue due to RFA, no vascular occlusion was observed. During the post-cautery course, thrombosis of the ablated blood vessels was observed in nearly all cases, likely causing blood flow obstruction.

Subject Areas

Surgery & Surgical Specialties

Keywords

Radiofrequency Venous Ablation, Vascular Occlusion, Great Saphenous Vein, Histological Finding

1. Introduction

Endovascular therapy gained popularity in the 2000s, leading to the worldwide adoption of endovenous laser ablation (EVLA) and RFA for the treatment of varicose veins.

In Japan, EVLA received insurance coverage in 2011, followed by RFA in 2014. In recent years, these procedures have become standard techniques and are safely performed.

Venous blood vessel occlusion may occur following EVLA [1]-[3], with two causative phenomena currently under consideration: laser irradiation of the blood and direct vein wall irradiation.

Conversely, RFA induces occlusion by passing a high-frequency current through a coil within the venous blood vessel, causing denaturation via Joule heat generated by coil resistance [4].

In vivo animal experiments have confirmed these ablation mechanisms [5], and one clinical report after EVLA examined pathological changes in vascular specimens from a single exposed ablated venous vessel [1]. However, no clinical reports have documented a cohesive number of observed and examined changes in blood vessels following ablation in actual clinical practice.

Assuming that heat-induced degeneration from RFA occurs in human venous blood vessels, we examined the pathological alterations and assessed whether these changes occur intravascularly.

RFA-ablated blood vessels were collected following high-level ligation, and their pathological changes were subsequently examined. We investigated the therapeutic mechanisms of RFA, including vascular occlusion and occlusion due to heat-induced vascular degeneration.

2. Materials and Methods

2.1. Study Design

This study involved 25 patients (men: women = 14:11; average age, 69.5 ± 13 years; total, 35 limbs), of which 27 had primary varicose veins of a unilateral limb, while eight were diagnosed with primary varicose veins of bilateral limbs. The current study was conducted at Amagasaki Central Hospital, Hyogo, Japan, between November 2017 and April 2018, with a 6-month follow-up period.

We investigated patient demographics, comorbidities, clinical manifestations of venous disease, preoperative venous duplex scanning findings, surgical procedures, and postoperative outcomes. All patients presented with symptomatic varicose veins and a preoperative Clinical-Etiology-Anatomy-Pathophysiology clinical classification of C2–C6 (**Table 1**). All patients were admitted and underwent clinical evaluations and routine hematological tests. They routinely underwent venous duplex scanning according to the Society of Vascular Ultrasound guidelines with Aloka prosound *α7* (Hitachi Aloka Medical, Japan). Saphenofemoral junction (SFJ) incompetence, GSV reflux, short saphenous vein (SSV) reflux, and deep venous reflux were assessed preoperatively in all patients. Valve closure times were assessed using the standing cuff deflation technique, with values of >0.5 s considered abnormal. Patients exhibiting abnormal results were scheduled for the intervention.

Table 1. Characteristics and comorbidity profile. 35 treated limbs in 25 patients who underwent radiofrequency venous ablation.

Variable		Total (n = 25)
Sex (male:female)		14:11
Age (y)		69.5 ± 13
Foot	unilateral:bilateral	27:08:00
Vein	GSV:SSV	35:00:00
CEAP classification:		
C1:C2:C3:C4:C5:C6 = 0:23:8:3:0:1		
GSV diameter (mm)		
Origin		7.5 ± 2.0
Knee		4.8 ± 0.7
Ankle		2.2 ± 0.3
Follow-up, months		6.5 (IQR, 79 - 359 days)
Hypertension		18
Diabetesmellitus		8
Ischemic heart disease		11
Smoking history		12

2.2. Study Device

All procedures were performed using segmental RFA with the ClosureFast™ system (Medtronic, USA), along with a ClosureRFG™ radiofrequency generator and a ClosureFast™ Endovenous catheter, and were performed by a trained consultant surgeon experienced and proficient in the system.

2.3. Intervention

The procedure was performed under regional anesthesia using routine intravenous preoperative antibiotics. Saphenous vein mapping was conducted in the operating room before the procedure with the patient in a standing position.

Initially, high ligation of the GSV at the SFJ was performed in all cases. Before RFA introduction, high ligation and devascularization of the varix were performed in certain cases due to recurrence associated with SFJ branching [6]. Although this has not been a standard procedure since the introduction of RFA, high ligation and devascularization were additionally performed alongside RFA in selected cases.

Ligation was performed approximately 1.5 cm distal to the SFJ using No.1 silk thread with 3-0 silk thread penetration; however, the GSV was not divided at this stage. Next, in the reverse Trendelenburg position, GSV access was achieved below the knee with duplex guidance using a microneedle, after which a 6F sheath was inserted, except in some patients who underwent venous cutdown. The RFA catheter was inserted 1.5 cm distal to the ligation site. Tumescent local anesthesia (TLA), which included 40 mL of 1% lidocaine with 1 mL epinephrine (1:100,000) in 500 mL of normal saline neutralized with 10 mL of 7% sodium bicarbonate, was administered under duplex guidance via a 20-gauge needle around the GSV extending from the sheath to the required area for cauterization.

Ablation was performed in 20-second cycles, with settings ranging from 40 W down to 10 - 15 W. The heating treatment was performed at 120˚C with a power setting of 40 W. Two cycles were applied to the initial 7 cm segment, followed by one cycle per subsequent segment. The catheter was withdrawn in 6.5 cm increments after each ablation cycle to allow a 0.5 cm segment overlap, following the manufacturer's guidelines.

Next, the catheter was removed, and the GSV, approximately 2 cm distal to the catheter insertion site, was ligated with a 3-0 silk thread and severed. Subsequently, a 2 cm segment of the cauterized vein was harvested as a pathological specimen after RFA.

The excised blood vessels were preserved in 10% buffered formalin and subjected to histopathological examination. After routine paraffin tissue processing, 5-micron-thick cross-sections of the varicose veins were stained with hematoxylin-eosin (HE) and Masson's trichrome. Thereafter, microphlebectomy was additionally performed to address the varicose veins, and each incision site was closed with sutures.

2.4. Outcomes and Follow-Up Protocol

All patients underwent a duplex ultrasonography of the treated extremities at the initial follow-up visit, conducted within a few weeks of the procedure. Duplex USG assessment results were classified as occluded vein (incompressible vein and no flow) and patent vein (partially incompressible vein and minimal flow pattern; compressible vein and presence of reflux for more than 2 s).

The GSVs were insonated to assess the results of RFA, such as changes in perivascular tissue, vessel walls, luminal structure, and the presence of thrombosis.

During the examination, the patients were positioned standing, and blood flow in the ablation area was observed using the milking technique. The patients were evaluated for possible adverse reactions to RFA during follow-up visits.

2.5. Statistical Analysis

Continuous variables are expressed as the mean ± standard deviation after the normality test (Kolmogorov-Smirnov test). If data were not normally distributed, the median and interquartile range (IQR) were reported instead.

3. Results:

A total of 35 RFA procedures were performed in 25 patients (14 men, 11 women) with a mean age of 69.5 ± 13 years (range, 31 - 85 years; **Table 1**). Thirty-three limbs had symptomatic varicose veins with or without skin changes (C2–C4), with one limb demonstrating a history of venous ulcers (C6). The etiology was primary valvular incompetence in all limbs. This study was performed in accordance with the surveillance for deep vein thrombosis and pulmonary embolism, and major complications, including thrombophlebitis, deep vein thrombosis, pulmonary embolism or skin burns, were not observed during RFA. The size of the catheter used was 6 Fr in all cases, with a hydrophilic GLIDEWIRE employed in five limbs to assist in catheter advancement through tortuous or spasmodic saphenous veins.

3.1. Histological Evaluation

In acute experiments, histological damage beyond the endothelial layer of the vessel wall was observed in all varicose veins. Vessel intima edema was observed in 85.7% (30/35) of veins treated with RFA, while microthrombi were detected in 88.6% (31/35; **Table 2**). Additionally, pathological changes, including loss of endothelial cells, vessel intimal thickening, vessel intimal-medial smooth muscle cell degeneration, and myxomatous degeneration of the intima-media, were also observed.

HE staining revealed a fibrin thrombus, medial structure degeneration (**Figure 1(A)**), and intimal hyperplasia in the vessel lumen. Masson's trichrome staining revealed rupture and intimal thickening of the medial smooth muscle (**Figure 1(B)**), cell infiltration of the adventitia, and partial neovascularization (**Figure 1(C)**). Similar to the macroscopic evaluation, the segments proximal to the large side branch were occluded.

Table 2. Histological evaluation in the acute experiments.

Loss of endothelial cell	8/35 (22.9%)
Vessel intimal edema	30/35 (85.7%)
Vessel intimal thickening	12/35 (34.2%)
Vessel intimal medial cell degeneration of smooth muscle	16/35 (45.7%)
Myxomatous degeneration of intimal media	8/35 (22.9%)
Microthrombus	31/35 (88.6%)

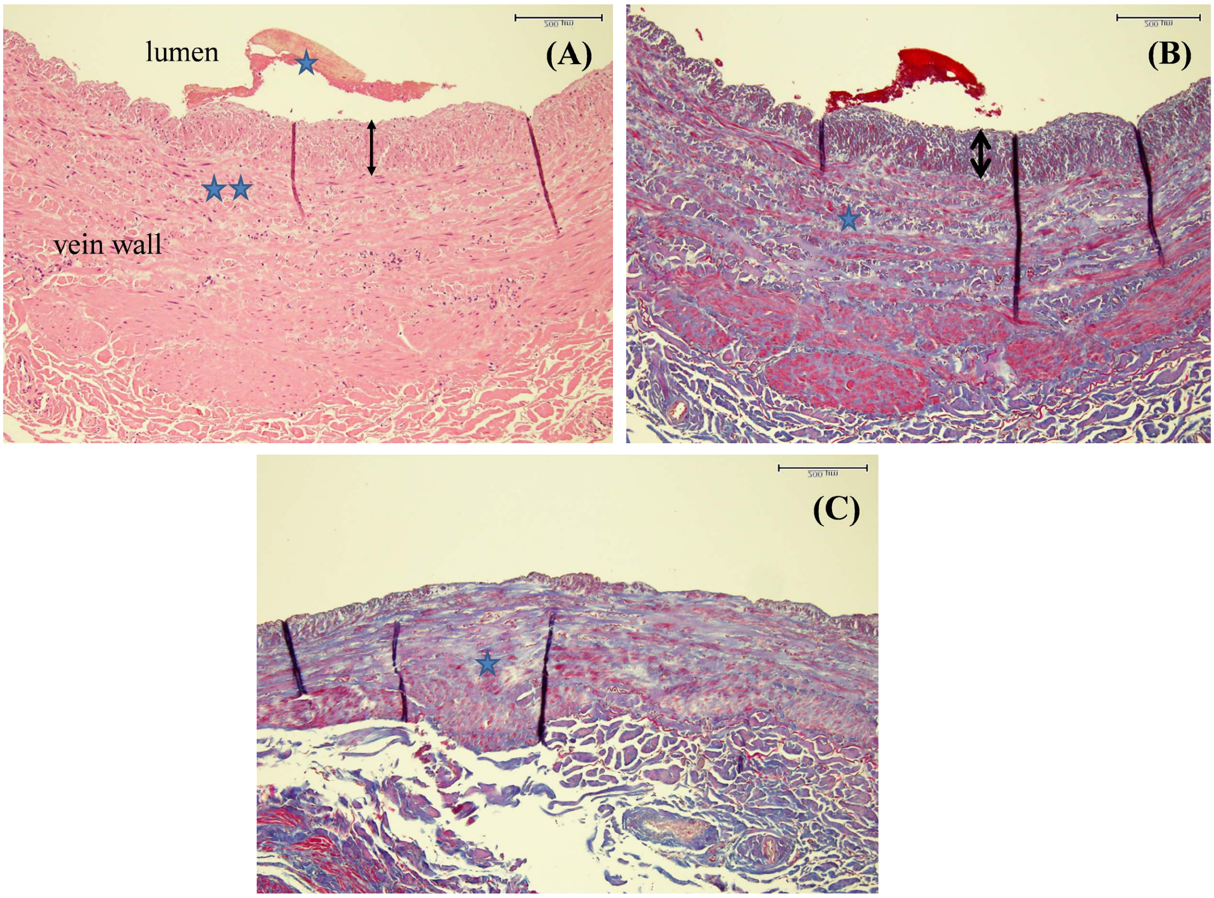

Figure 1. Microscopic hematoxylin-eosin stain and Masson's trichrome stain for histopathologic examination of GSV that has undergone RFA. (A) A fibrin thrombus (★) was found in the lumen. Intimal thickening (↕) and degeneration of the media structure (★★) were observed (×100, hematoxylin-eosin stain). (B) Medial smooth muscle rupture (★) and intimal thickening (↕) were observed (×100, Masson's trichrome stain). (C) Cell infiltration into the outer membrane and partial neovascularization (★) were observed (×100, Masson's trichrome stain).

3.2. Duplex Scanning Examination

Postoperative duplex scanning was performed in all cases within 7 - 59 days (median, 21.2 ± 19 days; Table 3). The aforementioned studies have demonstrated changes in the GSV, including intravascular thrombus, intimal thickening, and residual intravascular blood flow. Intravascular thrombi were observed in all cases, and intimal hyperplasia was observed in 80.0% of cases. Postoperative duplex scanning demonstrated blood flow in the penetrating branches of the varicose veins in 14 limbs (40.0%).

Table 3. Postoperative venous duplex scanning. The average day of postoperative duplex scanning: 21.2 ± 19 day.

Intravascular thrombus	35/35 (100%)
Vascular occlusion	26/35 (74.3%)
Thickening of vessel intima	28/35 (80.0%)
Blood flow presence of penetrating branches	14/35 (40.0%)

4. Discussion

With the increasing number of cases, varicose vein cautery has become a standard procedure. EVLA was first reported by Navarro *et al*. [7], and RFA was reported by Chandle *et al*. [8]. Other results comparing EVLA and RFA [9]-[12], postoperative outcomes have also been reported [13] [14].

Animal models have been utilized to examine changes in blood vessels following ablation. Schmedt *et al*. [15] reported structural findings using high-resolution optical coherence tomography in an animal experimental model.

EVLA findings included semicircular tissue defects, vascular wall perforation (+) in the blood vessel lumen, and a significant increase in lumen media thickness (+). The RFA findings were as follows: wall tissue defect (−), complete circular collapse of the inner media structure (+), a significant increase in intima-media thickness (+), and a significant decrease in vascular lumen diameter (+).

Thomis *et al*. [16] investigated changes in the luminal thrombus and vascular wall of ablated venous vessels in an animal model and discovered no significant differences between the two groups. However, histologically, they reported that RFA caused less damage to the perivenous tissue than did EVLA.

Each study was conducted as an animal experiment using the distal saphenous vein of an animal model. However, the environmental conditions differed from those in real-life clinical practice.

First, biological differences exist between human venous blood vessels and those of experimental animals.

Next, when comparing EVLA and RFA, the laser wavelength varied across studies, from 980 nm to 1470 nm. Thus, the effect of cauterization on the vascular tissue varied, making a direct comparison challenging.

In addition, tissues surrounding the venous blood vessels show differences, such as the attachment of muscles, amount of perivascular adipose tissue, vessel course, presence of perforating branches, and variation in their number. Furthermore, the extent of the anesthetized area may vary when local anesthesia is applied in humans versus experimental animals.

Due to these differences, clinical outcomes in humans are likely to differ significantly from those observed in animal experiments. Reports have highlighted the in vitro use of human venous blood vessels [1]. However, these studies involved venous blood vessels exposed outside the human body, resulting in varied environmental conditions.

Even before the introduction of RFA, our institution employed high ligation to treat varicose veins with venous reflux throughout the GSV. We examined cases of recurrent varicose veins in the lower extremities and identified dilation of various branch vessels at the root of the GSV as a likely cause of recurrence.

Recurrence of varicose veins in the lower extremities has been attributed to tactical failure, technical failure, or angiogenesis [17]-[20].

Based on our clinical experience to date, factors such as SFJ branching [6], angiogenesis, and duplicated GSV (D-GSV) [21] may be implicated in recurrence following treatment of varicose veins of the lower extremities.

Hence, even before the introduction of catheter-based endovascular treatment, we have been treating the blood vessels around the SFJ using high-level ligation in combination with sclerotherapy, depending on the clinical indications. Even following the introduction of catheter-based endovascular treatment, the indications have been examined for each case, with high-level ligation performed in combination as appropriate.

Before cauterization, TLA anesthesia was administered to the affected area to prevent pain and damage to the surrounding tissues, with a sufficient volume of TLA solution injected around the vessel targeted for cauterization. Consequently, the intravascular blood was typically encased by the low-temperature TLA solution surrounding the venous blood vessels. Although the catheter in the venous blood vessel was heated to 120˚C, a temperature gradient existed between the interior and exterior of the blood vessel, making it unlikely that the entire venous blood vessel was cauterized uniformly at 120˚C.

EVLA demonstrated remarkable loss of semicircular vascular wall tissue and complete vascular wall perforation, whereas RFA exhibited no vascular wall defects or symmetrical circular collapse of the intimal and medial layers [15].

Although the effects of both methods result from heat denaturation, histopathological changes occurred more gradually with RFA than with EVLA. This study demonstrated that ablated venous blood vessels exhibited histopathological changes consistent with the pathological effects of heat denaturation. Intimal and medial thickening, consistent with the findings reported by Schmedt *et al*. [15], was observed; however, no vascular occlusion resulting from tissue degeneration was detected.

Additionally, macroscopic intravascular thrombosis and histological microthrombosis were observed in approximately 89.0% of the cases, with postoperative duplex scanning of the lower extremities revealing intravascular thrombosis in all cases.

We believe that during ablation, especially with RFA, the primary mechanism may be intravascular thrombotic obstruction rather than systemic obstruction due to the thermal degeneration of venous blood vessels. This mechanism may be similar to that of phlebitis.

Clinically, the diameter of the ablated blood vessels varies, whereas the catheter diameter remains constant. Additionally, effective ablation requires transcutaneous compression of the target site during the surgery. As presented in **Table 3**, approximately 74.3% obstruction and 40.0% residual perforator blood flow were observed, further indicating uneven ablation during the process. Based on the outcomes of this study, further therapeutic effects are anticipated. Consequently, after this study, additional sclerotherapy has been incorporated after ablation.

5. Conclusion

We investigated the pathological changes in the GVS following RFA in clinical cases of varicose veins of the lower extremities. Thickening and edema of the vascular tissue were observed upon ablation; however, no vascular occlusion attributable to the vascular tissue was detected. During the post-cautery course, thrombosis was observed in most ablated blood vessels, which may be a contributing factor to blood flow obstruction.

Consent

Informed consent was obtained from the patients to report this cases.

Conflicts of Interest

All the authors have read the manuscript and have approved this submission. The authors declare no conflicts of interest regarding the publication of this paper.

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