Neovascularization and recurrent varicose veins: More histologic and ultrasound evidence

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Background: The recurrence of varicose veins is a common and costly consequence of varicose vein surgery. Despite the long history and vast experience of varicose vein surgery, the exact cause of recurrence is still unknown. This study aims to investigate the cause of recurrence further by correlating findings from duplex ultrasound scans, resin casts, and histologic investigation at the recurrence of the saphenofemoral junction. In particular, frequency and neovascularization are evaluated.

Method: Forty-nine saphenofemoral junctions (SFJs) from 42 patients who presented for re-operation on their varicose veins were examined with duplex ultrasound and physiologic air plethysmography tests before surgery. All patients had reflux at the groin for which surgery was carried out. Specimens taken during surgery were sectioned and stained for conventional histology and immunohistochemistry, and 5 specimens were infused with resin to form a cast of the venous vasculature.

Results: All but 3 re-operation specimens (94%) showed multiple vessels at the stump site of the previous SFJ ligation. Neovascular channels of variable size, number, and tortuosity accounted for the ultrasound appearances and reflux to recurrent varicosities in the vast majority of specimens. These new vessels connected to the common femoral vein at the site of the previous SFJ. In 2 incompetent junctions without femoral vein involvement, while small vessels were seen surrounding the femoral stump scar, ultrasound and histology confirmed both neovascular and residual (enlarged collateral) connections from epigastric and pudendal vessels into the thigh.

Conclusion: Neovascularization is the major cause for ultrasound-confirmed recurrence of reflux in the groin following varicose vein surgery. (J Vasc Surg 2004;40:296-302.)

Recurrence following surgery for varicose veins remains unacceptably high. Estimated rates of recurrence are as high as 40% at 5 years. As a result, surgery for recurrence represents a significant proportion of the workload of surgeons operating on varicose veins. The operations are also technically more demanding and complicated. Of the 100,000 varicose vein operations performed each year in the United Kingdom, at least 20% are performed for recurrent disease.

There has been long-standing speculation about the mechanism by which varicose veins recur, with poor surgical technique historically taking most of the blame. The complexity of the saphenofemoral junction (SFJ), as for example described by Royle3, along with the inexperience of more junior surgeons, is the often given reason for the inadequacy of the surgery. Anatomic variation is important and the advent of ultrasonography has shown how common these are. Persistent venous disease and incompetent perforators also may contribute to recurrence. In the past, a few investigators suggested neovascularization as a possible but infrequent cause of recurrence. This neovascularization has now been demonstrated on phlebography, ultrasonography, and histology. More recently, it has been suggested that recurrence due to the regrowth of veins at the SFJ after saphenofemoral ligation may be much more common than previously realized. However, many remain skeptical.

In order to overcome the problem of recurrent varicose veins, it is important to develop a clearer understanding of the cause of recurrence. In particular, the relevance of neovascularization needs to be clarified as well as the ability of ultrasonography and the surgeon’s perception at re-do surgery to discriminate this phenomenon. This study examines the histologic, immunohistochemical, and resin cast morphologic features of recurrent refluxing SFJs and correlates these with the duplex ultrasound and surgical appearances.

PATIENTS AND METHODS

Patients. Patients were an unselected consecutive group with recurrent varicose veins seen prospectively over a 4-year period at the vascular laboratory before surgery. These patients were clinically assessed by the standard classification recommended by the Society for Vascular Surgery and International Society for Cardiovascular Surgery. All had clinical classification class 3-6 disease. A clinical evaluation including surgical and medical history, risk factors for chronic venous insufficiency, type of previous superficial venous surgery, and time since initial surgery were recorded. Venous assessment included a full duplex ultrasound scan and physiologic tests with air plethysmography (APG).
Duplex scanning. Scanning was performed as described by Kalodiki et al. In brief, imaging of the groin and thigh veins was performed with the patients in a 30° reverse position. Examination of the popliteal and calf veins was carried out with the patients in a sitting position. A retrograde flow persisting for longer than 0.5 seconds was considered significant reflux. Particular attention was given to the recurrent SFJ by identifying the configuration and number of channels and their connections in the groin, primarily by using the Valsalva maneuver. The patterns of recurrent varicose veins in the groin were then classified as previously described. In brief, these included (1) single narrow channel (diameter ≤3 mm), (2) single large channel (>3 mm), (3) multiple small channels, and (4) no connection between common femoral vein (CFV) and superficial veins in the groin. In this latter group, reflux in the groin was from epigastric or pudendal veins down into the leg.

Physiologic tests. APG was performed with the technique as previously described by Christopoulos et al. and more recently by our own group. Measurements were completed with the patients lying, standing, and performing a series of tip-toe stands. Severity of venous reflux was primarily measured by venous filling index (VFI) and venous filling time (VFT).

Surgery. Each site of SFJ recurrence was approached through a transverse skin crease incision to expose the common femoral artery and dissecting medially from its anterior aspect towards the CFV. The vein was dissected from the common femoral artery (CFV) and dissecting medially from its anterior aspect towards the CFV. The vein was de-vascularized and included with the common femoral vein (CFV) and superficial veins in the groin. In this latter group, reflux in the groin was from epigastric or pudendal veins down into the leg.

Statistical analysis Data is presented as means ± 1 standard deviation. Analysis was performed on SPSS (Statistical Package in the Social Sciences, Chicago, Ill.).

RESULTS

Forty-two patients contributed a total of 49 limbs to the study. All 49 limbs had recurrent varicose veins in the groin, 47 with reflux into the CFV and 2 with non-saphenofemoral reflux. In addition, 3 recurrent saphenopopliteal junctions (SPJs) were resected and evaluated. The patients were aged 57.2 ± 18.2 years, with 52% requiring surgery on their left legs and with mean time since initial surgery of 9.6 years (range, 2 to 39 years). All patients had significant reflux (VFI, 5.4 ± 4.2 mL/s; VFT, 37.0 ± 21.4 s; 31% with a history of venous ulcers).

Ultrasonography identified 2 major patterns of reflux connected to the CFV: (1) apparent single large channels either with a direct course or a more tortuous one (38%) or (2) tortuous multiple small channels (62%). In either case, these vessels appeared to connect to the femoral vein as a single channel at the point of previous ligation (Fig 1). There did not appear to be a strong correlation between ultrasound patterns and the number of vessels observed histologically. Although the severity of reflux at the recurrent site was worse in those with the single-channel appear-
ance (VFI, 6.6 ± 5.7 mL/s vs 3.4 ± 2.4 mL/s for multichanneled, \( P = .03 \)), there was no correlation with the clinical severity (class 5 or 6, 38.5% in single channel, 40.0% in the multiple channels). Other sites of incompetence were present in the majority of the legs.

Surgical dissection confirmed that the site of reflux from the CFV was always from the previously ligated SFJ stump. The base of the stump was always wide necked at the femoral vein and frequently looked almost as if a surgeon had not been there before (Fig 2). Alternative connections to the CFV through other branches were not seen in any dissection. In the 2 limbs without ultrasound reflux from the CFV, surgery confirmed reflux to be from the abdominal wall and pudendal vessels. As in the other limbs, this reflux continued into the large refluxing vessels in the leg and was a contributor to the recurrent varicosities. Surgery also confirmed that there were no channels in continuity with the CFV, and the residual stump site scar tissue was excised and submitted for histologic evaluation.

Histology and resin casting of the 49 recurrent SFJ specimens did not reveal any intact unligated GSV at the SFJ. The histological orientation and the location of the previous site of surgical ligation at the SFJ was readily confirmed by the characteristic residual disrupted elastic lamina of the transected and ligated stump of the GSV and the presence of sutures and associated fibrosis.

In 46 specimens the dominant picture was of multiple neovascular channels through which the reflux had been shown to occur. The number of vessels observed by histology averaged approximately 30 and was often greater than 60, though it must be noted that the tortuous nature of these vessels made the possibility of repeat counting highly likely. These channels varied greatly in size, often with several large channels being surrounded by numerous small vessels. The channels were all lined with endothelium (von
Willebrand positive) and possessed a wall containing smooth muscle cells (α-smooth muscle actin positive). Most had the appearance of primitive vessels, lacking both clearly defined intimal and adventitial layers and medial and adventitial elastic tissue (Fig 3, A, B). A small minority of the multiple small vessels observed appeared to be distended native vessels with disrupted mural elastic fibers. (Fig 3, C).

Further out from the stump, these intact and at times overdistended residual veins were more evident (Fig 3, D). In only 3 specimens were these channels the dominant feature and in 1 specimen this was seen as a single dominant channel, but in each instance these were connected to the SFJ stump by neovascular channels. One of these specimens contained a medium-sized residual vein containing valve leaflets. The new vessels occurred in abundance throughout this material, in some cases even invading the suture material. The new channels were also seen to pervade the small arteries ligated and thrombosed at initial surgery. The extent of these neovascular channels was significant and could be followed to their connection to native varicose veins. Some of the larger vessels were seen to pass through adjacent lymph nodes and down the thigh to varicosities. Connections to recanalized, previously ligated thrombosed GSVs at least 5 to 10 cm away as well as to new channels within the tracts of the stripped GSVs could be observed (Fig 4). In cases in which the ultrasound reflux had not been shown to be in continuity with the CFV, the scar tissue at the previous ligation was shown to still contain some neovascular vessels. These were all microvessels as

**Fig 3.** Vessel morphology within recurrent saphenofemoral junctions. A and B, Abundant neovascular channels, by far the most common vessel type observed. These vessels were lined by a simple squamous endothelium overlying a medial layer consisting of 2 to 5 layers of vascular smooth muscle. The wall of these vessels lacked elastic fibers, intramural nerves (identified by PGP 9.5 immunostaining), and a distinct adventitia. C, Small residual vessels. These vessels contained distinct medial and adventitial layers. Fine (arrowheads), though often disrupted (arrow), elastic fibers were also present within the vein wall, particularly along the intimal medial border. D, Distended (residual) vessels. These vessels also contained distinct intimal, medial, and adventitial tunica. Abundant elastic tissue (stained black) was present both along the intimal-medial border and within the adventitia. Some regions of the vein wall contained relatively normal structure, including intact medial and adventitial elastic tissue and only modest intimal thickening (arrows). In contrast, other segments of the same vein contained medial atrophy, elastic tissue disruption (arrowheads), and extensive compensatory intimal thickening (asterisk). Verhoeff’s elastic tissue stain counterstained with van Gieson’s. Scale bars: A and D, 200 μm; B and C, 50 μm.
different areas of the complex (Fig 5, these recurrent vessels as well as the variation in their size in recurrent SPJs were similar to those in SFJ recurrence. In diameter. The appearances of the specimens from the deeply or about the CFV or artery but always into the subcutaneous tissue. It never appeared to extend downward into the leg connecting to varicosities or residual great saphenous vein when present. The direction of the neovascular channels from the stump was always outward laterally, and cephalad, but the dominant direction was toward the resin was from a variable number of vessels medially, (Fig 3, they matched the tortuous multichanneled appearance observed on ultrasound (Fig 1, B). Clearly, the ultrasound discrimination of these vessels is limited and not surprisingly underestimates the number of channels present in histologic sections.

DISCUSSION

Although the possibility of new vessel formation and reconnection resulting in recurrent saphenofemoral reflux has long been suggested, it has largely been considered to be an infrequent event and a rare cause for recurrent varicose veins. This study shows that there is no doubt that this does occur and that it is the most common cause of recurrence at the site of ligation. This is not the first description of neovascularization at the SFJ following ligation for reflux in varicose veins. Previous descriptions have been based on phlebography, colour duplex ultrasonography, and histology. We have also inferred its frequent occurrence from the clinical observation that, despite ultrasound proven ligation, the SFJ and SPJ vessels reconstitute and reflux after 5 years in 32% and 50% respectively. The very high frequency that has now been shown in this study is far greater than ever thought in the past. It is consistent with the suggestion from similar work by Nyamekye et al and the result of a long-term clinical follow-up by Fischer. The most prevalent view has been that recurrence is the result of surgical error. Failure to ligate the major refluxing veins would not be surprising considering the anatomic complexity and has most often been attributed to the efforts of the less experienced surgeon in training. This does occur but infrequently at the SFJ (1%). Some researchers have suggested that recurrence may be the result of dilatation of pre-existing small collateral veins that were missed during the original operation. In this study, there is a surprising lack of occurrence of these vessels with intact connections to the SFJ or to the CFV that would indicate previous inadequate surgery. Examples would be expected if, as has been suggested, most SFJ recurrence is due to incomplete surgery, particularly as the patients in this study came from a wide range of surgeons and a wide period of time. We were surprised that in the resected recurrences not one example of the typical histologic features of intact native long saphenous varicose vein with patent lumen was observed. Our own experience has shown a 1% ultrasound proven failure rate for SFJ ligation. This would at least imply that incomplete surgery is not the most common cause for recurrence.

Elements of neovascularization appear to occur following every ligation, and some of these new veins go on to reconnect with the main venous channels and establish channels of sufficient caliber to become clinical recurrences. Neovascularization is part of the normal sequence in the healing wound. The new connecting veins arise due to angiogenic stimuli produced during the surgical procedure. Ligation of the vessel causes hypoxia-induced activation of endothelial cells distal to the stump; this process is mediated by a number of different growth factors, such as vascular endothelial growth factor and fibroblast growth factor. The vessels may go on to develop arteriovenous connections and allow perfusion of the wound. Why this process leads to venovenous reconnection and reflux in varicose veins is not known. It appears not to be a general feature of venous surgery as suggested by its absence after SFJ ligation for harvesting the GSV for femoral-popliteal arterial bypass. It does occur with recanalization of venous thrombosis. This suggests that persistent venous abnormality is a factor.

This study also shows that these new channels are not merely a histologic curiosity but account for the reflux observed on ultrasonography. It might be imagined that the multiplicity of relatively small channels would not be sufficient to allow significant reflux in the low-pressure venous system. This is clearly not the case. The observation
of a single large channel by duplex ultrasonography may still be consistent with neovascularization. Unfortunately, ultrasound has limited discrimination and large channels and significant reflux observed on ultrasound assessment can correspond to several larger and smaller new, thin-walled vessels clustered together. Ultrasound appearance of an apparent single large refluxing channel without discrimination of neovascularized channels may suggest that there are a few larger dominant new vessels. The severity of the reflux is greater with this appearance and more commonly contributes to severe venous insufficiency as a consequence of neovascularization. Despite the inability of ultrasound to distinguish individual neovascular channels at the site of recurrence at the SFJ, appearances more suggestive of a complex network of vessels can be observed and are described as either a narrow tortuous vessel or a “sparkling” cluster on color duplex scan. This particular appearance is unlikely to be due to a residual varicose vein. Significant reflux contributing to venous insufficiency with ulceration can also result from this pattern of recurrence. It is likely that not all neovascular channels will result in clinically relevant reflux. These new vessels are present from the early days of wound healing. There will be a critical requirement for reconnection to venous channels and the stimulus to enlargement. This happens to varying degrees. We have shown that, for patients without any demonstrable reflux, these new vessels remain undeveloped within the SFJ scar.

These observations are significant because they change the understanding of the relative importance of the causes of recurrence of SFJ reflux. Inadequate or wrong surgery, while still an important consideration, can no longer be considered the primary cause. The study of limbs immediately following surgery with ultrasound is an effective approach to auditing the efficacy of surgery. Surgical shortcomings then are of the order of 1% to 7%. However, when completeness of SFJ ligation has been shown by ultrasound, recurrence still occurs thereafter in 32% at 3 years. This has been interpreted by us to be primarily due to neovascularization. We observed neovascularization in the excised SPJ recurrences in this study, which fits well with the development of recurrence at this site also. Neovascularization also appears to extend significant distances and may reconnect with the GSV ligated high in the thigh. That stripping of the GSV reduces recurrence may simply be that it reduces the likelihood of reconnection by neovascularization.

Unfortunately, a limitation in this study is the impossibility of detailing the exact surgical techniques and materials used nor the range of skills and training of the surgeons at the time of the original surgery. Such information is difficult to obtain for surgery done years back, in several
institutions, big and small, throughout New Zealand, with surgeons forgotten by patients, and operative records and patient descriptions of limited value. Clearly, there was much variation. Despite this and to our surprise, there was no specimen with histologic evidence of an intact inadequately ligated GSV to suggest technical failure. GSV stripping, which is well known to reduce recurrence, had been carried out in many of the limbs and yet neovascular reconnection to leg varicosities still occurred. Moreover, it should be noted that this study is less about surgical technique and more about the biology of the healing vein and its impact on the treatment of varicose veins.

Having shown that neovascularization is a major cause of recurrence, we now need to develop new approaches for its prevention. While high-quality surgery will always remain an essential, this does not stop neovascular refluxing vessels from forming. Inhibition of this phenomenon may be achieved by a number of different approaches. Mechanical obstruction by placing an interposing layer over the vessels from forming. Inhibition of this phenomenon may be achieved by a number of different approaches. Mechanical obstruction by placing an interposing layer over the ligated stump such as fascia or a synthetic patch has been suggested.\textsuperscript{17,20-22} The effectiveness of this has not been proven. Suppression of vascular growth factors by the local application or slow release of appropriate inhibitors of angiogenesis may be an attractive approach. Closure of the SFJ by an endovascular route without inducing a vigorous angiogenic response is an attractive option. Early results of endovascular procedures do suggest recurrence is reduced. Even so, there are reports of SFJ recurrence and neovascular ultrasound appearances.\textsuperscript{2,3} The simple closure with sclerotherapy, with its endoluminally induced fibrosis and often associated thrombosis, is unlikely to succeed given the inevitability of recanalization, which is itself a form of neovascularization, within the vessel lumen.

However, it is our impression that this phenomenon occurs not only at sites of major ligations but also where superficial vessels are stripped out, avulsed, or injected, or where perforators are disrupted. These new vessels can extend significant distances in the lower limb to make their reconnections and form varicosities. To control such a widespread response may require rather different approaches. Other questions then come to mind that remain as yet unanswered, including whether underlying persistent venous disease encourages neovascularization or whether patients who develop recurrence have a previous abnormality of angiogenesis.

REFERENCES