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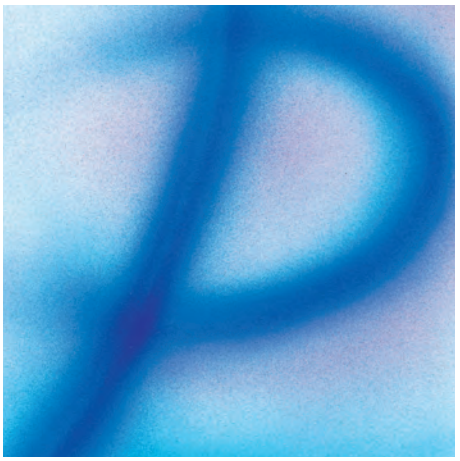

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AIMS AND SCOPE

Phlebolymphology is an international scientific journal entirely devoted to venous disease.

The aim of *Phlebolymphology* is to provide doctors with updated and interesting information on phlebology and lymphology written by well-known specialists from different countries worldwide.

Phlebolymphology is scientifically supported by a prestigious editorial board.

Phlebolymphology has been published four times per year since 1994, and, thanks to its high scientific level, was included in the EMBASE database in 1998.

Phlebolymphology is made up of several sections: editorial, articles on phlebology and lymphology, news, review, and congress calendar.

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CONTENTS

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PHLEBOLYMPHOLOGY

This issue of Phlebolymphology *will provide you with much information that is not readily available elsewhere.*

Were you aware that an incompetent small saphenous vein with centrifugal reflux is only rarely associated with a high junction above the popliteal fossa? One has to admit that this fact has considerable practical consequences with regard to surgery. Do you know why most high junctions are competent, or sometimes show centripetal reflux? You will find out when you study the comprehensive article by ***Denis Creton****.*

I am sure that you have already heard about the new catheter techniques utilizing radiofrequency or various laser probes for the treatment of great or small saphenous vein incompetence. If you would like an updated review of these topics then read the survey by ***Michel Perrin****, and you will find out everything you need to know about indications, techniques, and the latest results with these promising new endovenous procedures.*

You are probably already aware of the fact that compression is the most important treatment modality for the management of patients with venous leg ulcers. But did you also know that often this remains just mere theory, and that noncomplicance of ulcer patients with wearing bandages or stockings is a reality. In the article by ***Nicholas Fassiadis****, you will find a review of the literature on this important practical issue.*

Are you interested in lymphological research, with regards to the latest insights into lymphangiogenesis and lymphatic regeneration? Then I reommend the paper by Prof ***Seiji Kato****, which presents fascinating data on various newly discovered lymphatic endothelial cell markers.*

Enjoy reading!

Dr Hugo Partsch

Endovenous treatment of lower-limb varices by laser and radiofrequency

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Vascular Surgery
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SUMMARY

Endovenous treatment of varicose veins is an old technique, but new procedures using both radiofrequency and laser have markedly revived interest in it. In effect, the thermal energy these procedures generate and deliver to the venous wall results in vein wall fibrotic retraction and its subsequent occlusion. Details are given on the material and techniques used, including the difficulties, incidents, and accidents that can be encountered during the procedure.
The indications and contraindications concerning endovenous treatment of varicose veins are still debatable. Assessment of results remains difficult, particularly in laser procedures, since the material and technique used is variable and the techniques nonstandardized. Besides long-term results are not yet available.

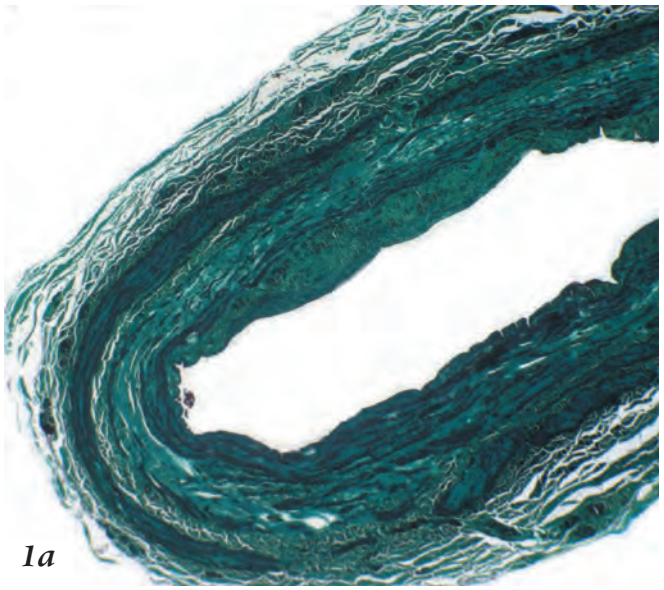
INTRODUCTION

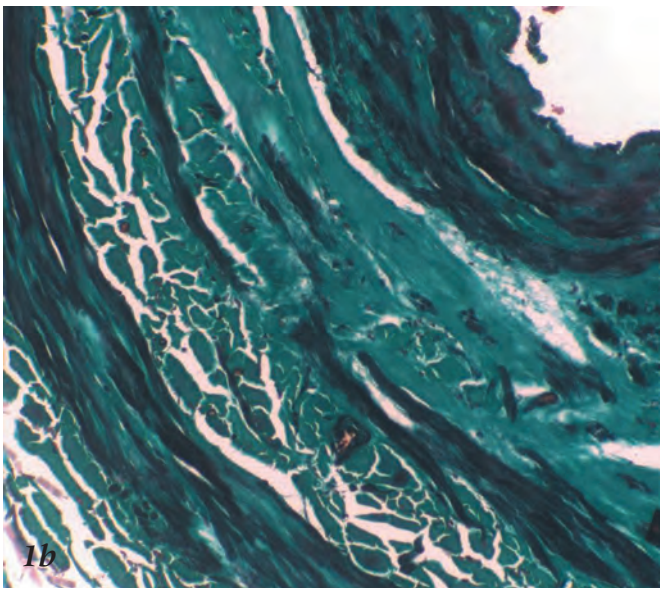
Varicose veins are subcutaneous veins that are permanently dilated, exhibit vessel wall alterations, and have a diameter greater than 3 mm when the patient is standing. They are usually tortuous, but the fact that they are a site of reflux is rarely mentioned.
Endovascular treatment means any therapeutic procedure carried out from the lumen of the vein that results in occlusion of the diseased vein, without anatomical excision, excluding sclerotherapy. Until the last few years, endovascular treatment of varicose veins had only seen limited development.

Principle and mode of action of radiofrequency (RF) and endovenous laser treatment (EVL)

Radiofrequency generates controlled thermal energy that raises the temperature of the vascular wall. It destroys the intima and causes contraction and thickening of the collagen fibers within the adventitia and especially the media *(Figures 1a, 1b, 1c)*.

Keywords:
Varices-Venous surgery - Varicosities endovenous treatment - Radiofrequency obliteration - Endovenous laser





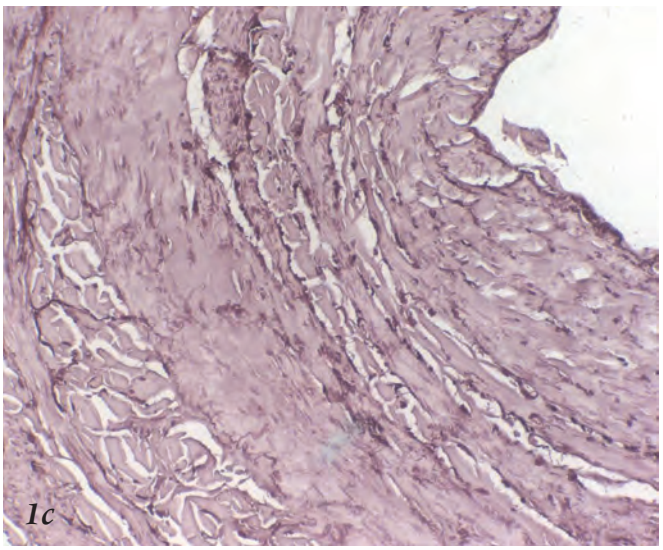


Figure 1a. Histopathology of the saphenous vein after Closure. Coagulation necrosis of the intima and media. Trichrome-light green stain.
Figure 1b. Histopathology of the saphenous vein after Closure. The myocytes of the media are stretched and flattened within the necrotic area. Trichrome-light green stain.
Figure 1c. Histopathology of the saphenous vein after Closure. The elastic fibers are fragmented. Orcein.

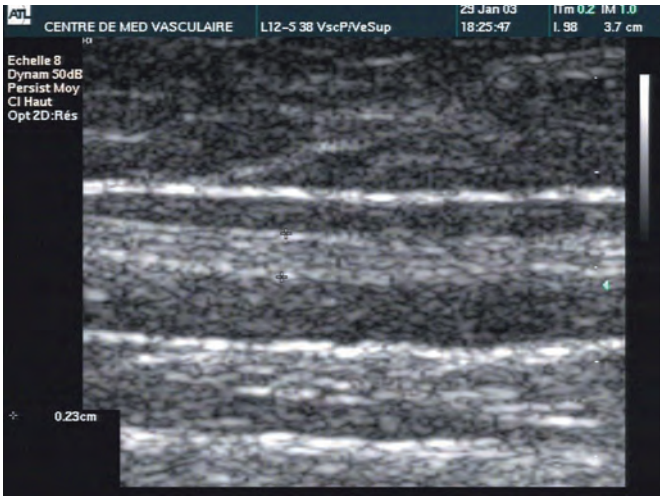


Figure 2. Fibrous transformation of the main body of the great saphenous vein. Doppler sonography checkup 4 years after the Closure procedure.

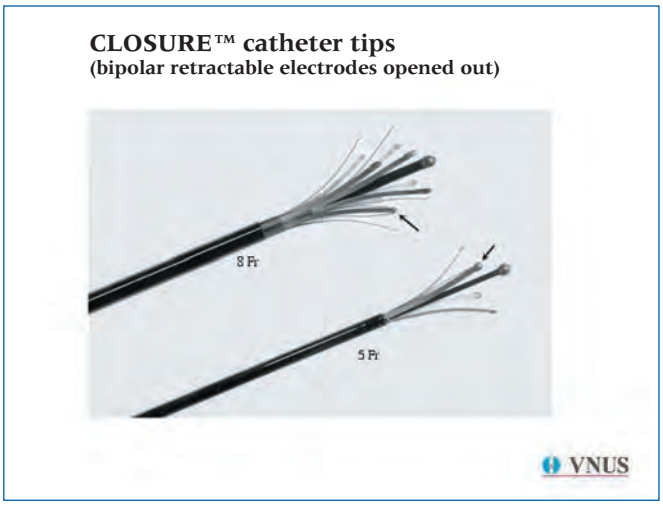


Figure 3. Tip of the Closure 5 and 8 F catheters.

The diameter of the vein is thus greatly reduced by contraction and thickening of collagen fibrils and also by the spasm induced by the increased temperature. These phenomena cause secondary fibrous changes, which are usually gradual, and result in the occlusion of the venous lumen *(Figure 2)*. Thrombus formation is reduced to a minimum, since the procedure is performed on a vein containing no blood, eliminating the risk of recanalization by thrombolysis.

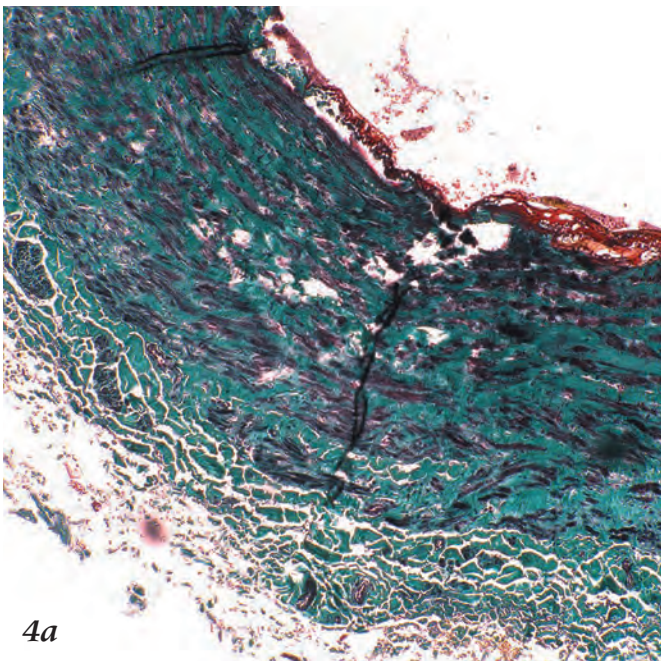
Radiofrequency produces this controlled thermal effect through a generator connected to a catheter. The catheters used have bipolar electrodes that deliver a temperature of 85°C at their tip *(Figure 3)*. This temperature increase is obtained over a cylindrical zone, 6 to 8 mm long. The diffusion of this heat depends on the distance from the electrode, the temperature decreasing progressively with the distance from the point of contact between the catheter and the vein, falling to values in the region of 43°C at a distance of 2 mm. This is why the thermal energy is diffused continuously.

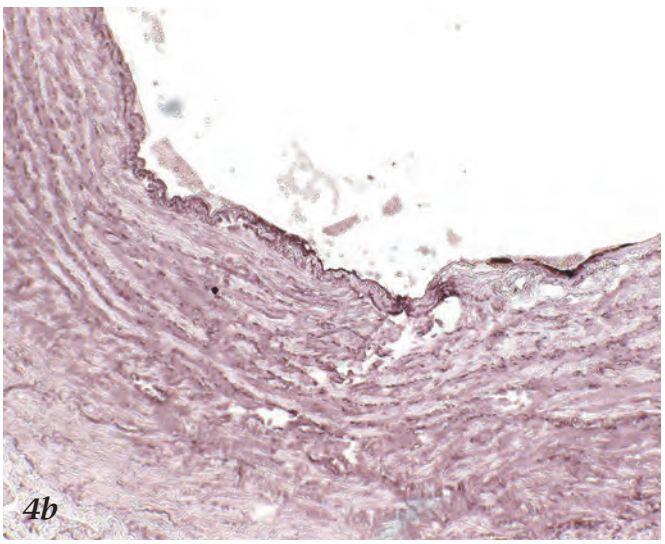
Lasers. Laser treatment uses thermal energy, acting through three complex and successive stages:

- Conversion of light to heat by optical diffusion, which varies according to the medium in which the light is delivered. As Proebstle[1,2] convincingly showed when studying the effects of the diode laser (810 nm, 940 nm, and 980 nm), the effect differs according to whether the light is delivered in normal saline, plasma, or blood. The laser energy, delivered into the blood with a 600-µm fiber using successive pulses of variable length, creates bubbles of vapor generated by the hemolyzed blood. Indeed, the treated vein is not collapsed during this procedure.

Three successive stages have been identified:

- Conversion of laser light into heat by optical diffusion. The volume heated, which in this case is blood, is termed the "primary heated volume."
- Transfer of the heat by conduction into the surrounding tissues, ie, the venous wall. The bubbles transmit the thermal energy to the entire circumference of the internal venous wall, the "secondary heated volume." The average temperature measured at the tip of the laser fiber is on average 729°C.[3] The thermal effect is diffused weakly in





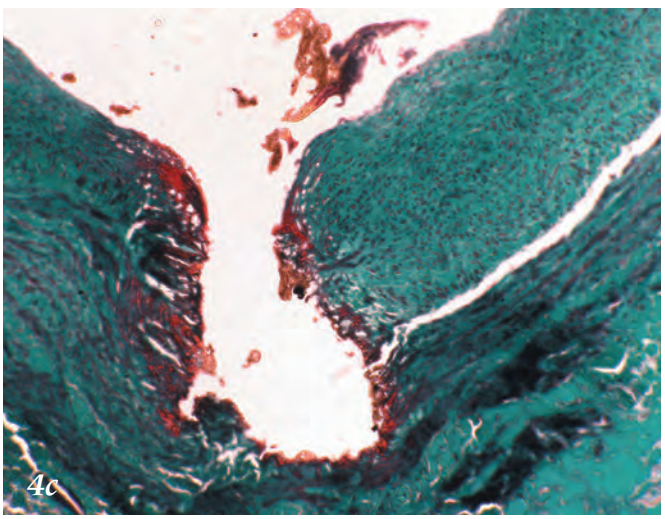


Figure 4a. Histopathology of the saphenous vein after laser procedure. Trichrome-light green stain.
Figure 4b. Histopathology of the saphenous vein after laser procedure. Coagulation necrosis involving the intima. Orcein.
Figure 4c. Histopathology of the saphenous vein after laser procedure. Localized coagulation necrosis involving the intima. Trichrome-light green stain.

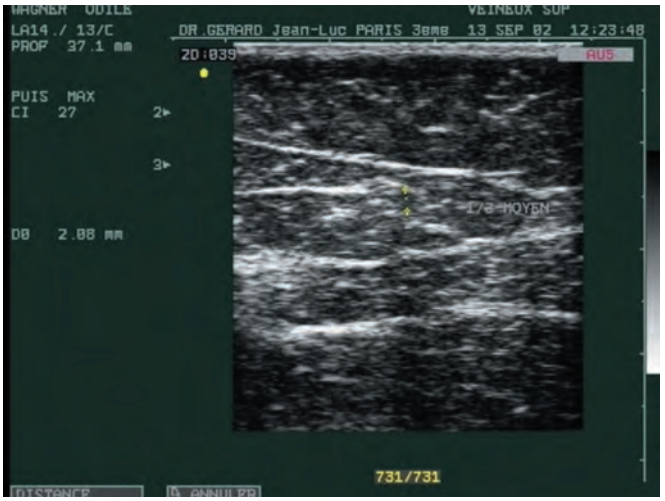


Figure 5. Atrophic appearance on Duplex sonography of the treated vein, 6 months after laser treatment.

blood: its penetration ability in the tissues is 0.3 mm[2].
- The 3rd stage is thermochemical. It results in the destruction of tissue. The appearance on histopathology[3] *(Figures 4a, 4b, 4c)* and ultrasound *(Figure 5)* of the treated vein is well documented. If applied continuously, the laser can cause perforation of the venous wall.[3]

Equipment

Radiofrequency The equipment is currently marketed by a single company, and is called Closure™.

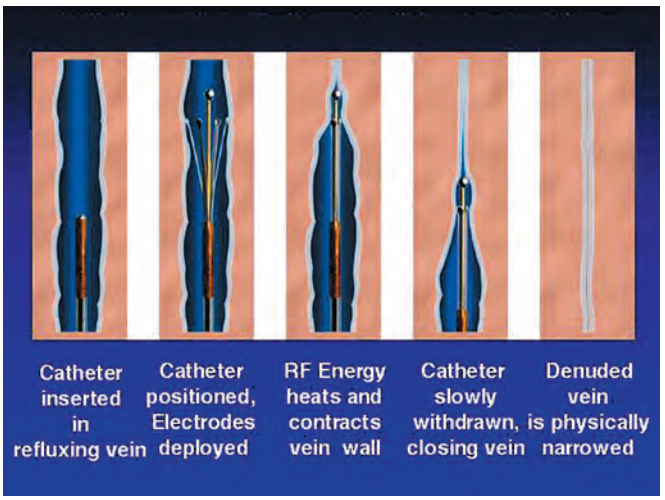
Endovenous laser The equipment is currently marketed by several companies, providing diode lasers, which have supplanted the YAG laser.

Technique

1) **RF and EVL procedures** have a number of points in common. They are usually performed under local (tumescent in most cases) or locoregional anesthetic, and require:
- either a tiny surgical incision, with limited exposure of the distal part of the vein to be treated, which can be accessed using a phlebectomy hook, then opened by phlebotomy;
- or percutaneous puncture.
In practice the vein is accessed below the segment to be treated (usually at the knee for the great saphenous vein) after localization using Duplex sonography (DS). A straight or J-shaped metal guidewire or better still a hydrophilic guidewire is then passed along the vein, and its position is checked using DS. An introducer is then passed over the guidewire.

After the procedure, the (Closure) catheter or the fiber-catheter unit (EVL) is withdrawn from the lumen of the vein. The cutaneous incision is closed and a compression bandage or an elastic stocking is put on the treated limb immediately.

2) **Closure™ procedure**
The choice of catheter depends on the caliber of the vein to be treated: 6 F (1.7 mm) is used for veins with a diameter less than or equal to 8 mm, measured in the supine position, and 8 F (2.7 mm) for veins of more than 8 mm. The catheter is connected to the generator and to a heparinized saline infusion that is maintained throughout the procedure to prevent thrombus formation inside the catheter. The catheter is passed along the vein with the electrodes folded *(Figure 6a)* as far as the upper segment of the area to treat. The blood is expelled from the limb by applying an Esmarch bandage, with additional manual compression over the tip of the catheter, the patient lying with the legs higher than the head at an angle of about 20°. The electrodes are deployed to make contact with the wall of the vein *(Figure 6b)*. The position of the catheter is then determined accurately (using ultrasound or fluoroscopy). It is important that the limb is not moved after this step. The various parameters are displayed on the screen of the generator: power (6 watts), temperature (85°C) and the duration of the procedure (999 s). Before commencing the procedure, the impedance is measured. The value displayed should be greater than or equal to 200 ohms, indicating that there is adequate contact between the electrodes with the venous wall. The treatment is then started. The catheter, with its electrodes deployed, is slowly drawn back from the proximal to the distal end of the area to be treated *(Figures 6c, d)* at a speed of 2 to 3 cm/minute



Figures 6 a, b, c, d. Closure procedure.

and the various parameters are monitored constantly. The maintenance of a constant temperature (85±3°C) determines the speed at which the Closure catheter can be advanced.

As mentioned above, two localization techniques may be used. The choice is usually made on the basis of the local availability of these investigations. Radiological techniques are rarely favored.

Duplex sonography (DS)[4] is the most commonly used investigation. Initially, it is used while entering the catheterized vein and later to monitor the progression of the catheter and its final position before radiofrequency is delivered, and finally to check the efficacy of the procedure after the intervention (absence of flow). The use of DS requires the presence of an operator who is experienced in this investigation, but it is faster than radiological mapping and is easy to repeat.

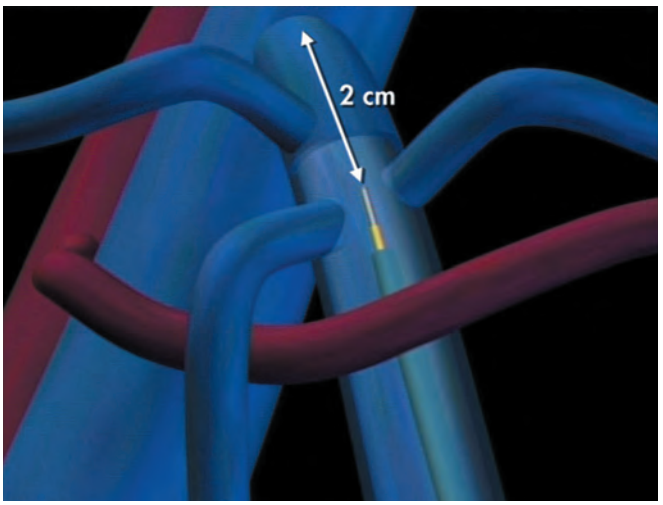


Figure 8. The tip of the laser fiber is positioned 2 cm below the saphenofemoral junction. It protrudes from the catheter by 2 cm.

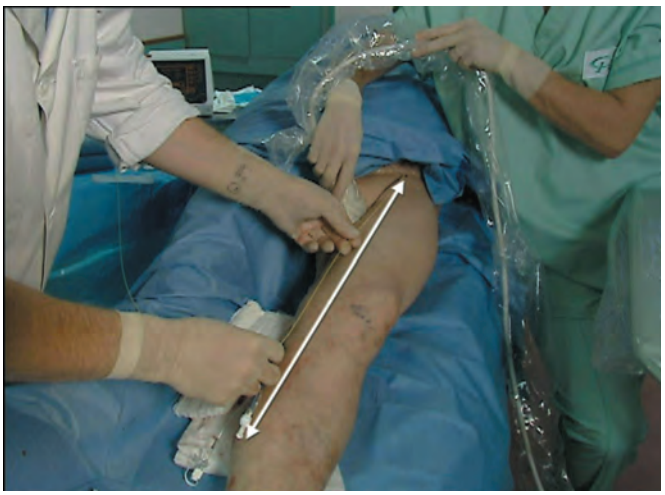


Figure 7. Laser procedure: the distance between the point where the fiber was introduced and the upper part of the vein to be treated is measured and marked on the laser fiber.

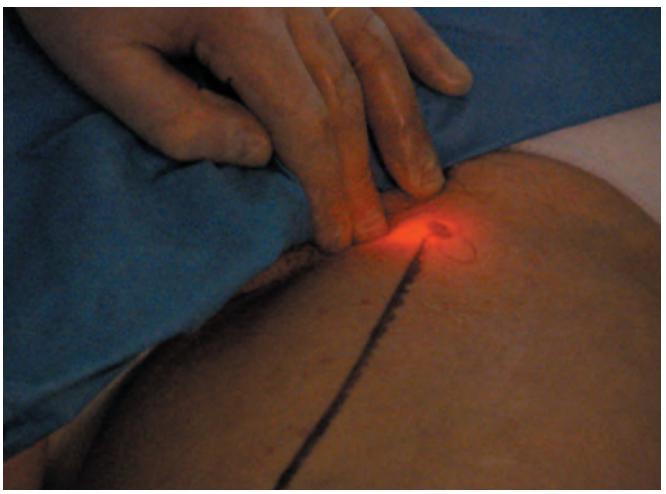


Figure 9. Laser procedure: the laser fiber is easily identified when it protrudes from the proximal tip of the catheter.

3) **Laser procedures**

Initially, the length of the vein to be treated is marked on the catheter using a Steri-strip or Steri-stip. It corresponds to the distance between the point of introduction and the point corresponding to the upper limit of the vein to be treated *(Figure 7)*. Similarly, the length of the catheter plus 2 cm is marked on the laser fiber. The catheter is then introduced into the lumen of the vein and moved along the guidewire using the introducer, which is left in place after any verification of reflux and rinsing with normal saline. Its tip must be positioned 4 cm below the upper limit of the vein to be treated *(Figure 8)*, its position is easily checked by DS, whereas the laser fiber is difficult to identify. The guidewire is then withdrawn and the laser fiber connected to the generator in standby position. The laser fiber is then introduced into the lumen of the catheter and pushed in until its tip (the sighting beam) is visible, which happens as soon as it emerges from the catheter as it is luminescent *(Figure 9)*. The tip of the fiber is therefore positioned 2 cm below the vein to be treated *(Figure 8)*. The fiber and the catheter are locked together. If the local anesthetic that was given at the point of introduction is used, it is then adminis- tered along the entire course of the vein to be treated. Tumescent anesthesia is the most common method. Everyone present in the theater wears protection glasses. The fiber-catheter unit is then withdrawn and disconti- nuous laser pulses are fired along the vein *(Figure 10)*. The various parameters (duration of

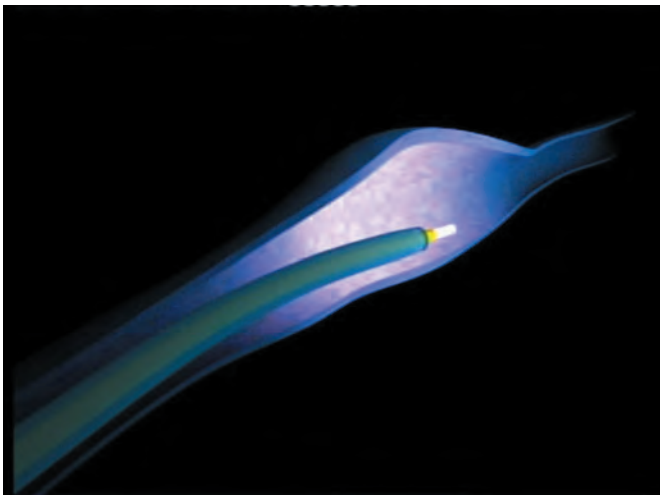


Figure 10. Laser procedure: laser pulses are delivered as the fiber is withdrawn.

the pulses, distance between two pulses, etc) vary according to the type of laser used. However, certain types deliver laser energy continuously.

The sighting beam can be seen through the skin, which means that in most cases the tip of the laser fiber can be visualized during the procedure as it moves along the vein.

4) **Additional procedures:** when the treated vein is the trunk of the great or small saphenous vein a number of further procedures can be performed.

Saphenofemoral junction ligation. This is no longer performed in the Closure technique because a study[5] showed that the results were equivalent with or without saphenofemoral junction ligation. It can be associated with the laser procedure[6] or not.[2,7-9]

Phlebectomy or sclerotherapy of collateral vessels. In the Closure technique, phlebectomy using mini-incisions along the collateral or tributary veins is usually combined with treament for venous insufficiency of the main vessel. As a rule, sclerotherapy is used postoperatively.

In EVL techniques, phlebectomy was performed in one series with a laser fiber.[6] In the others, the diseased collaterals were treated during the same operation by multiple-incision phlebectomy[8] or later by sclerotherapy.[2,9]

Intraoperative problems, incidents and accidents. Very few were reported in the various series analyzed.[2,6,7,10,11]

Postoperative complications. These are presented in *Tables I, II, and III*.

Contraindications. There are some contraindications to the Closure technique: presence of thrombus in the vein to be treated or extreme tortuous vein. A very superficial GSV is not a contraindication, because the risk of cutaneous burning can be avoided.

For EVL, the various companies mention no specific contraindications related to the morphology or diameter of the vein to be treated.

Procedure	No of limbs (GSV or SSV)	DVT Number (%)	PE Number (%)
RF			
Merchant[12]	319 GSV - SSV	3 (1)	1 (0.3)
Kistner[13]	300 GSV	2 (0.7)	0
Weiss[14]	140 GSV	0	0
EVL			
Min[9]	499 GSV	0	0
Anastasie[15]	232 GSV + 79 SSV	2 (0.9)	0
Proebstle[16]	37 SSV	1 (2.7)	0
Gerard[7]	20 GSV	0	0

Abbreviations: ST = superficial thrombophlebitis; GSV = great saphenous vein; SSV = small saphenous vein; DVT = deep vein thrombosis; PE = pulmonary embolism

Table I. Thromboembolic complications (excluding ST) after RF and EVL.

Therapeutic indications

1) Dependent on the anatomical or topographic localization of the varicose veins to be treated.

The GSV is generally treated by surgery, and is usually limited to the portion situated above the knee due to the risk of damage to the saphenous nerve in the crural segment. This same neurological risk means that the SSV has been treated less frequently using endoluminal procedures, but one series of SSVs was nevertheless treated by diode laser.[16] Where there is no venous insufficiency of the saphenofemoral junction or the main trunk, the collaterals of varicose saphenous veins can also be treated using EVL.[19]

2) Dependent on the clinical context. In theory they are identical to those for conventional surgical treatment of varicose veins.

Results

The results of surgery on varicose veins are difficult to judge at the clinical level. We will discuss briefly the main reasons for this.

Depending on the series, treatment of the saphenous trunk was either supplemented or not supplemented

Complication type	1 week (n=286)	6 months (n=223)	1 year (n=232)	2 years (n=142)	3 years[19] (n=68)
Hematoma	14 (5%)	1 (0.4%)	0	0	0
Infection	0	0	0	0	0
Superficial vein thrombosis	6 (2.1%)	1 (0.4%)	0	0	0
Dysesthesias	43 (15%)	24 (9.4%)	9 (3.9%)	8 (5.6%)	0
Skin burns	6 (2.1%)	0	0	0	0

Table II. Complications following Closure.[12,17]

Author	No of limbs treated	Complications expressed in %								
		Infect	Hem	Pain (mean duration in weeks)	Pain requiring an analgesic (mean duration in weeks)	Induration of treated vein (mean duration in weeks)	SVT	Dysesthesias (duration in weeks)	Swelling (duration in weeks)	Pigmentation (duration in weeks)
Anastasie[15]	311	0	2.1	NI	NI	NI	1.7	NI	NI	NI
Chang[6]	252	NI	0.8	NI	NI	NI	1.6	36.5 (1) 2.8 (24) 0 (52)	100 (1) 0 (24)	23 (1) 0.8 (24) 0 (52)
Min[9]	499	0	24	NI	NI	90	5	0	NI	NI
Proebstle **GSV**[18] **SSV**[16]	 104 41	 0 0	 45 46	 67 (1) 54 (1)	 51 49 (1)	 45 (3) 38 (4)	 10 8	 NI 11 (6.5)	 NI NI	 NI 0

Abbreviations: Infect=Infection; Hem=Hematoma; SVT=superficial venous thrombophlebitis, NI=no information

Table III. Complications after EVL.

No of limbs assessed and duplex US results No and (%)	Signs (%)			Symptoms (%)			Absence of reflux on Duplex US in the vein (to be treated or treated) (%)
	Varices	Pigmentation	Derma l sclerosis	Edema	Pain	Fatigability	
Preoperation 319	95	21.6	6.6	30.4	83.1	76.2	0
Postoperation 1 year: 232							
CO 194 (83.6)	5.7	9.3	1.5	0.5	3.1	1	100
NCO 13 (5.6)	15.4	7.7	0	0	0	0	85
REC 25 (10.8)	40	16	4	4	12	12	28
Postoperation 2 years: 142							
CO 121 (85.2)	8.3	7.4	0.8	4.1	3.3	1.7	100
NCO 5 (3.5)	0	0	0	0	20	20	100
REC 16 (11.3)	46.2	18.8	12.5	12.5	25	18.8	13

Table IV. Radiofrequency clinical results and duplex US.[12]

Abbreviations: CO: complete occlusion, NCO: near complete occlusion (patent<5 cm), REC; recanalization (patent >5 cm)

during the initial procedure by high ligation or/and stab avulsion of the branches. Whether or not these adjunctive procedures had influence on the treatment results is not clear.
Similarly, it is difficult to determine the importance of the additional procedures performed or not performed during the follow-up period.
Finally, the result can be judged on the basis of different criteria: clinical (symptoms, signs, quality of life) or hemodynamic criteria. However, not all of these criteria can be quantified easily.

Traditionally, the result is assessed in terms of:

The symptoms, which remain a subjective element. To quantify each symptom, a descriptive term can be used, generally 3 or 4 adjectives: absent, moderate, (significant), severe. The same scale may be used for each symptom.
A visual, analogue pain scale appears, however, to be more quantitative.
Whatever tool is used, it must be remembered that the so-called "venous" symptoms do not correlate well with presence or absence of varicose veins.[20]

Clinical signs. There are different classifications; the most commonly used classification is the CEAP,[21] but it is relatively unsuitable for varicose veins when there is no skin or subcutaneous change. The *European phlebological file*[22] recently renamed *Computerized Venous Registry* is probably a more precise tool. However, if the esthetic result is considered above all, it has been shown that this result is assessed differently by the physician and the patient.[23]
The venous clinical severity scores incorporating both signs and symptoms are certainly more suitable for judging the effectiveness of a treatment.[24] This score was validated,[25] but it seems more suitable for severe forms of chronic venous disease, ie, chronic venous insufficiency.[26]

- Quality of life is measured by generic and specific questionnaires.[27] Quality of life has already been used to estimate the results of conventional varicose vein surgery at 2 years.[28]

All of the comments just made concerning the difficulty of judging the results clinically are also valid for conventional surgery and sclerotherapy.
The results judged by means of the duplex US investigation, namely complete obliteration of the vein treated by EVL or RF appear equivalent percentage-wise, of the order of 90% at 3 years. In Merchant's series, we note a satisfying correlation between the clinical result and duplex US when the result is judged by the physicians, undoubtedly influenced by their knowledge of duplex US *(Table IV)*.
It must also be noted that in the absence of long-term results for the endoluminal methods, we do not know what the future holds, clinically speaking, for patients with a patent SFJ stump (patient listed near complete obliteration (NCO) following RF). It is known only that at 2 years, there is no significant difference between the complete obliteration and NCO groups).[12]
Finally, we do not currently have at our disposal controlled studies allowing us to judge mid-term or long-term endoluminal surgery versus conventional surgery.
However, indirect comparison is possible using data from different studies as a reference, in which patients have benefited from systematic preoperative duplex US. For this purpose, we have the Rutgers[29] series, which analyzed at 3 years the results for 69 limbs treated by crossectomy (high ligation) + stripping of the trunk±phlebectomy of the branches. It appears that there is no significant difference in terms of clinical results with RF used alone where the results published provide 3 years of hindsight.[17]
One point is worth highlighting: the absence of the phenomena of neovascularization, in particular at the level of the saphenofemoral and saphenopopliteal junctions following endoluminal surgery. It is known that this phenomenon, which occurs frequently after conventional surgery, plays an important role in the incidence of recurrence.[30-35]
It is difficult to determine whether not using high ligation can alone explain the absence of neovascularization. It is also possible that maintaining the patency of the saphenous termination tributaries plays a favorable role in that they drain physiologically.[36,37]

CONCLUSIONS

The following conclusions may be drawn after analysis of the publications on RF and EVL:
Many articles have been written on endovenous laser and venous ablation using radiofrequency.
Whereas the RF procedure has been standardized, the protocols for EVL vary because of the diversity of the equipment used.
The time to resumption of normal activities and the length of the convalescence period are considerably shorter after endoluminal procedures. The type and number of complications associated with these interventions are well documented. They appear to be transient and mild.
The medium-term clinical results for RF are better

documented than those of EVL. In terms of hemodynamics, ablation of the treated vein is identified by Duplex sonography in 90% of cases after 3 years for both procedures.

The absence of controlled long-term studies compared with sclerotherapy and conventional surgery means that grade A or B recommendations cannot currently be formulated for them.

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REFERENCES

1 Proebstle TM, Sanhofer M, Kargl A, et al. Thermal damage of the inner vein wall during endovenous laser treatment: key role of energy absorption by intravascular blood. *Dermatol Surg*. 2002;28:596-600.

2 Proebstle TM, Lehr HA, Kargl A, Espinosa-Klein C, Rother W, Behtge S, Knop J. Endovenous treatment of the greater saphenous vein with a 940-nm diode laser: thrombotic occlusion after endoluminal thermal damage by laser-generated steam bubbles. *J Vasc Surg*. 2002;35:729-736.

3 Weiss R A. Comparison of endovenous radiofrequency versus 810-nm diode laser occlusion of large veins in an animal model. *Dermatol Surg*. 2002;28:56-61.

4 Pichot O, Perrin M. Aspects échographiques de la jonction saphéno-fémorale après oblitération de la grande veine saphène par radiofréquence (Closure®). *Phlébologie*. 2002;55:329-334.

5 Chandler JG, Pichot O, Sessa C, Schuller-Petrovic S, Osse FJ, Bergan JJ. Defining the role of extended saphenofemoral junction ligation: a prospective comparative study. *J Vasc Surg*. 2000;32:941-953.

6 Chang CJ, Chua JJ. Endovenous laser photocoagulation (EVLP) for varicose veins. *Lasers in Surgery and Medicine*. 2002;31:257-262.

7 Gerard J-L, Desgranges P, Becquemin J-P, Desse G, Mellière D. Peut-on traiter les grandes saphènes variqueuses par laser endoveineux en ambulatoire? Résultats à 1mois d'une étude de faisabilité sur 20 patients en salle de consultation. *J Mal Vasc*. 2002;27:222-225.

8 Guex JJ, Min RJ, Pittaluga P. Traitement de l'insuffisance de la grande veine saphène par photo coagulation laser endoveineuse: techniques et indications. *Phlébologie*. 2002;55:239-243.

9 Min RJ, Zimmet SE, Isaacs MN, Forrestal MD. Endovenous treatment of the incompetent greater saphenous vein. *J Vasc Interv Radiol*. 2001;12:1167-1171.

10 Lebard C, Zucarelli F. Intérêt de l'angiographie de la jonction saphéno-fémorale au cours de la destruction de la grande veine saphène par le système Closure. *Phlébologie*. 2002;55:263-268.

11 Navarro L, Boné C. L'énergie laser intraveineuse dans le traitement des troncs veineux variqueux : rapport sur 97 cas. *Phlébologie*. 2001;54:293-300.

12 Merchant RF, DePalma RG, Kabnick LS. Endovenous obliteration of saphenous reflux: a multicenter study. *J Vasc Surg*. 2002;35:1190-1196.

13 Kistner RL. Endovascular obliteration of the greater saphenous vein: The Closure procedure. *Jpn J Phlebol*. 2002;13:325-33.

14 Weiss RA, Weiss MA. Controlled radiofrequency endovenous occlusion using a unique radiofrequency catheter under duplex guidance to eliminate saphenous varicose vein reflux: A 2-year follow-up. *Dermatol Surg*. 2002;28:38-42.

15 Anastasie B, Celerier A, Cohen-Solal G, Anido R, Boné C, Mordon S , Vuong PN. Laser endoveineux. *Phlébologie*. 2003;56:369-382.

16 Proebstle TM, Doendue G, Kargl A, Knop J. Endovenous laser treatment of the lesser saphenous vein with a 940-nm diode laser: Early results. *Dermatol Surg*. 2003;29:357-361.

17 Perrin M. Varices des membres inférieurs traitées par radiofréquence (CLOSURE®). Contrôle annuel des résultats : un suivi sur 3 ans. *Phlébologie*. 2004;57:69-73.

18 Proebstle TM, Gül D, Lehr HA, Kargl A, Knop J. Infrequent early recanalization of greater saphenous vein after endovenous laser treatment. *J Vasc Surg*. 2002;38:511-516.

19 Navarro L, Min RJ, Boné C. Endovenous laser: a new minimally invasion method of treatment for varicose veins–preliminary observations using an 810 nm diode laser. *Dermatol Surg*. 2002;27:117-122.

20 Bradbury A, Evans CJ, Allan PL, Lee AJ, Ruckley CV, Fowkes FGR. What are the symptoms of varicose veins? Edinburgh vein study cross sectional population survey. *BMJ*. 1999;318;353-356.

21 Porter JM, Moneta GL, and an international consensus committee on chronic venous disease. Reporting standards in venous disease: an update. *J Vasc Surg*. 1995:27:635-645.

22 Uhl JF, Cornu-Thénard A, Carpentier PH, Chleir F. Le Dossier Médical Phlébologique Européen (DMPE) : Son fonctionnement et ses applications passées, présentes et futures. *Phlébologie*. 2002;55:121-125.

23 Jakobsen BJ. The value of different forms of treatment for varicose veins. *Br J Surg*. 1979;66:182-184.

24 Rutherford RB, Padberg FT, Comerota AJ, Kistner RL, Meissner MH, Moneta GL. Venous severity scoring: An adjunct to venous outcome assessment. *J Vasc Surg*. 2000; 31:1307-1312.

25 Meissner MH, Natiello C, Nicholls SC. Performance characteristics of the venous clinical severity score. *J Vasc Surg*. 2002;36:889-895.

26 Perrin M, Dedieu F, Jessent. Une appréciation des nouveaux scores de sévérité de la maladie veineuse chronique des membres inférieurs. Résultats d'une enquête auprès d'angiologues français. *Phlébologie*. 2003;56:127-136.

27 Perrin M. Qualité de vie et chirurgie veineuse. *Phlébologie*. 2003;56:151-155.

28 MacKenzie RK, Paisley A, Allan PL, Lee AJ, Ruckley CV, Bradbury AW. The effect of long saphenous vein stripping on quality of life. *J Vasc Surg*. 2002;35:1197-1203.

29 Rutgers PH, Kitslaar PJEHM. Randomized trial of stripping versus high ligation with sclerotherapy in the treatment of the incompetent greater saphenous vein. *Am J Surg*. 1994;168:311-315.

30 Glass GM. Neovascularization in recurrence of the varicose great saphenous vein following transection. *Phlebology*. 1987;2:81-91.

REFERENCES

31 Darke SG. The morphology of recurrent varicose veins. *Eur J Vasc Surg*. 1992;6:512-517.

32 De Maesener MG, Ongena KP, Van den Brande F, Van Schill PE, De Hert SG, Eyskens EJ. Duplex ultrasound assessment of neovascularization after sapheno-femoral or sapheno-popliteal junction ligation. *Phlebology*. 1997;12:64-68.

33 Jones L, Braithwaite BD, Selwyn D, Cooke S, Earnshaw JJ. Neovascularisation is the principal cause of varicose vein recurrence: results of a randomized trial of stripping the long saphenous vein. *Eur J Vasc Surg*. 1996;12:442-445.

34 Nyamekye I, Shepard NA, Davies B, Heather BP, Earnshaw JJ. *Eur J Vasc Endovasc Surg*. 1998;39:412-415.

35 Van Ryj AM, Jiang P, Solomon C, Christie RA, Hill GB. Recurrence after varicose vein surgery: a prospective long-term clinical study with duplex ultrasound scanning and air plethysmography. *J Vasc Surg*. 2003:38:935-943.

36 Pichot O, Kabnick LS, Creton D, Merchant RF, Schuller-Petrovic S, Chandler JG. Duplex ultrasound scan findings two years after great saphenous vein radiofrequency endovenous obliteration. *J Vasc Surg*. 2004;39:189-195.

37 Pichot O, Perrin M. Aspects échographiques de la jonction saphéno-fémorale après oblitération de la grande veine saphène par radiofréquence (Closure®). *Phlébologie* 2002;55:329-334.

Saphenopopliteal junctions are significantly lower when incompetent. Embryological hypothesis and surgical implications

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SUMMARY

The aim of this study was to compare the levels of the small saphenous junction with deep veins in a control group versus a group of patients with insufficient short saphenous junctions. A comparative meta-analysis of the data from 15 series shows that the incompetent junctions were significantly lower in the control group. The comparison with anatomy and embryology shows that the functional and phylogenetic regression of the small saphenous vein might correspond to its embryologic regression. This embryologic regression is associated with a progressive descent of the anastomosis in the deep vein. This descent might also induce poorer competence of the small saphenous junction.

INTRODUCTION

Experience gained from surgical removal of the small saphenous vein (SSV), and repeated surgery following recurrence of a popliteal varicose vein after surgery of the SSV has shown us that the junction of the SSV was usually easily accessible via an incision in the popliteal fossa.[1,2] In contrast, anatomical dissections of the SSV have revealed a high degree of variability in the level of anastomosis with the popliteal vein.[3,4] This variability is not reflected in surgery for incompetent SSV. This discrepancy led us to speculate that the level of the junction could be linked with an incompetence of the SSV.

MATERIALS AND METHODS

In a meta-analysis of the various sets of anatomical data in the literature reporting the frequency of the different levels of the SSV junction, we have compared a control group with subjects presenting incompetent SSV. The classification used for this comparison was based on that of Kosinski *(Figure 1)*.[5] For our study *(Figure 2)* we defined type I anastomoses as located either at the popliteal bend level or between 0 and 7 cm above this bend, type II anastomoses

Keywords:
small saphenous vein - Surgery - Embryology - Anatomy - Reflux.

as located more than 7 cm above the popliteal bend, and type III anastomoses as located below the popliteal bend. To compare the various classifications reported in the literature, the data for some groups were reassigned according to this classification. Apparently healthy saphenopopliteal junctions were studied in several sets of anatomical dissections[5-7] and on the basis of Doppler scans of healthy patients *(Table I)*.[8] Incompetent junctions were defined on the basis of the surgical findings or, in the preoperative phase, on the basis of the Doppler ultrasound scan or by venography *(Table II)*.[9-19] The distributions were compared by independent χ_2 tests, with α set to 5%. The degree of significance, *P,* for the χ_2 test and the value of the reduced deviation μ (square root of χ_2) are reported for each comparison.

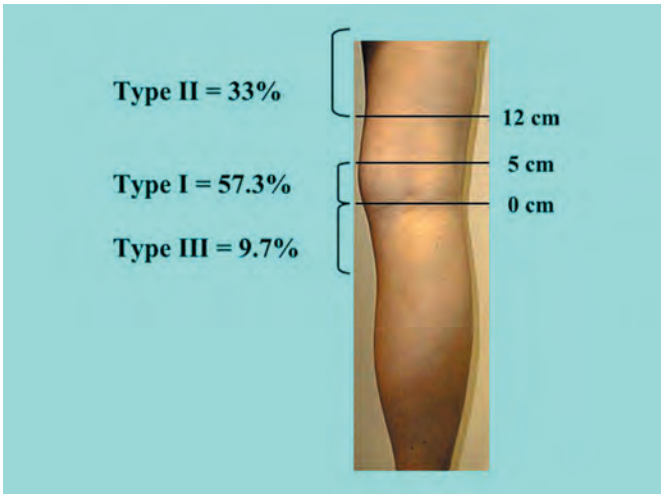

Figure 1. Classification used by Kosinski.[5]

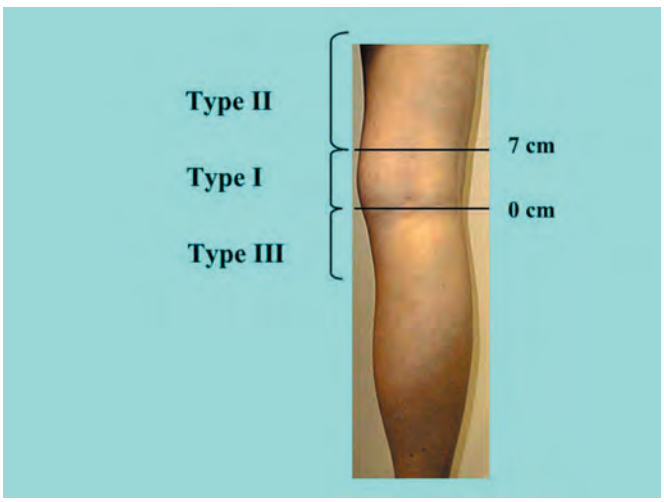

Figure 2. Classification used to compare the data in the various studies. Height in cm from the popliteal bend (0 located within the popliteal bend). (Type I, II, III).

RESULTS

1/ Anatomy of the saphenopopliteal junction in the subjects in the control group presenting with an apparently healthy saphenopopliteal junction *(Table I)*.[5-8] This has been very well documented by Kosinski[5] on the basis of 124 dissections. In this group *(Figure 1)*, 57.3% of SSV anastomoses were located between 0 and 5 cm above the popliteal bend (less than half of them had a considerable postaxial extension which constituted a Giacomini vein (GSV) when it rejoined the great saphenous vein. Thirty-three percent of the cases were high anastomoses, located at least 12 cm above the popliteal bend. In two out of five cases, there was a single anastomosis with the femoral vein, in two out of five cases there was a single anastomosis with the GSV, and in one out of five cases there was a composite anastomosis. From the low junctions, ie, those located below the popliteal bend, 9.7% consisted mainly of direct or indirect junctions with the GSV below the knee. A junction with the gastrocnemius veins was seen only occasionally. Cibor[7] found very similar percentages following cadaver dissections. On the basis of ultrasound scans of healthy subjects, Engel[8] reported virtually the same percentages, with 52.4% of junctions located in the popliteal fossa, 46.6% high junctions, and 1% low junctions. According to Moosman,[6] 17.5% of SSVs dissected in cadavers had no connection with the popliteal vein.

2/ Anatomy of the saphenopopliteal junction in subjects presenting with an incontinent saphenopopliteal junction *(Table II)*.[9-19] In patients with varicose veins, studies of the anatomy of the incompetent saphenopopliteal junction using Doppler ultrasound or venography, or observed intaoperatively, have shown that 80.1% of the popliteal junctions were located between the popliteal bend and a level 7 cm above it. High refluxes, more than 7 cm above the popliteal bend, were only found in 13.9% of cases.

3/ Statistical comparisons of the percentages in the varicose patient group and control subject group. These percentages are reported in *Figure 3*, which compares the junctions located within the popliteal fossa (type I), and in *Figure 4*, which compares the high junctions (type II) in these groups.

The meta-analysis of these various pooled sets shows that the mean frequencies of the various locations were significantly different between the groups with and without junctional incompetence. The incompetent junctions were more often located between the popliteal bend and a level 7 cm above than the junctions in the control group (80.1%

Table I. Type of saphenopopliteal junction in the control group subjects. These various groups correspond to SSVs investigated by anatomical dissection [5-7] or by Doppler ultrasound explorations (DUS) in groups of healthy subjects.[8] Type I corresponds to an anastomosis located within the popliteal fossa (0 and 7 cm), type II to an anastomosis located more than 7 cm above this fossa, type III to an anastomosis located below the popliteal bend. ? = Values not reported by the author.

		n	Method of exploration	Type I	Height (in cm)	Type II	Type III
KOSINSKI[5]	1926	124	Anatomical	57%	0-5	33%	9.7%
MOOSMAN[6]	1964	200	Anatomical	74.5%	?	17.5%	8%
CIBOR[7]	1968	200	Anatomical	59.5%	2-5	30.5%	10%
ENGEL[8]	1992	104	DUS	52.4%	0-5	46.6%	1%

Table II. Type of saphenopopliteal junction in subjects presenting with incompetence of the SSV. The various groups correspond to incontinent short saphenous veins. Method of exploration: DUS = Doppler ultrasound, Surgery = peroperative assessment, Venogr = Venography. Type I corresponds to an anastomosis located within the popliteal fossa (between 0 and 7 cm), type II to an anastomosis located more than 7 cm above it, type III to an anastomosis located below the popliteal bend. ? = Values not reported by the author .

		n	Method of exploration	Type I	Height (in cm)	Type II	Type III
HAEGER[9]	1963	35	Surgery	82%	0-5	8.5%	8.5%
DODD[10]	1965	444	Surgery	80.8%	?	15.2%	4%
GUILLAND[11]	1987	30	DUS – Venogr	90%	0-5	0%	0%
LEA THOMAS[12]	1988	145	Venogr	84%	0-7.5	16%	0%
SUGRUE[13]	1988	21	DUS	80%	0-5	19%	0%
VASDEKIS[14]	1989	64	Venogr	70%	0-7	30%	0%
ENGEL[15]	1991	62	DUS	78%	?	15.4%	6.6%
GORNY[16]	1994	225	Surgery	72%	?	28%	0%
LEMASLE[17]	1995	83	DUS – Surgery	57%	0-6	17%	26%
CORCOS[18]	1996	528	DUS – Venogr	70%	?	18.3%	11.5%
SAHARY[19]	1996	638	DUS	93.7%	0-6	2.2%	?

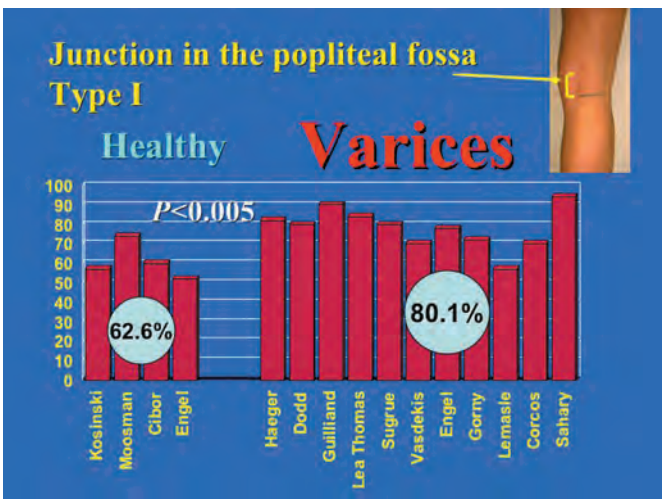

Figure 3. Comparison of type I junctions in the incompetent group and in the control group. Pairwise comparison of each of the 4 data sets comprising the control group of type I junctions (saphenopopliteal junction located within the popliteal fossa) with the 11 data sets from groups with type I saphenopopliteal junction incompetence (saphenopopliteal junction located in the popliteal fossa) shows that the junctions located within the popliteal fossa were usually incompetent (P<0.05).

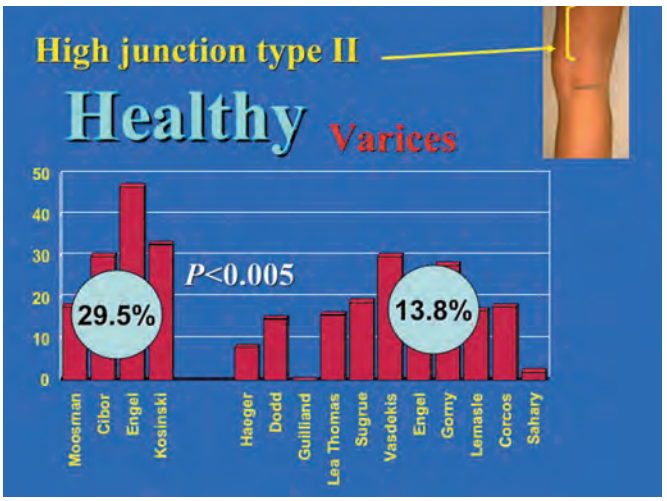

Figure 4. Comparison of type II junctions in the incompetent group and in the control group. The pairwise comparison of each of the 4 data sets for the type II control group (high saphenopopliteal junction) with the 11 data sets of the type II saphenopopliteal junction incompetence group shows that the high junctions were usually competent (P<0.05).

versus 62.6%, *P*<0.05). The junctions in the control group were more often located more than 7 cm above the popliteal bend than those in the incontinent junction group (29.5% versus 13.8%, *P*<0.05). This difference in frequency found by the meta-analysis of the type I and type II groups was significant *(P*<0.05) *(Table III)*, μ (reduced deviation) = 1.978, *P*<0.05 for the comparison of the type I groups, and u = 1.997, *P*<0.05 for the comparison of the type II groups.

DISCUSSION

With regard to the junctions located in the popliteal fossa *(Figure 3)*, only the percentages reported by Vasdekis[14] and Lemasle[17] and, to a lesser extent, those reported by Sugrue[13] showed no highly significant difference from the control group *(P*>0.01, or not significant). With regard to the high junctions *(Figure 4)*, only the percentages reported by Vasdekis[14] and Sugrue[13] showed no highly significant difference from the control group *(P*>0.01 or not significant). The data sets reported by Vasdekis[14] and Sugrue[13] in fact correspond to only 85 out of 2275 cases (3.7%), and their findings probably reflect recruitment bias. The data of Kosinski,[5] Moosman,[6] and Cibor,[7] based on cadaver dissections, do not specify what percentage (if any) of the cadavers displayed incontinence of the SSV. In fact it is unlikely that the frequency of SSV incompetence (10% to 12% of the population with varicose veins) could affect the degree of significance, *P*, which is often less than 0.01 for pairwise comparisons of data sets. In contrast, the differences in the percentages reported for the two groups by Engel[8,15] (normal SSV and incontinent SSV) and obtained using the same methodology are highly significant (*P*<0.01). A previous anatomical study of 41 incontinent saphenopopliteal junctions following surgery[1] gave similar percentages. Although the determinations of the height above or below the popliteal fossa were not exactly the same, 78% of the junctions were located at the level of the popliteal fossa, versus only 12% located higher up. These figures match those of Van der Stricht, who found only 29 junctions at the level of the popliteal fossa out of 60 dissections of unselected cadavers.[20]

	Saphenopopliteal junction (control group) n=628[5-8]	Incontinent saphenopopliteal junction n=2275[9-19]
Type I Popliteal fossa	62.6% (α)	**80.1%** (α)
Type II High junction	**29.5%** (Ψ)	13.8% (Ψ)
Type III Low junction	7.8% (φ)	6.5% (φ) (n=1637)

Table III. Comparison of the height of the junction in incompetent and competent SSV.
The meta-analysis of the group (α) and (Ψ) has demonstrated a significant difference in the type-I and type II junctions (P<0.05). The difference was not significant for the comparison of groups (φ) with a type III junction. The type III, incompetent saphenopopliteal junction group included a different number of subjects, because one study[19] was incomplete.

The notion that the lower the saphenopopliteal junction, the greater the risk of incompetence, could be explicable in terms of the embryology of the popliteal vein system.

1/ Embryology of the popliteal vein

In general, during the embryological development superficial veins appear prior to deep veins with a thicker histological texture. Similarly, the SSV appears before the great saphenous vein, also with a thicker histological texture. Initially, the venous return system is superficially located on the limb bud and only later is transferred to a deeper level. Finally, the residual superficial system is used for superficial cutaneous draining and thermoregulation.

In a 6-week-old embryo, the first collector networks can be clearly observed. The first venous network is superficial. It goes from the extremity to the root bud where the first anastomosis will be created with the deep venous pelvic system, and later the inferior vena cava. Later on, the preaxial superficial venous network will appear and form a secondary circulatory system.

In a 9-week-old embryo, deep veins appear in the form of an endothelialized vascular lacuna surrounding the arteries. These three networks– superficial primary postaxial, superficial secondary preaxial, and deep ones– prefigure globally and respectively the SSV, the long saphenous vein and femoral vein and the deep popliteal and femoral veins.

With time, anastomosis will occur between the superficial pre- and postaxial and the deep axial networks to facilitate blood draining at the limb root thanks to the deep network. Progressively, flow will be transferred from the limb extremity to its root, a transfer from the postaxial to the preaxial network that will finally ensure the main venous communication between the limb and the pelvis.[21-26]

In a 10-mm embryo, draining occurs via the postaxial system. The SSV is prolonged by the postaxial vein that drains into the pelvis through the ischiatic vein.[27]

With ingrowths of the femoral vein, the axial vein normally involutes. It corresponds to limb elongation and to medial rotation of the anterior side of the limb. The

anterior preaxial system becomes medial on the lower part of the limb.
In a 15-mm embryo, a secondary path appears to the detriment of the preaxial system. The superficial and deep femoral veins meet the long saphenous vein and flow into the iliac vein.
In a 20-mm embryo, with pelvic rotation and lower limb elongation, the draining system moves forward, from the ischiatic postaxial system to the iliac vein preaxial system.
In a 25-mm embryo, numerous anastomoses can be observed. Flow transfer is organized to the fore between the postaxial and axial systems and the axial and preaxial system.
In a 35-mm embryo, after the thigh has extended from the trunk, the SSV individualizes and loses its connections with the ischiatic system to flow into the great saphenous vein.
The final stage will only be achieved in the 50-mm embryo. The SSV connection with the deep vein moves progressively downward during the embryologic and fetal period.

Three nerves control vessel development:

1- The axial nerve or sciatic nerve that gives the tibial and common fibular nerves at knee level. It is followed by the axial venous plexus.
2- The preaxial or femoral nerve that gives a sensory branch called the saphenous nerve. It is followed by the preaxial venous plexus.
3- The postaxial or small sciatic nerve that is more particularly represented by its sensory branch: the posterior cutaneous nerve. It comes down along the posterior aspect of the lower limb. The postaxial venous plexus follows the posterior cutaneous nerve down to the calf tip.
Anastomosis between these different plexuses yields the final venous system.[28]

A/ The first anastomosis is ventral. It establishes a connection between the axial plexus below knee level with the preaxial plexus above knee level. This anastomosis generates the popliteal vein that drains all the deep leg axes to the thigh axial plexus.
B/ The second anastomosis is dorsal. It establishes a connection between the postaxial plexus and the axial plexus, at knee level. It is facilitated by the joining of the plexuses derived from knee bending. This anastomosis ensures all or part of the postaxial plexus superficial blood draining of the leg towards the thigh axial plexus, and becomes the saphenopopliteal junction.
1/ A complete anastomosis will give a SSV junction of terminal type that connects with the popliteal vein at the popliteal fossa level (50%). The upper postaxial extension, a satellite of the posterior femoral cutaneous nerve, will not grow. In this case, it is possible to say that the typical saphenopopliteal junction development as a cross is a "chance mishap" on the long postaxial axis of the embryo. The lack of conductor nerves along this cross may explain its extreme variability.[22-24]
2/ A partial anastomosis will give, on the one hand, after the branching of the short saphenous trunk, a deep anterior collateral which, with a normal cross, connects with the popliteal vein at the popliteal fossa level. On the other hand, it also gives a more superficial trunk that follows the posterior femoral cutaneous nerve on the fascia deep side. This postaxial extension is called the Hyrtl vein or Stolic vein. When it perforates the fascia at the upper level thigh part, crosses the medial thigh part to join the long saphenous vein at the femoral triangle level, it is called the Giacomini vein. It then creates an anastomosis between the postaxial leg plexus and the upper level thigh preaxial plexus. At the branching level, a deeper ascending collateral can appear; in this case the axial extension that follows the back of the sciatic nerve to join the axial trunk (sciatic nerve vein) constitutes another axiopostaxial anastomosis *(Figure 5)*.[29]

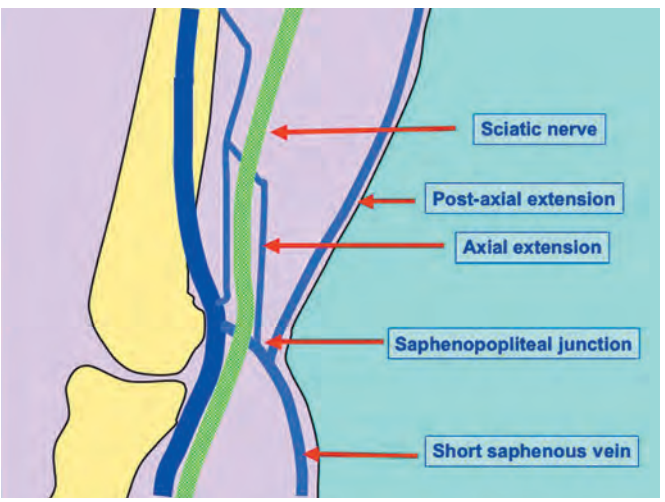

Figure 5. The SSV and its venous extensions.

3/ The lack of anastomosis entails a SSV without a junction. In this case, the trunk extends into a canal filled with the postaxial extension *(Figure 6)*.[30]
Embryology can explain the great diversity of types and heights of the saphenopopliteal junction.[31] The classically described modal junction only represents roughly 50% of cases. It then comes with a diameter of about 5 mm, is straight and 3 to 5 cm long, slightly bent forwards, ascending with a thick white multivalved wall (two to

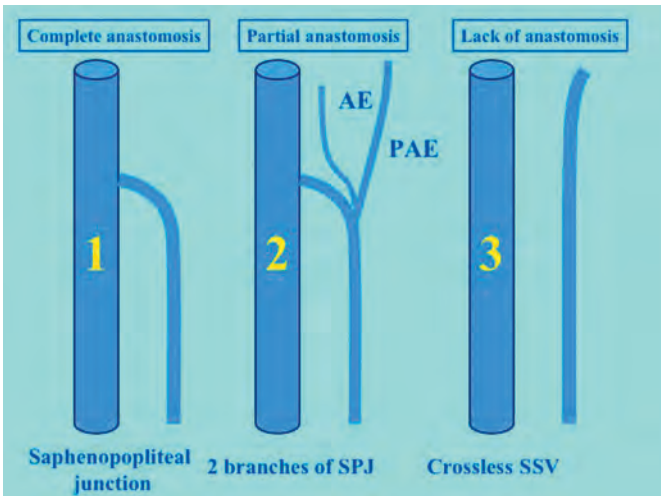

Figure 6. Different types of saphenopopliteal junction. 1: regular junction. 2: saphenopopliteal junction with axial and postaxial extensions. 3: small saphenous vein without a junction.

three valves). This SSV directly joins the popliteal vein some centimetres above the condyles, on the posterior side of the popliteal vein. The other endings reflect the major embryologic stages. The SSV often splits into three branches at the popliteal fossa: the postaxial extension upwards, subfascial, touching the posterior femoral cutaneous nerve; the axial extension at the rear of the tibial nerve and touching it; and the real saphenopopliteal junction that is a residue of the axiopostaxial anastomosis of the embryo. By this definition, postaxial extension characterized by the following features: a diameter exceeding 2 cm, a straight course, vertical, in the limb posterior axis, completely subfascial, accompanying the posterior femoral cutaneous nerve, in direct continuity with the SSV trunk and anatomically with valves oriented to allow centripetal reflux.

2/ Phylogenesis and ontogenesis

In phylogenesis and ontogenesis, superficial veins appear prior to the deep veins. Important differences can be observed between the evolution, the anatomy, the structure and the function of both superficial and deep veins and also between the great and short saphenous veins.[32,33] In mammals, the latter develop differently and can vary within the same group. From an embryological point of view, the SSV, which is a primary vein, comes with a thicker and better-developed wall and is therefore better prepared for its superficial vein function than a secondary vein.[34] SSV insufficiency only accounts for 15%.

3/ Comparative anatomy of the short saphenous vein

Embryologic downward regression of the SSV can also be found in mammalian phylogenetic evolution. Actually, in quadrupedal mammals, ungulate and plantigrade animals, the SSV is the most important hind vein whereas, in apes, it becomes much smaller and less functional. In big mammals, the short saphenous vein is generally the greater collecting vein toward the pelvis.[24] It usually connects at a very high level and frequently in the middle of very large muscular masses. The lower junction of the short saphenous vein in the popliteal fossa (a specific adaptation in large bipedal mammals) may be an adaptation to lower-limb elongation. In big mammals, the great saphenous vein is regularly connected according to the same pattern, whereas the short saphenous vein connection can present several different patterns, each of them specific to a mammal species and corresponding to each embryologic stage.[35] Therefore, in the different mammal species, normally and specifically, it is possible to observe the different kinds of connections in human beings.[36,37]

In quadrupedal mammals, the short saphenous vein is a very important vein with a usually double connection situated much higher than the knee-bending level, and is always embedded into large muscular masses. In pigs, a triple connection can be observed: in the circumflex femoral vein, in the gluteal veins and in the codalis femoralis vein (the equivalent of a deep femoral vein). In dogs, the short saphenous vein connects to the popliteal vein to form the femoral vein. In equids, it flows very high into the posterior thigh side or into the sciatic vein. In bovids, its two similar branches flow into the deep femoral vein and into the superficial femoral.

Embryologic and phylogenetic regression of the small saphenous vein may induce its functional regression. Actually, short saphenous vein dysfunction or reflux is a frequent disorder in human beings but has never been observed in animals. The theory of small saphenous vein phylogenetic functional regression accompanying the progressive lowering of its connection into the axial plexus could explain the relation between the small saphenous vein caliber and the height of its connection in the popliteal vein. According to Gillot,[23] the higher the small saphenous vein popliteal connection, the greater its caliber.

The SSV is a particular superficial vein.[34] Unlike the great saphenous vein, the SSV is the oldest vein. It is a primary vein with a primary pattern (and has never been accompanied by an artery during embryologic development). It is the most important vein in mammals with a stronger and thicker wall than the GSV and is better prepared for its superficial vein function.

4/ Surgical implications

Incompetent saphenopopliteal junctions are in fact usually easily accessible during surgery. Out of 41 operated

junctions, 78% were located within the popliteal fossa.[1] It is unusual to have to operate on incompetent junctions located far above the popliteal fossa. When these high refluxes are associated with varices, they are often linked to pelvic vein insufficiency that follows the trajectory of the axial venous system. These are indeed high refluxes, but they are not directly transmitted by the short saphenous vein. They are not directly accessible for local surgery. When a very high junction does present with a reflux, this reflux is often centripetal and so it flows in the same direction during both compression and decompression movements of the calf muscles *(Figure 7)*. This is in fact a venovenous shunt running alongside the path of the deep vein in the thigh. Varices of the popliteal fossa are usually collaterals of this second pathway. Surgery is not, of course, appropriate for a pathway displaying a systolic reflux with a very high junction.

With regard to popliteal recurrences after surgery of the short saphenous vein, the long stumps responsible for recurrences[2] are also very often located within the popliteal fossa. Long stumps that have been inadequately resected because they were inaccessible will probably not give rise to a recurrence, when they were not incompetent at the time of the first operation.

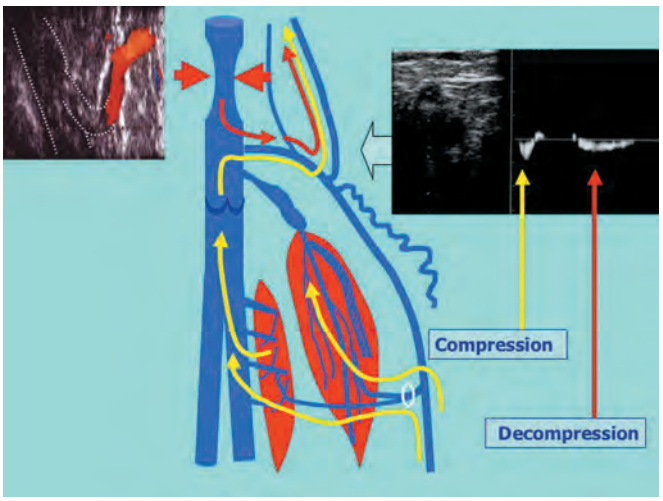

Figure 7. Systolic reflux at the saphenopopliteal junction. If the flow through the saphenopopliteal junction or through a proximal extension occurs in the same direction during both compression and decompression of the calf muscles, this indicates the presence of a physiological shunt. A very high junction of the small saphenous vein or of an axial or postaxial extension of this vein corresponds to the upper anastomosis of a deep vein-vein shunt.

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REFERENCES

1 Creton D. Etude anatomo-chirurgicale de 41 jonctions saphéno-poplitées incontinentes. Peut-on réduire le risque de récidive poplitée ? *Phlébologie*. 1998;51: 457-463.

2 Creton D. 125 réinterventions pour récidives variqueuses poplitées après exérèse de la petite saphène. (Hypothèse anatomique et physiologique du mécanisme de la récidive). *J Mal Vasc*. 1999;24:30-36.

3 Mercier R, Fouques PH, Portal N, Vanneuville G. Anatomie chirurgicale de la veine saphène externe. Conséquences chirurgicales dans le traitement radical des varices du membre inférieur. *J Chir*. 1967;93:59-70.

4 Payen B. Rappel anatomique de la veine saphène externe. *Phlébologie*. 1985;38: 453-461.

5 Kosinski C. Observations on the superficial venous system of the lower extremity. *J Anat*. 1926;60:131-142.

6 Moosman DA, Hartwell SW. The surgical significance of the subfascial course of the lesser saphenous vein. *Surg Gynecol Obstet*. 1964;115:761-766.

7 Cibor Z, Cencora A. La veine saphène externe: données anatomiques. *Phlébologie*. 1968;2:257-265.

8 Engel AF, Davies G, Keeman JN, Dorpt A. Colour Flow Imaging of the normal short saphenous vein. *Eur J Vasc Surg*. 1994;8:179-181.

9 Haeger K. The surgical anatomy of the sapheno-femoral and the sapheno-popliteal junctions. *J Cardio Vasc Surg*. 1963;3: 420-427.

10 Dodd H. The varicose tributaries of the popliteal vein. *Br J Surg*. 1965;52:350-354.

11 Guilland EL, Gerber CJ, Lewis JD. Short saphenous vein surgery, pre-operative Doppler ultrasound marking compared with on-table venography and operative findings. *Phlebology*. 1987;2:109-114.

12 Lea Thomas M, Chan O. Anatomical variations of the short saphenous vein; a phlebographic study. *Vasa*. 1988;17:51-55.

13 Sugrue M, Stanley S, Grouden M, Feeley M, Moore DJ, Shanik DG. Can pre-operative duplex scanning replace pre-operative short saphenous venography as an aid to localizing the sapheno-popliteal junction? *Phlébologie*. 1988;41:722-725.

14 Vasdekis SN, Clarke GH, Hobbs JT, Nicolaides AN. Evaluation of non-invasive and invasive methods in the assessment of short saphenous vein termination. *Br J Surg*. 1989;76:929-932.

15 Engel AF, Davies G, Keeman JN. Preoperative localisation of the saphenopopliteal junction with duplex scanning. *Eur J Vasc Surg*. 1991;5:507-509.

16 Gorny PH, Payen B, Reinharez D, Hutinel B, Blanchemaison P, Tran-duy S. Chirurgie de la saphène externe: conclusion pratique d'après une étude de 225 interventions. *Phlébologie*. 1994;47:383-388.

17 Lemasle PH, Uhl JF, Lefebvre Vilardebo M, Tamisier D, Baud JM, Cornu Thenard A. Confrontation écho chirurgicale de la terminaison de la saphène externe dans le cadre de la chirurgie d'exérèse, résultats préliminaires. *Phlébologie*. 1995;48:321-327.

18 Corcos L, Macchi C, De Anna D, Procacci T, et al. The anatomical variables of the sapheno-popliteal junction visualization by radiological and echographic examinations. *It J Anat Embryol*. 1996;101:15-28.

19 Sahary M, Farrah J, Shields RA, Summerville K, Riazuddin N, Coleridge Smith PD. Anatomical location of the sapheno-popliteal junction incompetence by duplex ultrasonography. *Dermatol Surg*. 1996;22:396. (Abstract).

20 Van der Stricht J. La petite saphène existe-t-elle ? *Phlébologie*. 2001;54:309-315.

21 Van der Stricht J. Petite veine saphène et angiodysplasies. In: Davy A, (ed) *La petite veine saphène. Phlébologie*. 1997;suppl: 521-525.

22 Gillot C. Formes anatomo-cliniques. In: Davy A, (ed) *La petite veine saphène. Phlébologie*. 1997;Suppl:511-515.

23 Gillot C. Dispositifs veineux poplités: hypothèses et certitudes. *Phlébologie*. 1998;51:65-74.

24 Bouchet A. Anatomie comparée et embryologie des systèmes veineux et lymphatiques des membres inférieurs. *Phlébologie*. 1999;52:9-18.

25 Lemasle P, Uhl JF, Lefebvre-vilardebo M, Gillot C, Baud JM, Vin F. Veine du nerf sciatique et maladie variqueuse. Aspects écho-anatomiques et hémodynamiques. *Phlébologie*. 2001;54:219-228.

26 Parry DJ, Aldoori MI, Hammond RJ, Kessel DO, Weston M, Scott DJA. Persistent sciatic vessel, varicose veins, and lower limb hypertrophy: an unusual case or discrete clinical syndrome? *J Vasc Surg*. 2002;36:396-400.

27 Kandel R. Chirurgisch-anatomische Eigentumlichkeiten bei der Behandlung der varikösen V. Saphena parva. *Zbl Phleb*. 1967;6:313-324.

28 Gillot C. Dispositifs veineux poplités: Hypothèses et certitudes. *Phlébologie*. 1998;51:65-74.

29 Gillot C. La veine décrite par Giacomini: Essai de systématisation et approche de la terminologie des veines fondée sur l'embryologie et la fonction. *Phlébologie*. 2001;54:425-429.

30 Gillot C. Le prolongement post-axial de la petite veine saphène. Etude anatomique-considérations fonctionelles-intérêt pathologique. *Phlébologie*. 2000;53:295-325.

31 Gillot C. Les veines profondes de la cuisse et du genou. In: Bonnel F, Chevrel JP, Outrequin G. *Anatomie clinique*. Paris, France: Springer-Verlag; 1991:437-442.

32 Ivan V, Icma I, Tilinca G, Tilinca M, Tudose N, Dema A. Le système veineux des membres inférieurs. Etude embryologique préliminaire. *Phlébologie*. 2002;55:9-14.

33 Kokova J, Horakova M, Horakova MA. L'évolution des veines pré- et post-natales. *Phlébologie*. 1993;46:241-252.

34 Horakova MA, Kokova J, Prokopova V, Horakova E. Etude comparative chez l'adulte de l'évolution des petite et grande veines saphènes à travers l'observation et l'analyse de la varicose de ces deux types de veines. *Phlébologie*. 2000;53:147-153.

35 Schummer A, Wilkens H, Vollmerhaus B, Habermehl KH. Kreislaufsystem Haut und Hautorgane. In: Nickel R, Schummer A, Seiferle E, eds. *Lehrbuch der Anatomie der Haustiere*. Band III, Berlin, Verlag Paul Parey, 1976;249-257.

36 Popesko P. *Topografisch-anatomische Atlas van de Huisdieren*. In: Popesko P, Wensing CJG, Hasselaar JC, De Vos N, Simoens P, eds, *Lehrbuch der Anatomie der Haustiere*. Utrecht, Bohn Scheltema & Holkema, 1982: 61-181.

37 Evans HE, Christensen GC. Veins of the pelvic limb. In: Evans HE, Christensen GC, eds. *Anatomy of the Dog*. Philadelphia: Saunders, 1979;782-787.

Patient compliance with venous leg ulcer treatment

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SUMMARY

Lower-limb venous ulceration is a common affliction, affecting particularly the elderly population, which significantly reduces quality of life. Compression treatment, in the form of hosiery, bandaging, or intermittent pneumatic compression devices (IPC) is regarded as standard in the management of venous leg ulceration (VLU). This article reviews the literature on patient compliance with such compression modalities, a factor often not paid much attention to clinical practice.

INTRODUCTION

Chronic lower-limb ulceration affects approximately 1% of the population.[1,2] The majority of these ulcers are of venous etiology[3] but a variety of other causes have been identified which might coexist.[4,5] The prevalence increases to more than 2% in those aged over 80 years[6] with demographic studies suggesting a significant increase over the next 85 years.[7] VLU is a considerable source of morbidity reducing patients' quality of life[8] and is estimated to cost the UK National Health System £300 to £600 million per year.[9]

The majority of VLUs are managed in the community and district nurses spend up to half of their time treating VLUs[3] because leg ulceration is characterized by a cyclical pattern of healing and recurrence[10] with recurrence rates ranging between 45% and 70%.[3,11,12] Therefore, community leg ulcer clinics have been established in order to deliver specialized treatment in the form of weekly application of high compression bandaging, which improves the healing rate.[13,14] Healed ulcer groups have also been suggested in the past, once healing of the ulcer has been achieved, mainly to educate patients, thus increasing their compliance with compression hosiery and in turn reducing the incidence of ulcer recurrence.[15,16]

PATIENTS' COMPLIANCE WITH COMPRESSION TREATMENT

Inelastic bandages

The goals of treating VLUs include counteracting the effects of venous hypertension and reduction of edema in order to heal and prevent ulcer recurrence. Edema is a significant problem, and the simplest way to reduce this is bedrest

Keywords:
Compression hosiery - Compression bandages - Venous leg ulcer - Compliance.

combined with leg elevation.[17,18] Regular daily elevation above the heart level has been shown to improve swelling and venous microcirculation[19] but this might be difficult for patients suffering from arthritis or heart failure.

Compression therapy is the oldest form of effective treatment for VLUs, as described by Hippocrates.[20] The most effective method of compression remains an issue of controversy, but in general there are three forms of compression applied in clinical practice: inelastic, elastic, and intermittent pneumatic compression (IPC).

Two main prototypes of inelastic bandages are available: a short-stretch bandage (eg, Comprilan, Beiersdorf) which is favored in mainland Europe, and the traditional Unna boot, a moist zinc oxide-impregnated paste bandage used mainly in the USA.[21] Such rigid bandages apply low resting but high working (walking) pressures, and require a patient who is not only mobile but also available; in particular the Unna boot requires frequent application because of its limited absorptive capacity for highly exudative wounds, which leads to a foul smell and might affect patients' compliance with such bandages.[22]

Elastic hosiery

Elastic compression therapy can be subdivided into compression hosiery and bandaging. Compression stockings utilize locally graduated pressure on the calf muscle and have been effective not only in treating[23,24] but also in preventing recurrence of venous ulcers.[25] Compression hosiery can be divided into three classes (British standard, I: 14-17 mm Hg, II: 18-24 mm Hg, III: 25-35 mm Hg at ankle), each class utilizing different pressure gradients with their own recommended indications for their use. A common problem related to compression hosiery is patient compliance and adherence to treatment protocols.[26,27] Patients' compliance to treatment with stockings can be affected by skin allergies (Elastane, Nylon, or Lycra contained within hosiery), cosmetic considerations, ill-fitting stockings causing pressure necrosis, patients' age-related dexterity, or any other disability which might cause difficulty in applying the stockings.[28,29,30] All of the above potential problems can be rectified, eg, patch tests can be performed to identify allergies[31] and if positive the use of a cotton lining under the stockings can overcome this problem. Accurate measurement of the calf is important to ensure that the type of compression hosiery chosen fits well and various aids are available from manufacturers which help patients with the application of stockings but occasionally assistance from a relative or carer needs to be organized.[32]

Furthermore, pain caused by venous ulcers can be severe and needs to be addressed appropriately, which in turn can improve patients' compliance with compression stockings.[33] Patients require two pairs (one to wear and one to wash) and a new pair is needed in approximately 4 to 6 months as they wear out[10] which means that costs have to be kept low by companies in order not to deter patients from buying them.

Awareness of all the above factors which can influence compliance will contribute to a successful treatment of VLUs, and in some cases it should be remembered that a lower level of compression is better than none.[34]

Compression bandaging

There are a variety of bandages available and they can be divided into multilayer or single-layer bandage systems.[35] The Charing Cross four-layer bandage which is widely used in the UK achieves venous ulcer healing rates of 69% at 12 weeks.[13] These multilayer bandages are designed to apply 40 mm Hg pressure at the ankle graduating to 17 mm Hg at the knee sustainable for a week.[21] Multilayer systems might be more expensive than single-layer bandages, but in the long run they work out cheaper as they achieve higher and faster healing rates.[10,21] Patients find the four-layer system comfortable and the once-weekly changing regimen less disruptive to their lives than other systems.[35,36] Nevertheless, on occasions it is required to change the bandage two or three times a week because of the excessive exudates, and address patients' discomfort with adequate analgesia.[33] The main disadvantage of the four-layer compression system is that a certain expertise is needed, with most nurses having to complete a course on management of leg ulcers [ENB N18] with additional training days to practise adequate application.[37] Nurses who specifically promote the delivery of four-layer high compression treatment achieve higher healing rates compared with results obtained by community nurses.[38]

Long-term continuation of compression in form of hosiery following healing of VLUs is the most effective preventive method for recurrence[39] and patients' compliance has been a concern.[15,25] Previous studies demonstrated that adherence to compression therapy reduces the recurrence rates.[40,41]

Compliance in general with treatment has already been identified as a problem; eg, 50% to 60% of patients are not compliant with taking their medication[42] and it has been shown that the noncompliance rate increases in particular with long-term treatment where the aim is preventive.[43] Patients' nonadherence with compression hosiery is a complex issue, as it is difficult to measure[44] and affected by multiple factors as mentioned earlier.

Intermittent pneumatic compression devices (IPC)

IPC is a compression pump device designed to squeeze the leg intermittently, thus reducing venous stasis by promoting venous blood flow[45] and increasing systemic fibrinolytic activity.[46] This pump system has been demonstrated in earlier studies to aid venous ulcer treatment in combination with bandages, but unfortunately all these studies are characterized by small numbers.[47,48,49] These trials confirmed that patients' compliance was good, with compression regimens varying from hourly sessions weekly to 4 hours per day within a home setting. IPC might have a role to play in patients with reduced mobility and difficult-to-heal ulcers.[50]

CONCLUSION

Venous ulceration can be the result of isolated superficial venous incompetence, but most studies have demonstrated that deep venous reflux alone or in conjunction with superficial insufficiency are the main causes of VLU.[51,52] Superficial venous surgery is regarded as controversial in the presence of deep venous reflux[53,54] but is indicated in patients with isolated superficial venous incompetence.[55]Conservative management in form of compression treatment will therefore remain the cornerstone for VLUs, and compliance of patients with such modalities to achieve healing or avoid recurrence will remain a major concern to doctors and nurses treating patients with lower-limb venous ulcers. Compliance is a complex issue based on patient's attitude, their motivation and ownership of the problem[27] which requires education, supervision, and support so that patients become responsible for their own care, thus in turn increasing adherence to compression therapy.

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REFERENCES

1. Callam MJ, Harper DR, Dale JJ, et al. Chronic ulcer of the leg: clinical history. *BMJ*. 1987;294:1388-1391.
2. Hallboeck T. Leg ulcer epidemiology. *Acta Chir Scand*. 1998;544(Suppl):17-20.
3. Callam MJ, Ruckley CV, Harper DR, et al. Chronic ulceration of the leg: Extent of the problem and provision of care. *BMJ*. 1985;290:1855-1856.
4. Scriven JM, Hartshorne T, Bell PRF, Naylor AR, London NJ. Single visit venous ulcer assessment clinic: first year. *Br J Surg*. 1997;84:334-336.
5. Fassiadis N, Kapetanakis E, Law N. Etiology of leg ulcers, healing and recurrence rates in octo- and nonagenarians. *Int Angiol*. 2002;21:193-195.
6. MacKenzie RK, Bradbury AW. The management of venous ulceration. In: Earnshaw JJ Murie JA, eds. *The Evidence of Vascular Surgery*. Cheltenham TFM Publishing Ltd; 1999:151-158.
7. Department of Health Caring for people. Community care in the next decade and beyond. HMSO, London; 1989.
8. Phillips T, Stanton B, Provan A, et al. A study of the impact of leg ulcers on quality of life: financial, social and psychologic implications. *J Am Acad Dermatol*. 1994;31:49-53.
9. Coleridge Smith PD. Venous ulcer. *Br J Surg*. 1994;81:1404-1405.
10. Armstrong S. Compression hosiery. *Prof Nurse*. 1997;12:10-11.
11. Lees TA, Lambert D. Prevalence of lower limb ulceration in an urban health district. *Br J Surg*. 1992;79:1032-1034.
12. Nelzen O, Bergqvist D, Lindhagen A. Leg ulcer etiology – a cross sectional population study. *J Vasc Surg*. 1991;14:557-564.
13. Moffat CJ, Franks PJ, Oldroyd M, et al. Community clinics for leg ulcers and impact on healing. *BMJ*. 1992;305: 1389-1392.
14. Simon DA, Freak L, Kinsella A, et al. Community leg ulcer clinics: a comparative study in two health authorities. *BMJ*. 1996;312:1648-1651.
15. Fassiadis N, Godby C, Agland L, Law N. Preventing venous ulcer recurrence: The impact of the Well Leg Clinic. *Phlebology*. 2002;17:134-136.
16. Ruane-Morris M, Thompson G, Lawton S. Supporting patients with leg ulcers. *Prof Nurse*. 1995;19:765-770.
17. Gibson B. Use of compression in the treatment of leg ulcers. *Care, Science and Practice*. 1990;8:67-69.
18. Callam MJ, Lynch DJ. Preventing and treating leg ulcers. *Patient Care*. 1992;26:118-136.
19. Abu-Owen A, Scurr JH, Coleridge Smith PD. Effect of leg elevation on skin microcirculation in chronic venous insufficiency. *J Vasc Surg*. 1994;20: 705-710.
20. Browse NL, Burnand KG, Thomas ML. *Diseases of the Veins: Pathology, Diagnosis and Treatment*. Edward Arnold; 1988:1-21: 411-442.
21. Fletcher A, Cullum N, Sheldon TA. A systematic review of compression treatment for venous ulcers. *BMJ*. 1997;315:576-579.
22. DeAraujo T, Valencia I, Federman DG, Kirsner RS. Managing the patient with venous ulcers. *Ann Int Med*. 2003;138: 326-334.

REFERENCES

23. Hendricks WM, Swallow RT. Management of skin leg ulcers with Unna's boot versus elastic support stockings. *J Am Acad Dermatol*. 1985;12:90-98.
24. Horakova M, Partsch H. Venous leg ulcers: are compression bandages indicated? *Phlebology*. 1994;47:53-57.
25. Moffat CJ, Dorman MC. Recurrence of leg ulcers within a community ulcer service. *J Wound Care*. 1995;4:57-61.
26. Kiev J, Kerstein MD. Venous insufficiency and graded compression therapy. *Wounds*. 1993;5:280-283.
27. Moffat C, O'Hare L. Graduated compression hosiery for venous leg ulceration. *J Wound Care*. 1995;4:459-462.
28. Callam MJ, Ruckley CV. Hazards of compression treatment of the leg ulcer: an estimate from Scottish surgeons. *BMJ*. 1987;295:1382.
29. Edwards L, Moffat CJ. The use of compression hosiery in the care of leg ulcers. *Nursing Standard*. 1996;10:53-56.
30. Johnson S. Compression hosiery in the prevention and treatment of venous leg ulcers. *J Tissue Viability*. 2002;12:67-73.
31. Cowan T. Compression hosiery. *Prof Nurse*. 1997;12:881-886.
32. Williams C. Leg ulcer after care: the role of compression hosiery. *Br J Nursing*. 2000;9:822-888.
33. Mallett L. Controlling the pain of venous leg ulceration. *Prof Nurse*. 1999;15: 131-136.
34. Blair SD, Wright D, Backhouse C, et al. Sustained compression and healing of chronic venous ulcers.*BMJ*.1988;297: 1159-1162.
35. Thompson B, Hooper P, Powell R. Four-layer bandaging and healing rates of venous ulcers. *J Wound Care*. 1996;5: 213-216.
36. Loftus S, Wheattey C. Developing skills in leg ulcer nursing: the lessons learned. *J Wound Care*. 2000;9:483-489.
37. Morrell CJ, Walters SJ, Dixon S. Cost effectiveness of community leg ulcer clinics: randomised controlled trial. *BMJ*. 1998;316:1487-1491.
38. Palfreyman SJ, Lochiel R, Michaels JA. A systematic review of compression therapy for venous leg ulcers. *Vasc Med*. 1998;3: 301-313.
39. Weingarten MS. State of the art treatment of chronic venous disease. *Clin Infect Dis*. 2001;32:949-954.
40. Phillips TJ, Machado F, Trout R, et al. Prognostic indicators in venous ulcers. *J Am Acad Dermatol*. 2000;43:627-630.
41. Samson RH, Showalter DP. Stockings and the prevention of recurrent venous ulcers. *Dermatol Surg*. 1996;22:373-376.
42. Becker MH, Maiman L. Strategies for enhancing patient compliance. *J Community Health*. 1980;6:113-135.
43. Sackett DL, Snow JC. The magnitude of compliance and non-compliance. In Haynes RB, Taylor DW, Sackett DC, eds. Compliance in Healthcare. Baltimore, Md: John Hopkins University Press; 1979.
44. Furlong W. Venous disease treatment and compliance: the nursing role. *Br J Nursing*. 2001;10:18-32.
45. Coleridge Smith PD, Sarin S, Hasty J, Scurr H. Sequential gradient pneumatic compression enhances venous ulcer healing: a randomised trial. *Surgery*. 1990;108:871-875.
46. Tarnay TJ, Rohr PR, Davidson AG, et al. Pneumatic calf compression and fibrinolysis. *Surgery*. 1980;88:489-495.
47. Rowland J. Intermittent pump versus compression bandages in the treatment of venous leg ulcers. *Aust NZ J Surg*. 2000;70:110-113.
48. McCulloch JM, Marler KC, Neal MB, Phifer TJ. Intermittent compression improves venous ulcer healing. *Adv Wound Care*. 1994;7:224-226.
49. Kumar S, Walker A, Gates S, Walker MA. Intermittent pneumatic compression as an adjuvant therapy in venous ulcer disease: Randomised trial. *Phlebology*. 2001; 16:48-49.
50. Vowden K. The use of intermittent pneumatic compression in venous ulceration. *Br J Nursing*. 2001;10:491-509.
51. McEnroe CS, O'Donell TF Jr, Mackey WC. Correlation of clinical findings with venous haemodynamics in 386 patients with chronic venous insufficiency. *Am J Surg*. 1988;156:148-152.
52. Wilson NM, Rutt DL, Browse NL. Repair and replacement of deep vein valves in the treatment of venous insufficiency. *Br J Surg*. 1991;78:388-394.
53. Scurr JH. Varicose veins and the ulcerated leg. *Surgery*. 1996;14:40-41.
54. Padberg FT Jr, Pappas PJ, Araki CT, Back TL, Hobson RW. Haemodynamic and clinical improvement after superficial vein ablation in primary combined venous insufficiency with ulceration. *J Vasc Surg*. 1996;24:711-718.
55. Bello M, Scriven T, Hartshorne PRF, et al. Role of superficial venous surgery in the treatment of venous ulceration. *Br J Surg*.1999;86:755-759.

Benefit of Daflon 500 mg in chronic venous disease-related symptoms

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INTRODUCTION

According to the literature,[1] chronic venous disease (CVD) includes patients who present with so-called symptoms and/or signs of venous disease which characterize each class of chronic venous disease in the CEAP (Clinical, Etiological, Anatomical, and Pathophysioloical) classification,[2] from class C0 to class C6. The symptoms traditionally ascribed to chronic venous disease include aching, heaviness, a feeling of swelling, cramps, itching, tingling, and restless legs. These symptoms are nonspecific, and the relationship between symptoms and particular signs or stages of chronic venous disease according to the severity is controversial. In the Edinburgh Vein Study,[3] the occurrence of symptoms and the presence of trunk varices[3] or venous reflux assessed by duplex ultrasonography[4] was very weak, whereas in the recent study by Labropoulos,[5] symptoms increased with the severity of chronic venous disease.

Despite the fact that symptoms are common in the general population (especially in women),[3] little has been written on their prevalence and pathogenesis, the influence they may have on the quality of life, on the way to assess them, as well as on their management in practice.

PREVALENCE OF CHRONIC VENOUS DISEASE-RELATED SYMPTOMS

The grading of chronic venous disease has been facilitated and standardized by the introduction of the CEAP classification system, in 1995.[2] Among epidemiological surveys that have been performed since then, at least six have used the CEAP classification,[6-11] making comparison between studies much easier. Unfortunately, little is still known about the prevalence of symptoms in chronic venous disease, despite the introduction of the notion of symptoms in the CEAP classification. The percentage of symptomatic patients with chronic venous disease varied between 25% and 84%, depending on the population studied, the severity of the disease, and the mean age of patients *(Table I)*.

The French team of Carpentier found that symptoms increased with the CEAP classes. In this study, patients with symptoms went from 25% in C0 to 84.6% in C3, 82.2% in C4, and 77.5% in C5-6.[11] In the same way, Labropoulos[5] found a direct relationship between the amount of inflammatory mediators and the severity of the disease.

	Rabe[6]	Jawien[7]	Labropoulos[8]	Eklof[9]	Scuderi[10]	Carpentier[11]
Type of population	General populations of Bonn	Cross-sectional study of primary care center patients in Poland (conservative patients)	Cross-sectional study in Maywood (USA)	Straub clinic Hawaii (conservative patients)	General ambulatory population of the University Hospital Sao Paulo	Group of specialists (International)
Date of recruitment	October 2000 March 2002	2000	-	-	March 1998 Dec 2000	-
Mode of recruitment	Examination	Questionnaire by physicians and examination	-	-	Examination Questionnaire	Filling in of a computerized file
Use of the CEAP classification	Yes	Yes	Yes	Yes	Yes	Yes
Respondents (N)	3072	40 095	1000 limbs	166 limbs	2104	872
% in C_0-C_1	C_{0S} = 9.6 C_1 = 59.0	C_0 = 51.1 C_1= 16.5 symptoms = 48.4 (all patients)	C_0 = 1.3 C_1 = 5.9 Symptoms = 70-80% (all patients)	C_0 ? C_1 = 19	C_{0S} = 3.9 C_1+ ; C_1 = 49.6 Symptoms (all patients) 58.7	C_0, right L = 11.0 C_0, left L = 11.8 C_1, right L = 20.9 C_1, left L = 22.4 % symptoms C_0 = 25 C_1 = 53.3 C_2 = 67.5 C_3: 84.6 C_4 = 82.2 C_5-C_6 = 77.5

* Leg problems included: "pain in legs" and "presence of varicosities of the legs".

Table I. Summary of epidemiological survey using the CEAP classification (adapted from refs 6-11).

PATHOPHYSIOGENESIS OF CHRONIC VENOUS DISEASE-RELATED SYMPTOMS

Two major mechanisms may be responsible for pain in the absence of trophic changes:
- first, venous wall tension which results from dilatation of the vein in a normal subject in a standing position and valvular incompetence during dynamic movement in a standing position in a subject with valvular insufficiency;
- second, hypoxia of the tunica media of the venous wall due to alteration of the vasa vasorum. Pain seems more related to hypoxia; indeed, in the early stages of chronic venous disease, superficial venous distensibility is slight, while pain is more severe than in the advanced stages of chronic venous disease where venous pressure is elevated and therefore high venous wall pressure exists.[12] However, the venous remodeling phase that precedes the development of varicose veins, which is accompanied by the process of venous distension, hemorheological disturbances, and conditions of hypoxia, can be painful.

Pain and heaviness in the legs

Two specific targets for phlebotropic drugs exist which aim to decrease the sensation of heaviness in the legs and pain, and ankle edema at the end of the day. The first target of such therapy is increased venous wall pressure: indeed, it is known that distensibility is increased by 10% to 50% in patients[13] with chronic venous disease according to different studies, and that this is due to a decrease in venous tone. The second target is hypoxia of the tunica media related to disease of the vasa vasorum.[12]

Restless legs, nighttime cramps

These symptoms most often occur during the latter half of the night, but also can occur during a prolonged seated position. They can also be related to hypoxia of the tunica media, but more specifically may be associated with hemorheological disorders.[12] In fact, red blood cell hyperviscosity and hyperaggregation are constant findings in venous disease.[14,15] It appears likely that hemorheological disorders worsen the circulation in the vasa vasorum. Hypoxia in the tunica media, in turn, induces deterioration of the venous wall. Indeed, hypoxia has a potent effect in inducing metabolic disorders: triggering of enzyme activities, such as those of matrix metolloproteinases (MMP), and dedifferentiation and migration of smooth muscle cells which secrete growth factors. Fibrosis of the venous wall governs the development of the varicose vein. The

diseased venous wall generates several metabolic disorders, including hypofibrinolysis due to elevated levels of plasminogen-activating inhibitor (PAI-1). The links between pathophysiology, symptoms, and clinical signs of chronic venous disease are summarized in *Table II*.

Edema

The process of edema appears to be due to increased capillary permeability related to permanent venous hypertension,[12] whose mechanisms vary: reflux, obstruction.

Two stages should be distinguished in the progression of capillary disorders, as venous disease becomes progressively worse: first, the existence of a functional disorder followed by development of a lesional disorder, which characterizes chronic venous insufficiency.

ASSESSMENT OF CHRONIC VENOUS DISEASE-RELATED SYMPTOMS

It must be stated that since it is not possible to confirm whether such symptoms are related to venous disease, it appears important to try to characterize these symptoms based on the following secondary criteria:

- *Variability with position or physical activity*

Symptoms generally occur after prolonged standing, at the end of the day, and are diminished in the morning, by supine position, or with the legs elevated.

- *Variability with temperature*

Symptoms occur or are exacerbated by warmth, the summertime season, hot baths, hot waxing to remove body hair, floor-based heating systems, and regress or are diminished in winter and with cold temperatures.

- *Variability with levels of circulating sex hormones*

Symptoms fluctuate with the menstrual cycle; they can occur with hormonal therapy (estrogens or estrogen-progestin) and regress with discontinuation of such treatment.

The existence of at least two secondary criteria is necessary to confirm that symptoms are related to chronic venous disease, but the absence of such criteria does not rule out the possible venous-related origin of the symptoms.[16]

Pain and heaviness in legs are the symptoms that are the most often encountered in patients with chronic venous disease. Symptoms, and in particular, pain, can be evaluated with self-evaluation rating scales. Three types of scales have been validated[17]:

The visual analog scale most often used in the conventional format which consists of a 100-mm-long horizontal line. The patient indicates the level of his or her pain by drawing a mark on the line (paper format) or by moving a cursor along this line (mechanical ruler). It has a white background and has no words other than those at each end.

Numerical scales are also used for evaluation of pain intensity. Several types of numerical scales exist, generally with numbering of 0 to 10 or 10 to 100. The patient has to assign a score to his or her pain intensity.

Simple verbal scales also allow evaluation of pain intensity. They are based on the choice of an adjective to define pain intensity. A numerical value corresponds to each adjective. Measurement is limited to 5 or 6 levels. Generally, they are reserved for persons who have difficulty (limited capacity for abstraction) in using the previous two types of rating scales.

These scales are validated but are not specifically adapted to the chronic venous disease.

Some scales have been used to evaluate patient-reported symptoms. One has been recently set up for use across the range of CVD-related symptoms,[18] one for varicose veins,[3] and one for patient-reported measure of quality of life and symptoms.[19] The questions of the VEINES/QoL-Sym

Table II. Possible links between pathophysiological variables, symptoms and signs of chronic venous disease (adapted from ref 12).

Pathophysiology	Symptom	Sign
Leukocyte adhesion in subvalvular areas/areas of inflammation	Pain	Early valvular reflux
Valvular incompetence		Reflux
Venous hypertension and venous wall tension	Pain	Edema
Venous wall hypoxia	Pain	
Increased capillary pressure	Sensation of heavy legs and swelling	Edema
Hemorheological disorders and platelet hyperaggregability	Nighttime cramps Restless legs	

specifically devoted to symptoms are presented in *Table III*. Presentation of a scale to the patient has to be done in a relatively standardized manner (for example, always at the same time of the consultation).

All these scales evaluate pain intensity but do not provide information on the nature of the pain complaint. They make it possible to compare intraindividual variations between groups of patients in evaluation studies or longitudinal observational surveys.

THE BENEFIT OF Daflon 500 mg IN CHRONIC VENOUS DISEASE-RELATED SYMPTOMS

Evaluation of symptoms associated with CVD and the expected benefit of therapy with Daflon 500 mg (micronized purified flavonoid fraction, MPFF) is not easy because many intercurrent factors exist. However, randomized, controlled studies with a washout period have been conducted using measurable criteria for evaluation of symptoms in the legs.

Randomized, double-blind, 2 -month studies have evaluated the effectiveness of Daflon 500 mg twice daily in the treatment of symptoms and signs of the disease compared with placebo.[20,21] The long-term efficacy of 2 tablets of Daflon 500 mg daily has been studied in two nonblinded, multicenter trials of 6- (Reflux Assessment and Quality of Life Improvement with Micronized Flavonoids [RELIEF] study)[22] or 12 -months' duration.[23]

The efficacy of Daflon 500 mg was evaluated by the change in clinical symptoms in the legs, ankle and calf circumferences and/or plethysmographic parameters.

The number and type of clinical symptoms (eg, functional discomfort, sensation of leg heaviness, leg pain, nocturnal cramps, sensation of swelling, paresthesia, redness and/or cyanosis, sensation of heat and/or burning was different in each study. Patient response was determined by changes in symptom score on a 4-[20-22] or 5-point scale[23] where increasing numbers indicated increasing symptom severity. Pain[22] was measured on 10-point visual analog scale in some of the trials. A variety of noninvasive techniques, which correlate with changes in venous pressure, have been developed for objectively quantifying the severity of the disease. These include strain-gauge plethysmography (eg, venous distensibility at various venous occlusion pressures and duration of venous outflow after removal of venous occlusion)[20] and duplex ultrasonography (eg, venous reflux time).[22]

In the RELIEF study, the Chronic Venous Insufficiency Questionnaire (CIVIQ), the first health-related quality-of-life scale specific for CVD, to evaluate the effects of MPFF on health-related quality of life was used.[22] CIVIQ is a 20-question self-administered questionnaire with a range of scores from 0-100 where 100 indicates a very good health-related quality of life.

1. During the past 4 weeks, how often have you had any of the following leg problems?

(check one box on each line)	Every day	Several times a week	About once a week	Less than once a week	Never
1. Heavy legs	1	2	3	4	5
2. Aching legs	1	2	3	4	5
3. Swelling	1	2	3	4	5
4. Night cramps	1	2	3	4	5
5. Heat or burning sensation	1	2	3	4	5
6. Restless legs	1	2	3	4	5
7. Throbbing	1	2	3	4	5
8. Itching	1	2	3	4	5
9. Tingling sensation (eg, pins and needles)	1	2	3	4	5

Table III. Questions from VEINES/QoL-Sym for the evaluation of chronic venous disease-related symptoms (adapted from ref 19).

*2. At what time of day is your **leg problem** most intense (check one)*
1. On walking
2. At mid-day
3. At the end of the day
4. During the night
5. At any time of day
6. Never

*3. Compared with 1 year ago, how would you rate your **leg problem** in general now? (check one)*
1. Much better now than 1 year ago
2. Somewhat better now that 1 year ago
3. About the same now as 1 year ago
4. Somewhat worse now that 1 year ago
5. Much worse now than 1 year ago
6. I did not have any leg problems last year

▶ *Comparative trials with placebo*

Compared with placebo in two randomized, double-blind, placebo-controlled trials in 36 or 150 patients treated with Daflon 500 mg, 2 tablets daily for 2 months significantly decreased ankle or calf circumference,[20,21] improved many symptoms[19,20] and improved plethysmographic parameters.[20]

Mean ankle circumference significantly decreased from baseline at week 4 and week 8 in patients with symptoms. Mean calf measurements also decreased significantly from baseline with MPFF compared with placebo *(Table III)*; significant reductions after Daflon 500 mg treatment were shown in the trial by Gilly et al.[21]

▶ *Long-term treatment*

Two tablets of Daflon 500 mg daily maintained efficacy throughout the treatment in the long-term in patients with symptoms of chronic venous disease in two nonblinded, multi-center trials of 6[22] and 12 months'[23] duration, respectively. In the RELIEF study (n=4527 intention-to-treat population), patients receiving 2 tablets of MPFF daily showed progressive improvement in the symptoms of chronic venous disease, *(Figure 1)* which were paralleled by improvement from more severe (C3 or C4) to less severe (C0–C2) CEAP clinical classes of chronic venous disease.[22] After 6 months, patients in the per-protocol population showed significant improvement from baseline in the study outcome measures (ankle circumference, pain, leg heaviness, cramps, sensation of swelling; $P<0.012$). In the 1-year trial of 2 tablets of Daflon 500 mg daily in 170 patients,[23] a significant reduction from baseline values in physician-assessed clinical symptoms (functional discomfort, cramps, and evening edema), ankle and calf circumference, and patient overall assessment of symptom severity was demonstrated at each 2-month evaluation ($P<0.001$). The rapid reductions observed during the first 2 months of treatment represented approximately 50% of the total improvement ultimately observed after 1 year of treatment. Continuing improvement in all parameters, albeit less rapid, was reported at each time point from month 2 to month.[12]

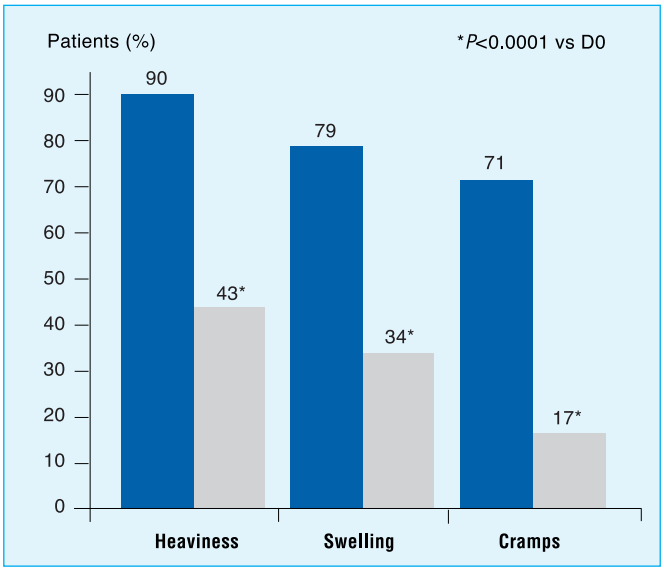

Figure1. Improvement in symptoms related to chronic venous disease with a 6-month Daflon 500 mg treatment (adapted from ref 22).

Daflon 500 mg: THE REFERENCE TREATMENT QUOTED IN GUIDELINES

In recent guidelines[24,25] or extensive reviews[26] on the treatment of chronic venous disease, the use of phlebotropic drugs is indicated to treat edema and the chronic venous disease-related symptoms (eg, edema, fatigue, nocturnal cramps, and heaviness) in any stage of the disease. In the more advanced stages of the disease, only Daflon 500 mg has demonstrated a significant acceleration of ulcer healing. Thus Daflon 500 mg should be used in advanced stages of the disease in conjunction with sclerotherapy, surgery and/or compression therapy, or as an alternative treatment when surgery is not indicated or is unfeasible, or when patients are unwilling or unable to use compression therapy (elastic stockings or compression bandages). These recommendations were supported by clinical trials of Daflon 500 mg, which has been extensively evaluated for use in patients with chronic venous disease.

REFERENCES

1. Ramelet AA, Kern P, Perrin M, eds. *Les varices et télangiectasies*. Paris: Masson; 2003:7-16.
2. Porter JM, Moneta GL. International Consensus Committee on chronic venous disease. Reporting standards in venous disease: an update. *J Vasc Surg*. 1995;21:635-645.
3. Bradbury A, Evans C, Allan P, Ruckley CV, Fowkes FGR. What are the symptoms of varicose veins? Edinburgh vein study cross sectional population survey. *BMJ*. 1999;318:353-356.
4. Bradbury A, Evans CJ, Allan P, Lee AJ, Ruckley CV, Fowkes FGR. The relationship between lower limb symptoms and superficial and deep venous reflux on duplex ultrasonography: the Edinburgh Vein Study. *J Vasc Surg*. 2000;32:921-931.
5. Labropoulos N. Inflammation parallels severity of chronic venous insufficiency. *Phlebology*. 2003;18:78-82.
6. Pannier-Fischer F, Rabe E. Epidemiology of chronic venous diseases. *Hautarzt*. 2003;54:1037-1044.
7. Jawien A, Grzela T, Ochwat A. Prevalence of chronic venous insufficiency in men and women in Poland: multicentre cross-sectional study in 40,095 patients. *Phlebology*. 2003;18:110-122.
8. Labropoulos N. Hemodynamic changes according to the CEAP classification. *Phlebolymphology*. 2003;40:130-136.
9. Masuda EM, Kisner RL, Eklof B, Yang D. Practical application of the CEAP classification. In: Ballard JL, Bergan JJ, eds. *Chronic Venous Insufficiency, Diagnosis and Treament*. London: Springer-Verlag London Limited; 2000:41-49.
10. Scuderi A, Raskin B, Assal FA, Scuderi P, et al. The incidence of venous disease in Brazil based on the CEAP classification. An epidemiological study. *Int Angiol*. 2002;21:316-321.
11. Carpentier PH, Cornu-Thénard A, Uhl JF, Partsch H, Antignani PL and the European working group on the clinical characterisation of venous disorders. Appraisal of the information content of the C classes of CEAP clinical classification of chronic venous disorders: a multicenter evaluation of 872 patients. *J Vasc Surg*. 2003;37:827-833.
12. Boisseau MR. Pharmacologie des médicaments veinotoniques : données actuelles sur leur mode d'action et les cibles thérapeutiques. *Angéiologie*. 2000;52:71-77.
13. Clarke GH, Vasdekis SN, Hobbs JT, Nicolaides AN. Venous wall function in the pathogenesis of varicose veins. *Surgery*. 1992;111:402-408.
14. Boisseau M, Freyburger G, Busquet M, et al. Hemorheological disturbances in venous insufficiency after induced stasis. *Clin Hemorheol*. 1989;9:161-163.
15. Le Devehat C, Boisseau MR, Vimeux M, et al. Hemorheological factors in the pathophysiology of venous disease. *Clin Hemorheol*. 1989;9:861-870.
16. Garde C, Perrin M, Chleir F, Henriet JP et al. First meeting of review and consensus on venoactive agents on the symptoms of chronic venous disease. (In French). *Phlébologie*. 2003;56:103-109.
17. Agence Nationale d'Accréditation et d'Evaluation de la Santé. Evaluation et suivi de la douleur chronique chez l'adulte. en médecine ambulatoire www.anaes.fr
18. Rutherford RB, Padberg FT Jr, Comerota AJ, Kistner RL, Meissner MH, Moneta GL. Venous severity scoring: an adjunct to venous outcome assessment. *J Vasc Surg*. 2000;31:1307-1312.
19. Lamping DL, Schroter S, Kurz X, Kahn S, Abenhaim L. Evaluation of outcomes in chronic venous disorders of the leg: development of a scientifically rigorous, patient-reported measure of symptoms and quality of life. *J Vasc Surg*. 2003;37: 410-419.
20. Chassignolle J-F, Amiel M, Lanfranchi G, et al. Activité thérapeutique de Daflon 500 mg dans l'insuffisance veineuse fonctionnelle. *J Int Med*. 1987;99(Suppl) 32-37.
21. Gilly R, Pillion G, Frileux C. Evaluation of a new venoactive micronized flavonoid fraction (S 5682) in symptomatic disturbances of the venolymphatic circulation of the lower limb: a double-blind, placebo-controlled study. *Phlebology*. 1994;9:67-70.
22. Jantet G. Chronic venous insufficiency: worldwide results of the RELIEF study. Reflux assEssment and quaLity of life improvEment with micronized Flavonoids. *Angiology*. 2002;53:245-256.
23. Guillot B, Guilhou JJ, de Champvallins M, et al. A long term treatment with a venotropic drug: results on efficacy and safety of Daflon 500 mg in chronic venous insufficiency.*Int Angiol*. 1989;8(4 Suppl):67-71.
24. Italian College of Phlebology. Guidelines for the diagnosis and treatment of chronic venous insufficiency. *Int Angiol*. 2001;20(2 Suppl 2):1-37.
25. Coleridge Smith PD. The drug treatment of chronic venous insufficiency and venous ulceration. In: Gloviczki P, Yao JST, eds. *Handbook of Venous Disorders: Guidelines of the American Venous Forum*. 2nd ed. London: Arnold; 2001:309-321.
26. Lyseng-Williamson KA, Perry CM. Micronised purified flavonoid fraction. A review of its use in chronic venous insufficiency, venous ulcers and haemorrhoids. *Drugs*. 2003;63:71-100.

Histochemical insight into lymphangiogenesis and lymphatic regeneration

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SUMMARY

The lymphatic vascular system consists of a network of thin-walled vessels, and it plays an important role in transportation of extravasated fluid and macromolecules from tissues back to blood circulation, as well as in many pathological processes, including tumor metastasis and lymphedema. The structural organization and fine distribution of lymphatic vessels in the tissues are very important in the pathophysiology of a variety of microcirculatory disorders, infectious diseases, and cancer. The lymphatic system develops through a process known as lymphatic development (lymphangiogenesis). However, studies of the lymphangiogenesis of the lymphatic system have been hampered by a lack of lymphatic specific markers. Recently, the molecules that specifically control lymphangiogenesis and lymphatic regeneration have been identified.[1] Thus, the discovery of new lymphatic endothelial cell (LEC)-specific markers has now provided new insights into the molecular mechanisms that control lymphatic development and function. We herein review the histochemical insight into the field of lymphangiogenesis, with special emphasis on the novel and reliable LEC markers, such as 5′-nucleotidase and VEGF-C/VEGFR-3, as well as on lymphatic regeneration after experimental injury.[2,3]

MARKERS OF LYMPHATIC ENDOTHELIAL CELLS

Enzyme histochemistry

5′-nucleotidase (5′-Nase), an important enzyme in the metabolism of nucleotides, has widely been employed as a marker of cell membranes. 5′-Nase histochemical staining has proved to be an effective method for differentiating initial lymphatics from blood capillaries, based on its much higher activity on lymphatic than on blood vascular endothelium *(Figure 1a, 1b)*.[4] In addition, blood endothelia, especially artery and arterial capillaries, have comparatively strong alkaline phosphatase (ALPase) activity. Thus, two types of vessels have been distinguished by using 5′-Nase-ALPase double staining *(Figure 1c)*.[5,6] Furthermore, dipeptidyl aminopeptidase IV (DAPase) activity is markedly higher in the endothelium of the venous part of the capillaries and venules *(Figure 1d, 1e)*. Thus, a differential staining method using DAPase-ALPase

Keywords:
Lymphatics - Lymphoangiogenesis - Histochemistry - Lymphatic endothelial cells - Lymphatic regeneration.

double or DAPase-ALPase-5′-Nase triple staining for venous and arterial capillaries and/or lymphatics is effective for several tissues, not only in laboratory animals but also in humans *(Figure 1f)*.[7]

SEM examination of 5′-Nase–stained tissues on whole-mount preparations or tissue blocks after cryosection allows precise analysis of the abluminal aspects and three-dimensional structure of the lymphatic network.[8,9] Adequate treatment with NaOH effectively removes connective tissue matrices from the specimens and enables clear visualization of the three-dimensional structures of the immuno-histochemical stained lymphatics in both secondary emission and backscattered images.[10]

Immunohistochemistry

5′-Nase mAb (JC815) and ecto-5′-Nase mAb (CD73): when 5′-Nase antigenicity, rather than its activity, is considered, 5′-Nase mAb specific for the lymphatic endothelium, instead of adenosine monophosphate, can serve immunohistochemically as a useful marker for cell selection and in vitro cultivation. Our 5′-Nase-mAb, (JC815) immunoreactivity was distinctly expressed on the lymphatic vessels of several tissues from mice and rats, in comparison with 5′-Nase staining controls.[11] In the pancreas, JC815 strongly stained the interlobular lymphatic endothelium and was similar to the 5′-Nase staining pattern *(Figure 2a, 2b)*. CD31 strongly stained arteries *(Figure 2 C)*. Furthermore, in the rat tongue, immunohistochemical analysis of ecto-5′-Nase (CD73) is assumed to provide not only reassessment of the validity of 5′-Nase as a lymphatic endothelial marker, but also new information, including technical benefit *(Figure 2d, 2e)*. The 5′-Nase activity, CD73 immunoreactivity and hybridization signals for its mRNA were colocalized in the lymphatic vessels, including central lacteals of the small intestine, suggesting that 5′-Nase is actually produced in lymphatic endothelial cells and allocated to their cell membrane as an enzyme to regulate lymph production and flow.[12] These findings support our view that 5′-Nase is potential marker of lymphatics, and indicates the usefulness of the histochemical methods for 5′-Nase not only for demonstration of lymphatics, but also for examining the functional roles and dynamics of 5′-Nase in lymphatic endothelial cells in physiological and pathological conditions.

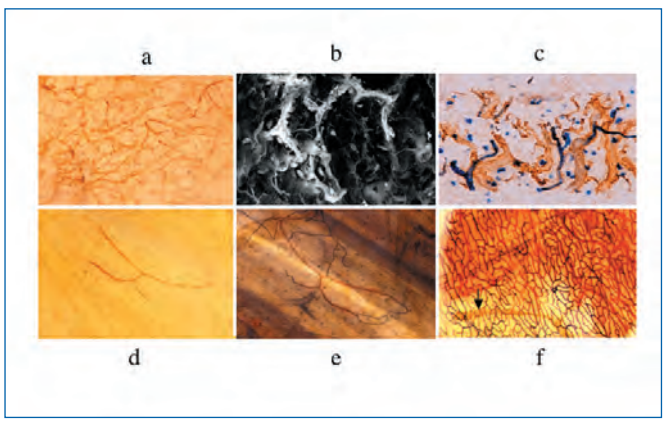

Figure 1. Light micrographs (a, c, d, e, f) and SEM-BEI view (b) of whole-mount preparations and cryosections stained with enzyme-histochemical staining. (a) 5′-Nase- positive lymphatics on a whole-mount preparation of rat skin. (b) The 5′-Nase-positive lymphatics are strongly highlighted. (c) 5′-Nase-positive lymphatics (brown) and ALPase-positive blood vessels (blue) on a rat skin cryosection stained with 5′-Nase-ALPase double staining. Figures 1d, e show a DAPase-positive vein and an ALPase-positive artery on a whole-mount presentation of the rat cecal wall stained with DAPase or DAPase-ALPase double staining. (f) Whole-mount preparation of the rat stomach wall stained with DAPase-ALPase-5′-Nase triple staining. a: x 45, b: x 200, c: x 250, d-f: x 100

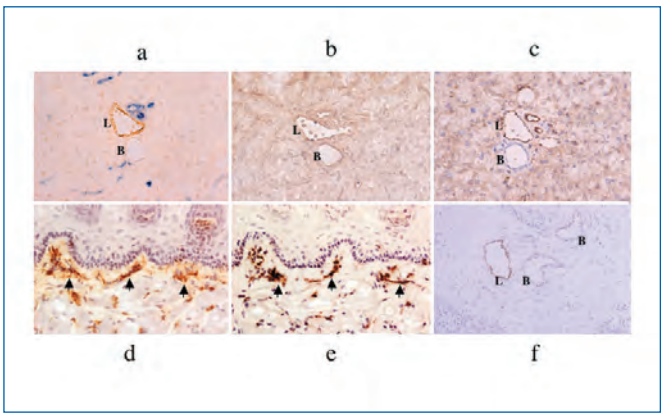

Figure 2. Enzyme- and immunohistochemical staining of the lymphatics and blood vessels of several mouse and rat tissues. (a-c) Serial sections of the mouse pancreas stained with 5′-Nase-ALPase (a), JC815 (b), and CD31 (c) staining, respectively. (d, e) Serial sections of the rat tongue stained with 5′-Nase (d) and CD73 (e) staining, respectively. (f) VEGFR-3-positive lymphatics. x 160

VEGFR- 3 Flt-4 and its ligand VEGF-C: a highly glycosylated class III cell surface tyrosine-kinase receptor, VEGFR- 3/Flt-4 was preferentially immunolocalized in the structures corresponding to the 5′-Nase-positive lymphatics in the lesion *(Figure 2f)*, and the immunoreaction products were ultrastructurally distributed on the cell membrane of lymphatic vessels. In contrast, immunostaining for VEGF-C demonstrated significant reaction products in many stromal cells, which predominantly demonstrated signals for VEGF-C mRNA in in situ hybridization. Therefore, the combination of 5′-Nase mAb with other lymphatic endothelial cell markers, and enzyme histochemistry with immunohistochemistry, may provide new possibilities for lymphatic investigation.

LYMPHOSTASIS BY TD BLOCKAGE

The effects of experimentally induced lymphedema have been studied in the extremities and internal organs of various animals and the results have been discussed in the light of the clinical understandings of lymphedema.[13,14] After thoracic duct (TD) blockage in rats, the mucosal and submucosal compartments of the small intestine in lymphostasis showed tortuous lymphatic networks and saccular dilations of the lymphatic vessels surrounded by fibrinoid materials, swollen collagen fibers, and focal accumulation of mononuclear cells. A tendency for reduced 5′-Nase activity in the TD endothelial cells became visible when the lymph flow was obstructed by TD blockage *(Figure 3a)*.[15] During TD blockage-induced lymphostasis, the 5′-Nase reaction product was almost undiscernible as a continuous demarcation of the endothelial layer within 2 weeks. Interestingly, the reduced 5′-Nase activity appeared earlier in the intramural intestinal lymphatics than in the TD *(Figure 3b)*. Prolonged obstruction of intestinal lymph flow will progressively aggravate peripheral lymphostasis and lymphatic incompetence. The effect of TD blockage on the endothelial cells of the intestinal lymphatics and TD was temporary, and lasted for about 6 weeks after ligation. The gradual recovery of the structure and function of the endothelial cells might be due to the observation that effective circulation is established by the marked regenerative capacity of smaller lymphatics, rapid development of collateral pathways around the blockage, and a relatively high rate of lymphovenous anastomosis formation.[16]

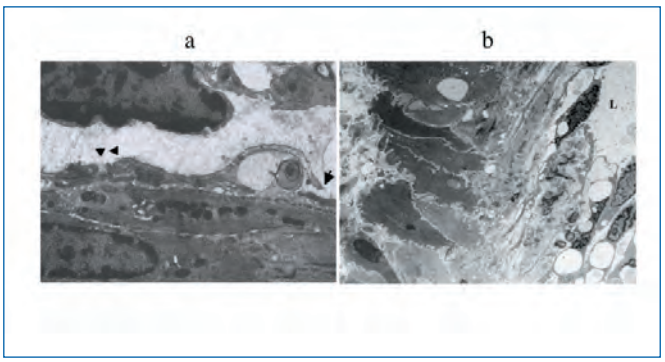

Figure 3. TEM views of small intestinal lymphatics in TD blockage animals. (a) After 2 days of TD blockage, endothelial cells frequently represent open intercellular (arrow) and interdigitating (arrowhead) junctions. (b) After 4 days of TD blockage, 5′-Nase activity of intramural intestinal lymphatics appears to reduce. a: x 15 000, b: x 2300

LYMPHATIC DEVELOPMENT (LYMPHANGIOGENESIS)

In early embryonic tissue, endothelial cells of newly formed lymphatic-like structures usually show extremely low 5′-Nase activity.[17] Interrupted weak or absent staining of endothelial cells was seen on newly formed lymphatic-like structures in the early stage, although no 5′-Nase reaction product was observed in blood vascular endothelial cells. Lymphatic vessels in the rat stomach revealed increased 5′-Nase activity as the animals grew.[18] Thus, 5′-Nase staining appears to be impractical for distinguishing developing lymphatics and blood vessels. On the other hand, in the early developing gastric wall, anti-VEGFR-3 was expressed in a cluster of circular lymphatic-like structures, which were gathered into several groups.[18] VEGFR-3 makes it possible to identify developing and regenerating lymphatic vasculature by localizing the antigen. VEGFR-3 binding to endothelial cells showed variations in staining intensity in the lymphatic wall and among samples. Developing endothelial cells of lymphatic and blood vasculatures react with VEGFR-3 during the early embryonic stage *(Figure 4a)*, although immature lymphatic vessels were usually stained with VEGFR-3 staining more intensely than typical lymphatic vessels. This suggests that lymphatic endothelial cells have similar molecular physiological features to blood vascular endothelia, and probably originate from the sprouting of small venous structures. The staining results for cell proliferation should be carefully analyzed. This opinion is not contrary to the observation that lymphangiogenesis originates from lymphatic vessels.[19] The present findings are in close agreement with the view that VEGFR-3 is

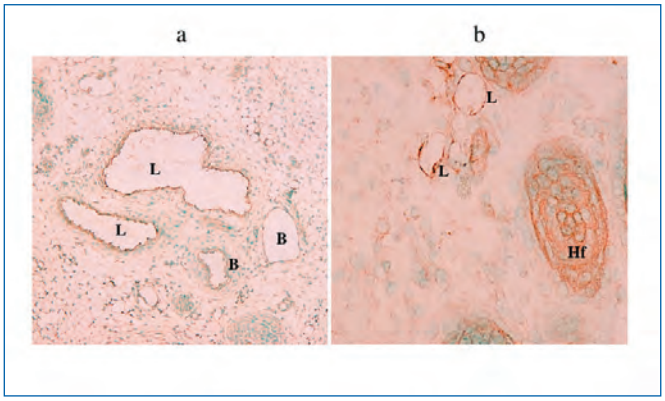

Figure 4. Photomicrographs of fetal monkey gastric cryosection (a) and an adult mouse skin section (b) treated with VEGFR-3 staining. The VEGFR-3-expressing lymphatic-like structures (L) are seen in the submucosa (a) and in the subcutaneous tissue (b). Hf: hair follicle x 100, x 400

widely expressed to the lymphatic endothelium at later developmental stages and in postnatal life.[20] Many circular and incomplete lymphatic-like structures expressing VEGFR-3 show an obvious accumulation, indicating that lymphangiogenesis occurs sequentially in definite regions in the early embryonic stage.

LYMPHATIC REGENERATION

Wound healing skin

Wound healing skins in mice were processed for 5′-Nase and VEGFR-3 histochemical staining to distinguish lymphatics from blood capillaries and analyze lymphangiogenesis.[3] In the wound skin of the mice 3 to 5 days after injury, anti-VEGFR-3 immunopositive signals unevenly appeared in 5′-Nase-positive lymphatic vessels in the subcutaneous tissue. On days 7 to 15, numerous accumulated vasculatures were stained for 5′-Nase and PECAM-1/CD31, extending irregularly along the wound edge. Ultrastructural changes in lymphatic vessels developed at different stages, from lymphatic-like structures to newly-formed lymphatic vessels with an extremely thin and indented wall. The generating signals of VEGFR-3 on lymphatic endothelial cells appeared as early as 3 days after injury in the subcutaneous tissue, much earlier than in the dermis. The expression pattern of VEGFR-3 in regenerating tissues was extremely uneven on the lymphatic wall, indicating that endothelial sprouting might begin from its up-expressing side. The most noteworthy finding was that numerous circular and irregular lymphatic-like structures with VEGFR-3 expression were distributed in the dermal and subcutaneous tissues along the wound edge *(Figure 4b)*. According to the maturation of lymphatic vessels, the lymphatic wall became slender and irregular, and the endothelium protruded into the lumen and adjacent connective tissue. Intercellular junctions underwent morphological changes, from simple end-to-end to overlapping and interdigitating. The simple junction might facilitate separation, spreading, and migration of endothelial cells during lymphatic remodeling in compliance with tissue repair patterns. This finding appeared to be in good agreement with our previous observations in the early embryonic tissues of the monkey.[18] However, 5′-Nase activity in the endothelial cells of newly-formed lymphatic vasculature is low during the wound healing process.
Combined histochemical staining for 5′-Nase and VEGFR-3 with multiple endothelial cell markers is useful for studying regenerating lymphatics and their relationship with blood vessels. VEGFR-3–expressing vasculatures occurred in the dermal-subcutaneous transitional area at the early stage of wound injury, whereas a 5′-Nase-positive lymphatic structure along the wound edge underwent morphological changes. These findings indicated that sprouting and growth of regenerating lymphatic vessels are active processes in the healing tissue response.

Regrowing an intestinal muscle coat after myectomy

The lymphatic regrowth from the surviving vessels in the severed stumps of the intestine occurred behind the regeneration of other tissue elements[21] including blood vessels. The vascular arcades and terminal expansions, which were observed in the present lymphatic regeneration, are presumed to serve as growth points in lymphangiogenesis, as in angiogenesis.[22] The unusual ultrastructural characteristics of the regrowing lymphatic endothelial cells, involving spindle-shaped and elongated cytoplasm, filopodium-like cytoplasmic projections and numerous intracellular thin filaments, probably indicate the high regenerative and migratory potential of the cells to establish new vascular channels.

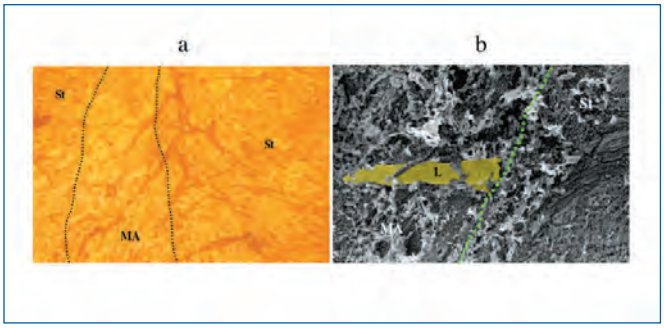

Figure 5. Light micrograph (a) and SEM view (b) of regrowing lymphatics from a postmyectomized week-4 rat jejunum with 5′-Nase staining. MA: myectomized area, St: stump. The broken line indicates the cut line of the muscle coat. a. Regrowing lymphatics (arrows) with 5′-Nase activity show thickening and varicose structures. b. Secondary emission image of regrowing lymphatics arising from a vascular arcade in the lesion. 5′-Nase-positive lymphatic endothelial cells are colored yellow. x 800

The enzyme histochemistry for 5′-Nase demonstrated the manner of lymphatic regrowth, which was established by vascular sprouting from preexisting lymphatics and structural changes in the endothelial cells indicating their high migratory potential *(Figures 5 a,b)*. The expression of 5′-Nase in the regenerating lymphatics was increased in proportion to their growth. These findings suggest that 5′-Nase may be correlated with the functional maturation of the regenerating lymphatics, because it is thought to facilitate membrane transport of lymph.[12]

VEGF-C was expressed in a subpopulation of regenerating interstitial cells which are close to the regrowing 5′-Nase-positive lymphatics. Furthermore, the cells with VRGF-C transcripts showed a marked increase in cell number and intensity of mRNA signals during progression of tissue regeneration. These findings imply that VEGF-C is upregulated upon lymphatic regrowth in the repairing intestinal tissue following myectomy. The VEGFR-3 immunoreactivity was preferentially distributed in the 5′-Nase-positive regrowing lymphatics in the lesion and restricted on the cell membranes of lymphatic endothelial cells. Thus, the VEGFR-3 immunoreactivity demonstrated in this study is considered to be expressed in the regenerating lymphatics, although the presence of VEGFR-3 in the blood vasculature has been reported in cutaneous wound healing.[23] The expression of 5′-Nase in the regenerating lymphatics was increased in proportion to their growth, VEGF-C, a highly specific lymphangiogenic factor, was highly expressed in a subpopulation of interstitial cells, being close to the regrowing lymphatics with the immunoreactivity of its receptor, VEGFR-3, in the regenerative area. The present findings suggest that transaction of the intestinal muscle coat affords a useful experimental model for the investigation of lymphatic regeneration in tissue repair, and that the interstitium may play a crucial role in lymphangiogenesis.

With regard to adhesion, differentiation, and migration of endothelial cells, some elements, including 5′-Nase and eNOS,[15] the extracellular matrix of the basement membrane, and components of the surrounding connective tissues, are very important factors in the formation of developing vasculature. More recently, several other proposed markers for lymphatic endothelial cells, eg, LYVE-1, a homologue of the CD44 hyaluron receptor,[24,25] podoplanin,[26,27] and Prox 1[28,29] have emerged, but there are still questions about their reliability and specificity, and they need further study. However, coexpression of these new markers with so-called routine differentiating reagents, such as laminin, collagen type IV, and α-smooth muscle actin, is definitely helpful in analyzing the functional-structural properties of endothelial cells of both lymphatic and blood vessels.

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REFERENCES

1. Kato S. Lymphatic endothelial cell markers and lymphangiogenesis. *Jpn J Lymphol*. 2003;26:1-20.
2. Shimoda H, Takahashi Y, Kato S. Regrowth of lymphatic vessels following transaction of the muscle coat in the rat small intestine. *Cell Tissue Res*. 2004;316:325-338.
3. Ji RC, Miura M, Qu P, Kato S. Expression of VEGFR-3 and 5′-Nase in regenerating lymphatic vessels of the cutaneous wound healing. *Microvasc Res Tech*. 2004;64:279-286.
4. Kato S. Enzyme-histochemical identification of lymphatic vessels by light and backscattered image scanning electron microscopy. *Stain Technol*. 1991;65:131-137.
5. Kato S. Organ specificity of the structural organization and fine distribution of lymphatic capillary networks: histochemical study. *Histol Histopathol*. 2000;15:185-197.
6. Kato S. Morphological characteristics of the lymphatics: enzyme-histochemical demonstration. *Phlebolymphology*. 2000;27:17-22.
7. Kato S, Itonaga I, Ji RC, Miura M. Enzyme triple staining for differentiation of lymphatics from venous and arterial capillaries. *Lymphology*. 1996;29:15-19.
8. Kato S, Miura M, Miyauchi R. Structural organization of the initial lymphatics in the monkey mesentery and intestinal wall as revealed by an enzyme-histochemical method. *Arch Histol Cytol*. 1993;56:149-160.
9. Kato S, Gotoh M. Application of backscattered electron imaging to enzyme histochemistry of lymphatic capillaries. *J Electron Microsc*. 1990;39:186-190.
10. Shimoda H, Kajiwara T, Takahashi Y, et al. Scanning electron microscopic observation of immunohistochemically specified stromal cells following the NaOH maceration method. *J Electron Microsc*. 2002;512:137-139.
11. Ji RC, Qu P, Kato S. Application of a new 5′-Nase monoclonal antibody specific for lymphatic endothelial cells. *Lab Invest*. 2003;83:1681-1683.
12. Shimoda H, Takahashi Y, Kajiwara T, Kato S. Demonstration of the rat lymphatic vessels using immunohistochemistry and in situ hybridization for 5′-nucleotidase. *Biomed Res*. 2003;24:51-57.
13. Casley-Smith JR, Clodius L, Foldi-Boresok E, Gruntzig J, Foldi M. The effects of chronic cervical lymphostasis on regions drained by lymphatics and by prelymphatics. *J Pathol*. 1978;124:13-17.
14. Olszewski WL. The treatment of lymphedema of the extremities with microsurgical lymph-venous anastomoses. *Int Angiol*. 1988;7:312-321.
15. Ji RC, Kato S. Histochemical analysis of lymphatic endothelial cells in lymphostasis. *Microsc Res Technique*. 2001;55:70-80.
16. Piller BB, Clodius L. Experimental lymphoedema: its applicability and contribution to our clinical understanding. In: Johnston MG, eds. *Experimental Biology of the Lymphatic Circulation*. Amsterdam: Elsevier; 1985:189-230.
17. Ji RC, Kato S. Enzyme-histochemical study on postnatal development of rat stomach lymphatic vessels. *Microvasc Res*. 1997;54:1-12.
18. Ji RC, Kato S. Lymphatic network and lymphangiogenesis in the gastric wall. *J Histochem Cytochem*. 2003;51:331-338.
19. Witmer AN, van Blijswijk BC, Dai J, et al. VEGFR-3 in adult angiogenesis. *J Pathol*. 2001;195:490-497.
20. Wilting J, Neeff H, Christ B. Embryonic lymphangiogenesis. *Cell Tissue Res*. 1999;297:1-11.
21. Takahashi Y, Shimoda H, Kato S, Noguchi T, Uchida Y. Immunohistochemical study on myenteric nerves following transaction of the muscle coat in the rat small intestine, with special reference to glial cells line-derived neurotrophic factor(GDNF) and its receptor (Ret). *Biomed Res*. 2002;23:73-83.
22. Rhodin JAG, Fujita H. Capillary growth in the mesentery of normal young rats. Intravital video and electron microscope analyses. *J Submicrosc Cytol Pathol*. 1989;21:1-34.
23. Paavonen K, Puolakkainen P, Jussila L et al. Vascular endothelial growth factor receptor-3 in lymphangiogenesis in wound healing. *Am J Pathol*. 2000;156:1499-1504.
24. Banerji S,Ni J,Wang SX et al. LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. *J Cell Biol*. 1999;1244:789-801.
25. Jackson DG, Orevo R. Clasper S, Banerji S. LYVE-1, the lymphatic system and tumor lymphangiogenesis. *Trends Immunol*. 2001;22:317-321.
26. Breiteneder-Geleff S, Soleiman A, Kowalski H, et al. Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium. *Am J Pathol*. 1999;154:358-394.
27. Weninger W, Partanen TA, Breiteneder-Geleff S, et al. Expression of vascular endothelial growth factor receptor-3 and podoplanin suggests a lymphatic endothelial cell origin of Kaposi's sarcoma tumor cells. *Lab Invest*. 1999;79:243-251.
28. Wigle JT, Harvey N, Detmar M. et al. An essential role for Prox 1 in the induction of the lymphatic endothelial cell phenotype. *EMBO J*. 2002;21:1501-1513.
29. Hong YK, Harvey N, Noh YH et al. Prox 1 is a master control gene in the program specifying lymphatic endothelial cell fate. *Dev Dyn*. 2002;225:351-357.

Congress and conference calendar

SOCIETE FRANCAISE D'ANGEIOLOGIE (SFA)

This congress will be held in Paris (France) from March 18 to 19, 2005.

• *For further information, please contact:*

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VIth EUROPEAN AMERICAN CONGRESS ON VENOUS DISEASES XXXth ANNUAL CONGRESS OF THE CZECH SOCIETY OF PHLEBOLOGY

This congress will be held in in Prague (Czech Republic) from May 26 to 28, 2005.

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This congress will be held in Heraklion, Crete (Greece) from June 24 to 26, 2005.

• *For further information, please contact:*

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XLVIIth ANNUAL MEETING OF THE GERMAN SOCIETY OF PHLEBOLOGY

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E-mail: mail@phlebologie2005.de

XVth WORLD CONGRESS OF THE UNION INTERNATIONALE DE PHLEBOLOGIE

This congress will be held in Rio de Janeiro (Brazil) from October 2 to 7, 2005.

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