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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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PHLEBOLYMPHOLOGY

EDITORIAL

Chronic venous disease: chronic venous disorder or chronic venous insufficiency?

This issue of Phlebolymphology *contains some interesting articles, all dealing with chronic venous disturbances, each of them using different terminology.*

***Michel Perrin's** paper reports on the results of reconstructive surgery for the elimination of deep venous refluxes. There is no question, that these procedures are directed towards normalization of insufficient venous pumping function. The author states that "refluxes related to deep venous insufficiency" are corrected. In this functional context the expression of chronic venous insufficiency (CVI) is certainly justified.*
It is also made clear that the clinical signs of potential candidates for deep venous valve repair correspond to edema (CEAP: C3), skin changes (C4), and ulceration (C5 and C6), and not to simple varicose veins.
This is exactly the definition of how the term CVI was also understood by Leo Widmer, who differentiated three stages of CVI:
Stage I: edema (and corona phlebectatica paraplantaris), stage II: skin changes, and stage III: open or healed venous ulcer. Thus, the functional term "CVI" describing an abnormality of the venous pump, was used for the clinical description of severe stages of venous disease. Spider veins (C1) should certainly not be classified as CVI, and this is also true for varicose veins (C2).
*As **Tomasz Urbanek**, winner of the Servier-UIP award, correctly states in his basic research article, the "valve failure theory to explain the development of varicose veins is no longer satisfactory." Refluxes are rather the consequence than the primary cause of varicose veins. This also endorses the concept that varicose veins should not be classified as CVI.*

*The ischemia/reperfusion experiments of **Eliete Bouselka** and coworkers present an excellent model on some of the consequences of severe CVI on the microcirculation of humans. Translating the findings from the animal experiments to human pathology, beneficial pharmacological effects may be expected also in severe stages of CVI.*
Even when the disturbed pumping function is not restored, there is some hope that the consequences of chronic venous insufficiency (edema, skin changes, and ulceration) can be improved by the administration of venoactive drugs.

*In the first part of their Consensus papers entitled "Effects of venoactive agents on the symptoms of chronic venous disease" **Claude Garde** and coworkers concentrate on subjective symptoms in patients with mild venous pathology. In the chapter on "Review of epidemiology" it is stated that over 18 million adults in France – 57% of women and 26% of men – present with venous disease.*
Is it really true that more than half of French females suffer from chronic venous disease (CVD) or is there confusion due to the translation from French (maladie) into English (disease)? Shouldn't we rather speak about "chronic venous disorder (another "CVD"), at least in the majority of cases?

Is this just a game of words?
There could be a difference: a disease always needs to be treated, while this is not necessarily the case for a disorder. In this case, therapy can be administered but does not need to be. Of course this definition may be discussed.
When a patient consults the doctor because of chronic pain in the legs the doctor will probably react even if no venous pathology can be recognized. Is the prescription of a medicine by a doctor enough to define the condition as a "disease"?
These certainly are unsolved questions and hot topics, especially when it comes to reimbursement.

*The second part of the consensus document from **Claude Garde** et al deals with edema that would correspond to Widmer's stage I of CVI. A patient with chronic edema needs therapy so that the term "chronic venous disease" may be justified.*

I would propose that the international phlebological nomenclature in English texts should follow the last consensus statement of the CEAP group[1] which uses the term chronic venous disorders (CVD). This expression encompasses the broad spectrum of venous problems that can be observed in daily practice, including spider veins, and varicose veins with and without symptoms, but also the severe stages of CVI, which in fact are to be considered as a chronic disease needing sustained treatment.

Dr Hugo Partsch

[1] Eklöf B, Rutherford RB, Bergan JJ, et al. Revision of the CEAP classification for chronic venous disorders: consensus statement. *J Vasc Surg.* 2004;40:1248-1252.

Deep venous reconstructive surgery to treat reflux in the lower extremities

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Keywords:
deep venous reflux - deep venous reconstructive surgery - valvuloplasty - valve transfer - venous surgery

INTRODUCTION

The development of deep venous reconstructive surgery (DVRS) for reflux in the lower extremities has been far more limited than arterial reconstructive surgery. This discrepancy may be explained by a variety of factors:
- The importance of deep venous reflux (DVR) has been identified as a major pathophysiological factor only in the last 20 years.
- Indications for surgery are uncommon.
- The efficacy of surgical repair of the deep veins remains controversial.
Furthermore, many questions remain partially answered or unanswered. Is there a correlation between clinical and hemodynamic results? When superficial venous insufficiency (SVI) and/or perforator insufficiency (Pe I) are associated with DVR, should they be treated as a first step or in combination with correction of DVR?

ETIOLOGY

It must be kept in mind that DVR has different etiologies. The most common is secondary DVR, ie, post-thrombotic syndrome (PTS), followed by primary DVR. Congenital venous malformation may be identified in some cases.

Pathology and pathophysiology

PTS is the end result of thrombosis and the subsequent inflammation of the valve cusps and vein wall during the process of recanalization. This leads to scarring and shortening of the cusps.[1] Failure of the cusps to achieve normal coaptation results in reflux. Reflux secondary to the PTS is usually not amenable to direct surgical repair of the now-destroyed valve.
Primary reflux is the result of structural abnormalities in the vein wall and valve itself. Although the precise etiology has not been characterized, it is presumed to be congenital in origin.[2] Redundant, malopposed cusps and venous dilation permit valve prolapse and reflux. Unlike the PTS, there is no evidence of previous thrombosis or inflammation near the valve.[3]
A rare cause of congenital reflux is the complete absence of valves secondary to agenesis.

TECHNIQUES

Surgical techniques for treating DVR can be classified into two groups: those that do involve phlebotomy and those that do not involve phlebotomy.

Techniques with phlebotomy

- Internal valvuloplasty: since the first procedure using a longitudinal phlebotomy *(Figure 1)* described by Kistner in 1968[4] various procedures have been proposed.
- Venous segment transfer: *(Figure 2)* The purpose of venous segment transfer is to transpose the incompetent axial deep venous system, ie, the femoropopliteal axis into a competent valve-bearing system, namely the great saphenous vein or the deep femoral vein at the groin level. Devised again first by Kistner[5] several surgical variations of venous segment transfer have been employed. Unfortunately this technique can be used in only approximately 20% of the PTS, as there is no competent valve into the great saphenous vein or the deep femoral vein valve in their proximal segment in 80%.
- Vein valve transplantation: Taheri[6] deserves the credit for introducing the use of vein valve transplant *(Figure 3)*. A 2 cm to 3 cm segment of axillary vein is inserted as an interposition graft at the termination of the (superficial) femoral vein just below the junction of the deep femoral vein with the (superficial) femoral vein. O'Donnel[7] initiated transplantation to the above-knee popliteal vein as the gatekeeper role of the popliteal vein had been underlined.[8]

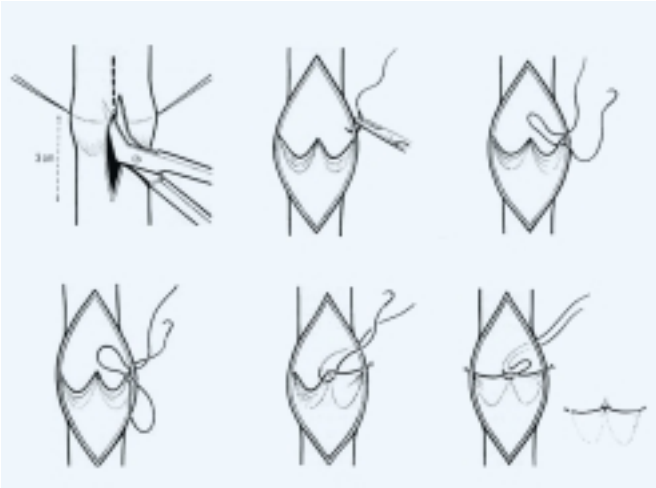


Figure 1.
Internal valvuloplasty according to Kistner. Using a longitudinal phlebotomy each valve is repaired by interrupting a series of sutures placed at the commissures. Each suture progressively shortens the leading edge of the cusp.

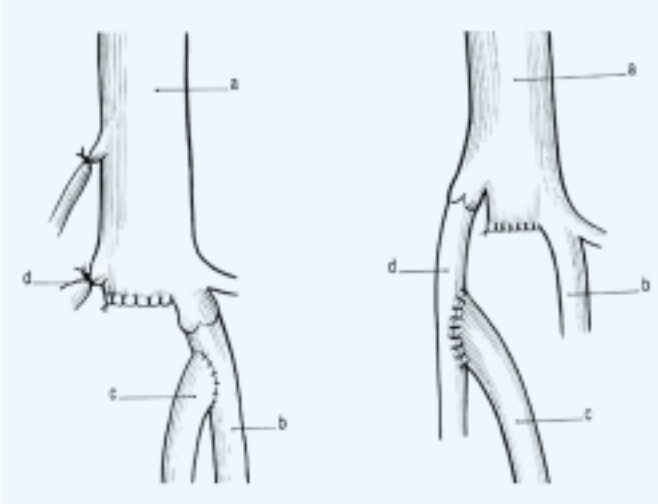


Figure 2. Venous segment transfer:
The refluxive (superficial) femoral vein (FV) may be transposed onto the great saphenous vein (GSV) or the profunda femoral vein (PFV) provided they have a competent proximal valve above the transposition.
a, common femoral vein; b, profunda femoral vein; c, (superficial) femoral vein; d, great saphenous vein.

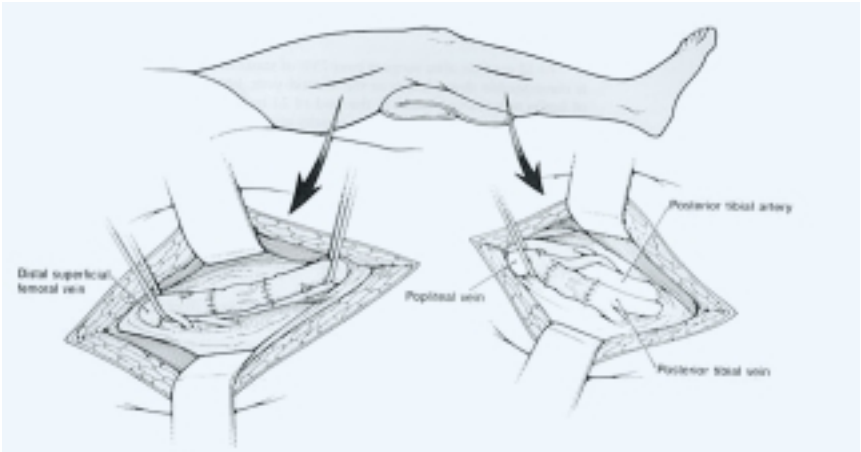


Figure 3. Above-knee or below-knee axillary vein-to-popliteal vein transplantation end-to-end anastomosis.

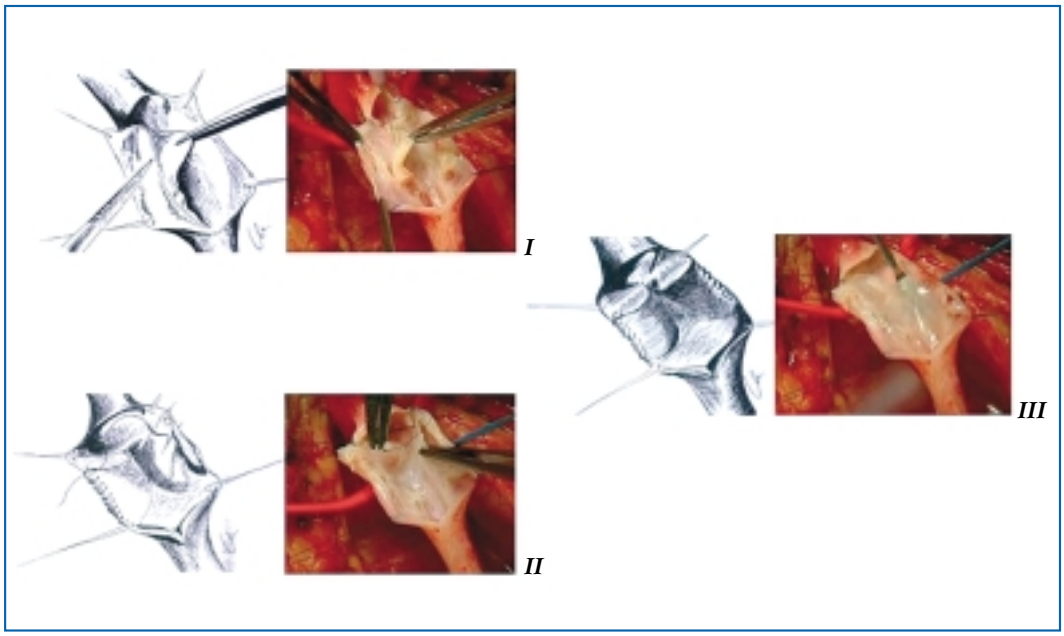


Figure 4. Neovalve according to Maleti.
***I** After a longitudinal phlebotomy a blade is used to perform a parietal dissection to create a neovalve which is derived from the union of the two dissection lines, transversal and longitudinal.*
***II** The second valve created is then fixed to the vein wall (bicuspid valve).*
***III** Bicuspid neovalve.*

- Neovalve: Various techniques for creating a neovalve have been developed. Plagnol[9] used the termination of the great saphenous vein to construct a neobicuspid valve by invagination. Maleti[10] created a valvular cusp by dissection of the femoral venous wall to obtain a single or a bicuspid valve *(Figure 4)*. Both these techniques have been used in PTS.

Techniques without phlebotomy

- Wrapping, banding, cuffing, and external stenting *(Figure 5)* were developed initially by Jessup and Lane[11] for saphenous vein incompetence, and later for primary deep vein incompetence.[12]

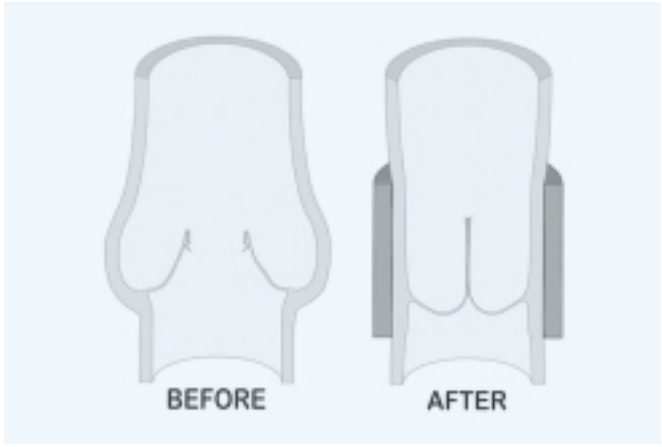


Figure 5. External stenting.
Schematics of incompetent venous valve demonstrating "floppy" free edge border (BEFORE) and competent valve following external stenting (AFTER).

- External valvuloplasty: The first step consists in advential dissection until the valve insertion lines are clearly identified as an inverted V. The commissural angle is normally acute and widened in the refluxive valve.

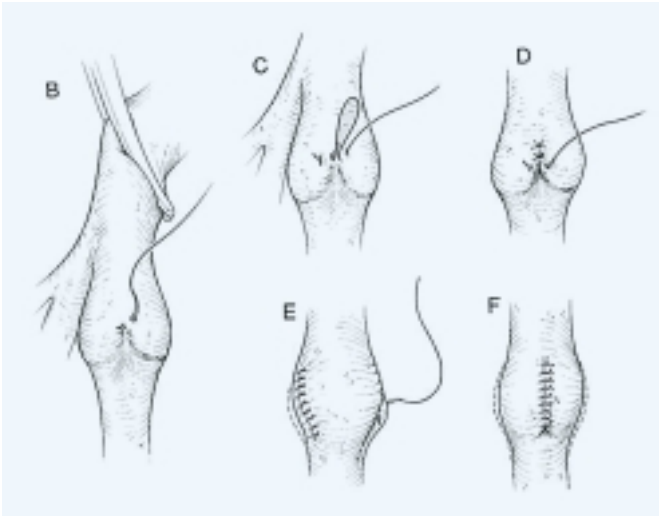


Figure 6. External transmural valvuloplasty.
The placating interrupted sutures are placed from outside the lumen to tighten the commissural angle until the valve becomes competent.

Transmural valvuloplasty: Kistner introduced external valvuloplasty *(Figure 6)* in 1990.[13] He placed an external row of sutures along the diverging margins of the valve cusp in the vein wall. The interrupted sutures are carried inferiorly along both commisures until the valve becomes competent by strip-testing. Transcommissural

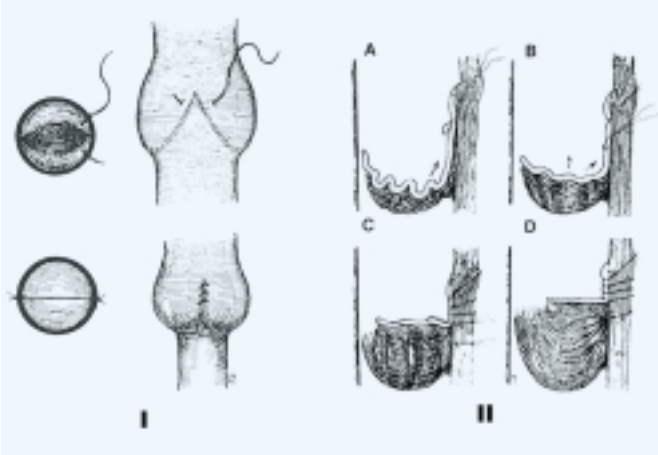


Figure 7. External transcommisural valvuloplasty.
I Correct suture placement narrows the angle between valve attachment lines and tightens cusps, resulting in good apposition.
II Transluminal sutures with each successive suture biting deeper and less obliquely than the suture above to pull up the valve, tighten the cusp edge, deepen the sinus and appose valve attachment lines. Each suture is tied up before the next is placed.

valvuloplasty was promoted by Raju.[14] Transcommissural valvuloplasty *(Figure 7)* differs from the transmural valvuloplasty described previously, as a transluminal suture is carried out. "A through-and-through transluminal resuspension suture (7-0 Prolene) is placed obliquely across the inserted commissural V, traversing the valve cusps blindly near their wall attachment to pull them up."
- Angioscopy-assisted external valve repair:[15,16] An angioscope is introduced through a saphenous side branch and advanced into the proximal (superficial) femoral vein and positioned directly above the valve. Prolene sutures are passed from outside to inside the lumen directed by video-enhanced, magnified angioscopic visualization allowing for precise approximation of the valve cusps.
- Percutaneously placed device: the Portland valve[17] consisting of a square stent and porcine small intestinal submucosa covering has initiated a clinical phase I study.

CLINICAL ASPECTS

Though there exist some clinical features that enable to distinguish superficial venous insufficiency from deep insufficiency, they are not reliable, particularly considering the fact that deep venous insufficiency is frequently combined with superficial insufficiency. In addition, primary deep vein reflux is difficult to distinguish from secondary deep vein reflux.
It is generally admitted that when deep venous reflux exists the chronic venous disease is more severe.

INVESTIGATIONS

- *Duplex scanning* (DS) provides both hemodynamic and anatomic information. Assessment of reflux is carried out using manual calf compression or inflating-deflating distal cuff and duration of reflux (normal less than 0.5 seconds) is measured at the femoral, popliteal, and tibial levels for the deep system and at similar levels for the superficial system.[18,19] Perforators are also investigated. DS allows discrimination of primary reflux from PTS and is also helpful for planning DVRS. Presence or absence of competent proximal valves at the termination of the GVS or the profunda femoris vein may allow consideration of venous segment transfer. DS of the axillary vein and brachial veins segments determines whether there is a segment containing a functioning valve, which can be used for transplantation.

- *Photoplethysmography,* with and without inflatable cuff compression of the superficial venous system, can help when superficial and deep venous refluxes are combined to identify the predominant pathophysiological components by measuring venous return time with and without compression. VRT is considered normal above 20 s and frankly severe when less than 12 s. However, the value of this investigation is debated.
It would seem logical to go beyond the two investigations described above only in those patients in whom DVRS may be considered. That means that continuing investigations are dominated by the clinical context and absence of contraindication to DVRS:
- uncorrectable coagulation disorder: antithrombin III or C and S deficiency, etc.
- ineffective calf pump: frozen or stiff ankle after physiotherapy, permanent muscular deficit of the triceps surae.

- *Volume plethysmography*. Air plethysmography[20] is one of the most useful currently available methods, identifying the various pathophysiological mechanisms involved. It enables the measurement of venous filling time, ejection fraction, and residual volume after dynamic test.

- *Pressure measurements*. The ambulatory venous pressure (AVP) measurement is supposed to be the "gold standard" for evaluating the global hemodynamic situation, providing pressure figures both before and after exercise and VRT. Improvement in VRT using a tourniquet was assumed to indicate isolated great or small saphenous vein incompetence[21] but this point has been questioned.[22]

- *Venography* remains essential when DVRS is considered, as it provides the necessary information accurately.

Ascending phlebography provides both anatomic and etiologic information but very few hemodynamics.

Descending phlebography is performed by puncturing the ipsilateral common femoral vein, or better, the contralateral one or the brachial vein. It is strongly recommended to videotape the procedure and obtain spot films during the course of the examination. The Valsalva maneuver is essential to the performance of accurate descending phlebography, and it can be standardized. Reflux grading is according to the system previously reported and scored from 0 to 4[23] *(Figure 8)*. Descending phlebography also gives precise information on the morphology of the valve and allows to determine whether a valve is reparable or not by valvuloplasty *(Figures 9, 10)*.

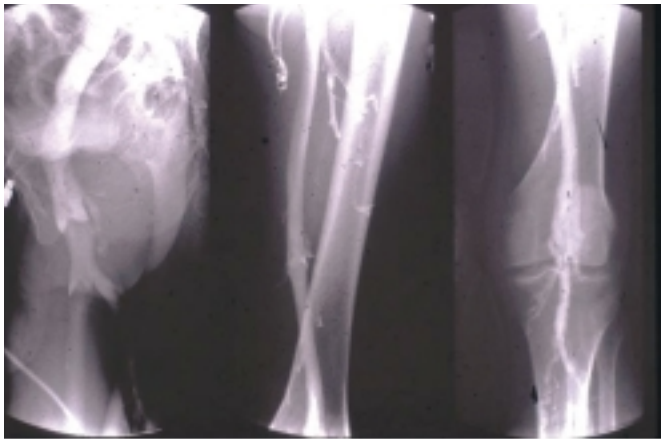


Figure 8. Descending phlebography.
Primary deep vein insufficiency. Reflux grade 4. Cascading reflux to the calf.

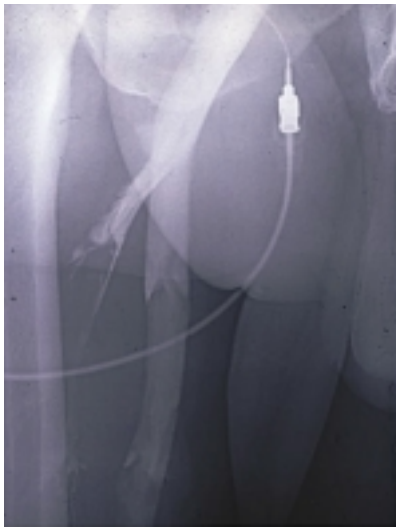


Figure 9. Descending phlebography.
Primary deep vein insufficiency.
The highest valve of the (superficial) vein is refluxive but obviously reparable by valvuloplasty.

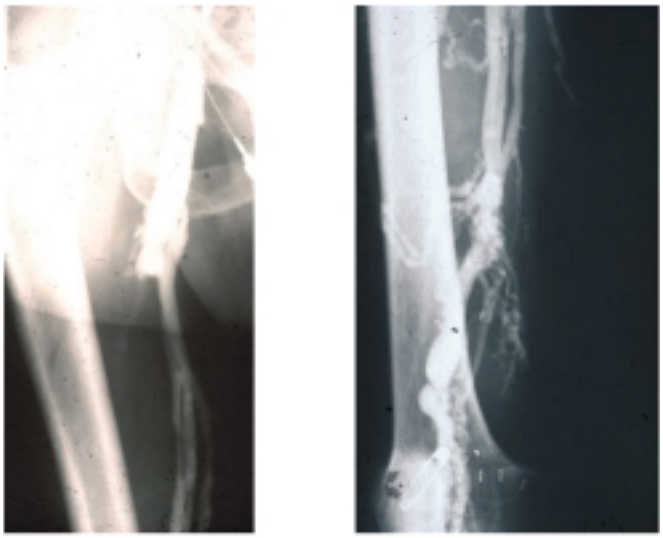


Figure 10. Descending phlebography.
Post-thrombotic syndrome. Reflux grade 4 (Calf not seen). No valve is reparable.

The objective of surgery for treating deep venous reflux

The goal of DVRS is to correct the reflux related to deep venous insufficiency at a subinguinal level. This reflux leads to a permanent increase in venous pressure, unaffected by the activity of the calf venomuscular pump. However, it must be kept in mind that DVR is frequently combined with superficial and perforator reflux; consequently all these mechanisms have to be corrected in order to reduce the permanent increased venous pressure.

RESULTS

DVRS results are somewhat difficult to assess as superficial venous surgery and/or perforator surgery have often been performed in combination with DVRS.

In primary deep reflux the most frequent procedure used is valvuloplasty. Results are summarized in *Table I.*[14,24,26-28] On the whole, valvuloplasty is credited with achieving a good result in 70% of cases in terms of clinical outcome, defined as freedom, ulcer recurrence from the reduction of pain, valve competence, and hemodynamic improvement over a follow-up period of more than 5 years. In all series, a good correlation was observed between these three criteria. External transmural valvuloplasty does not seem to be as reliable as internal valvuloplasty in providing long-term valve competence or ulcer-free survival.[25] Other procedures used in primary deep vein reflux including angioscopic repair[15,16] and wrapping are more difficult

to assess as they have a shorter follow-up, except in the series reported by Lane[12] *(Table II)*.[12,25,29,30]

In PTS long-term results are available for transposition *(Table III)*[24,26,28,31,32] and transplantation *(Table IV)*.[6,26,28,33-36]

In terms of clinical result and valve competence, a meta-analysis demonstrates that a good result is achieved in 50% of cases over a follow-up period of more than 5 years, with a poor correlation between clinical and hemodynamic outcome.

Authors	Surgical Technique	No of limbs (No of valves repaired)	Etiology PVI/total	Follow-up months (mean)	Clinical result Ulcer recurrence or non-healed ulcer (%)	Hemodynamic results	
						Competent valve (%)	□ AVP ■ VRT
KISTNER (24)	I	32	/	48-252 (127)	(28)	24/31 (77)*	□ ↗ 81% (m) ■ ↗ 56% (m)
RAJU (25)	I	68 (71)	/	12-144	16/68 (26)	30/71 (42)	/
RAJU (25)	TMEV	47 (111)	/	12-70	14/47 (30)	72/111	/
RAJU (14)	TCEV	141(179)	98/141	1-42	(37)	(59)	□ ↗ 15% (m) ■ Normalized 100%
SOTTIURAI (26)	I	143	/	9-168 (81)	9/42 (21)	107/143 (75)	/
ERIKSSON (27)	I	27	27/27	(49)	/	19/27 (70)	□ ↗ 81% (m) ■ ↗ 50% (m)
PERRIN (28)	I	85 (94)	65/85	12-96 (58)	10/35 (29)	64/83 (77)	■ Normalized 63% (m)

Abbreviations: PVI = Primary venous insufficiency; □ AVP = Ambulatory venous pressure; I = Internal valvuloplasty; ■ VRT = Venous refill time
TMEV = Transmural external valvuloplasty; ↗ = Improved; TCEV = Transcommissural external valvuloplasty; m = Mean; * = No reflux or moderate reflux (less 1 s)

Table I. Valvuloplasty results.

Authors Material used	No of extremities treated (No of valves repaired)	Site	Etiology PVI/Total	Follow-up Months (average)	Clinical result Non-healed or recurrent ulcer (%)	Hemodynamic results	
						Patent and competent valve (%)	□ AVP ■ VRT
AKESSON (29) Venocuff	20 (27)	F, P	7/20	5-32 (19)	2/10 (20%) both PTS	**PVI** 7/7 (100) **PTS** 7/10 (70)	**PVI** □ ↗ 10% (av) ■ ↗ 10% (av) **PTS** □ ↗ 10% (av) ■ ↗ 100% (av)
CAMILLI (30) Dacron sleeve	54	F	54/54	4-63		41/54 (76)	/
LANE (12) Venocuff II	42 (125)	F, P	36/42	64-141 (93)	(20)	(90)	□ ↗ ? ■ ↗ 100% (av)
RAJU (25) Dacron sleeve	? (96)	F, P, T	/	12-134	6/22 (27)	60/72 (83%)	/

Abbreviations: PVI = Primary venous insufficiency; □ AVP = Ambulatory venous pressure; ■ VRT = Venous refill time; ↗ = Improved; (av) = Average

Table II. Banding, cuffing, external stent, wrapping results.

Authors	No of extremities treated	Etiology PTS/total	Follow-up months (average)	Clinical result Non-healed or recurrent ulcer (%)	Hemodynamic results	
					Patent and competent valve (%)	☐ AVP ■ VRT
CARDON (31)	18	18/18	24/120	4/9 **(44)**	12/16 **(75)**	/
XJOHNSON (32)	16	16/16	12	4/12 **(33)**	3/12 **(25)**	☐ Unchanged ■ Unchanged
KISTNER (24)	14	/	48-252	7/14 **(50)**	10/13 **(77)**	☐ ↗ 70% (av) ■ ↗ 70% (av)
PERRIN (28)	18	16/18	12-168	2/8 **(25)**	9/17 **(53)**	/
SOTTIURAI (26)	16	/	9-149	9/16 **(54)**	8/20 **(40)**	/

Abbreviations: PTS = Post-thrombotic syndrome; AVP = Ambulatory venous pressure; VRT = Venous refill time; ↗ = Improved; (av) = Average
Transposition as isolated surgical procedure.

Table III. Transposition results.

Authors	No of extremities treated	Site	Etiology PTS/Total	Follow-up months (average)	Clinical result Non-healed or recurrent ulcer (%)	Hemodynamic results	
						Patent and competent valve (%)	☐ AVP ■ VRT
ERIKSSON (27)	35	F, P	35/35	6-60	/	11/35 **(31)**	■ Unchanged
MACKIEWICZ (33)	18	F	/	43-69	5/14 **(36)**	/	■ Improved?
NASH (34)	25	P	25/25	/	3/17 **(18)**	18/23 **(77)**	☐ ↗ 18% (av)
BRY (35)	15	P	/	15-132	3/14 **(21)**	7/8 **(87)**	☐ Unchanged ■ Unchanged
PERRIN (28)	32	F	31/32	12-124 **(66)**	9/22 **(41)**	8/32 **(25)**	■ ↗ 19% (av)
RAJU (36)	83	X F, P, T	83/83	12-180	**(40)** 6 yrs	**(38)** 4 yrs	☐ Unchanged
RAJU (36)	82	/	77/82?	12-180	**(25)** 6yrs	/	/
SOTTIURAI (26)	18	F, P	/	7-144	6/9 **(67)**	6/18 **(33)**	/
TAHERI (6)	71	F, P	/	/	1/18 **(6)**	28/31 **(90)**	☐ ↗ 15% (av)

Abbreviations: PTS = Post-thrombotic syndrome; AVP = Ambulatory venous pressure; VRT = Venous refill time; ↗ = Improved; (av) = Average
Axillary vein transfer in trabeculated (poorly recanalized) vein.

Table IV. Transplantation results.

Neovalve according to Plagnol[9] and to Maleti[10] had given interesting results but their follow-up is short.

Cryopreserved valve results were poor.[37,38]

INDICATIONS

DVRS indications for reflux rely on clinical severity, hemodynamics, and imaging.

▶ *Clinical severity*

Most of the authors recommend surgery in patients graded severe C4 and C 5-6. However, the results are better in primary reflux. DVRS must be considered in young and active patients reluctant to wear compression for all of their lifetime. When superficial and perforator reflux are associated they must be treated in association. For some authors as the first step, for others shortly before DVRS in the same hospitalization stay. Contraindications as previously stipulated have to be kept in mind.

▶ *Hemodynamics and imaging*

Only reflux graded 3-4 according to Kistner is usually treated with DVRS.[23]

It is generally recognized that, to be significantly abnormal, the values for VRT must be less than 12 s, and the difference between pressures at rest and after standardized exercise in the standing position must be less than 40%. The decision to operate should be based on the clinical status of the patient, not the noninvasive data, since the patient's symptoms and signs may no correlate with the laboratory findings.[39] In addition to meeting the clinical criteria, patients selected for surgery should be highly motivated to participate in their recovery since ultimate success is dependent on their compliance with postoperative management.

▶ *Indications according to etiology*

The indications for surgery can be simplified according to the clinical, hemodynamic, and imaging criteria described above.

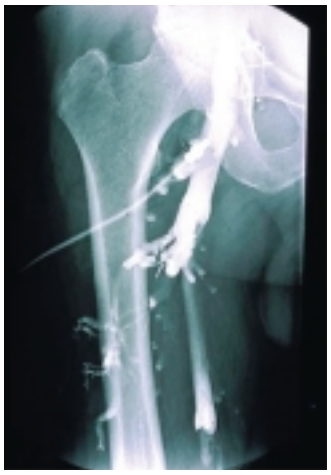
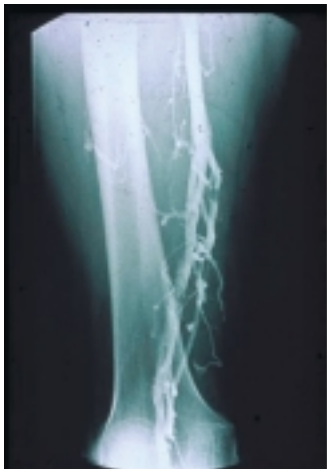


Figure 11. Descending phlebography. The common femoral vein and the upper half of the (superficial) femoral vein are refluxive and their appearance is in favor of primary reflux. Valvuloplasty seems feasible.



However, the lower half of the (superficial) femoral and popliteal veins are typically post-thrombotic.

In primary reflux reconstructive surgery is recommended after failure of conservative treatment and in young and active patients reluctant to wear permanent compression. Valvuloplasty is the most suitable technique, with Kistner, Perrin, and Sottiurai favoring internal valvuloplasty[24,26,28] and Raju transcommissural external valvuloplasty.[14]

Secondary deep venous reflux (PTS) may be treated only after failure of conservative treatment. As the results achieved by subfascial endoscopic perforator surgery associated or not with superficial venous surgery are not convincing,[40] it is recommended that this procedure might be carried out in combination with deep reconstructive surgery. The techniques to be used, given that valvuloplasty is rarely feasible *(Figure 11)*, in order of recommendation, are: transposition, transplantation, neovalve, and cryopreserved allograft.

In PTS obstruction may be associated with reflux; most of the authors agree that when significant obstruction is localized above the inguinal ligament, obstruction must be treated first. Patients must be informed that in PTS surgery for reflux has a relatively high failure rate. Contraindications to reconstructive surgery that must be taken into account include inactivity, stiff ankle, and severe coagulopathy disorder.

CONCLUSIONS

As large randomized control trials comparing conservative treatment and DVRS for DVR would be difficult to conduct, we must rely on the outcome of present series treated by DVRS. Their analysis provides grade C recommendation. Better results are obtained in the treatment of primary reflux compared with secondary reflux. Such surgery is not however, often indicated, and the procedure must be performed in specialized and centers with highly trained staff.

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First Consensus Meeting on Venoactive Agents and Review of their Clinical Benefits

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Organizer of the One-Day Conference, La Baule, France

CONSENSUS COMMITTEE N°1
"EFFECTS OF VENOACTIVE AGENTS ON THE SYMPTOMS OF CHRONIC VENOUS DISEASE"

The First Meeting of Review and Consensus on Venoactive Agents was organized, not as an attempt to revive interest in this therapeutic class, but rather in an effort to map out an uncontested path in the improvement in symptoms of chronic venous disease (CVD).
The following paper describes the work of the first Committee Meeting.

FOREWORD

Our first priority was to specifically determine the title of our conference. For reasons of harmonization with international terminology, it appears desirable to use the word "Symptoms" instead of "Functional signs." For the same reasons, it is preferable to use the term "chronic venous disease" (CVD) instead of "venous disease" or "chronic venous insufficiency."
Thus, the title of the Committee is as follows**: "Effects of Venoactive Agents on the Symptoms of Chronic Venous Disease."**

OBJECTIVE

After observing flaws in clinical trials of venoactive agents, our objective has been to define measurable criteria that allow evaluation of efficacy of venoactive agents on the symptoms of CVD.
This objective had to be reached by answering four questions:
- what is the specific effect of venoactive agents, and how can this effect be quantified?
- which study protocols should be proposed to validate the action of venoactive agents?
- what are the pitfalls of these protocols?
- what other therapeutic alternatives exist, and what are their advantages and disadvantages?

Therefore, we sought to identify the symptoms that characterize CVD, and then we tried to determine which are the most specific and/or the most common among those, which appear quantifiable.

Once this first step had been completed, we had to determine the threshold from which a real benefit versus placebo or compared with other previously evaluated therapies exists for these agents.
Lastly, depending on the problems encountered at each step of this process, we suggested possible study protocols to validate these hypotheses.

REVIEW OF EPIDEMIOLOGY

Demographic data supplied in 1996 by the French Institute of Demography[1] showed that over 18 million adults in France – 57% of women, and 26% of men – present with venous disease. In terms of economic aspect, the total cost of venous diseases in 1989 was put at about 800 million euros, approximately one third of which is related to hospital stay.[2] Thus, these figures demonstrate the usefulness of good practice in therapy of venous disease, in terms of public health and socioeconomic impact.
And yet, the most obvious question arises: what is the objective of all therapy? The objective is twofold: first, to eliminate symptoms, and second, to cure the patient or to slow the progression of the disease.
A disorder may be expressed clinically, by changes in laboratory test data, or by pathophysiological abnormalities. For example, in diabetes, the abnormal biological parameter is hyperglycemia. Almost no clinical symptoms exist apart from lack of control of blood glucose or complications of this disease. Therefore, treatment consists of normalizing blood glucose. But is this all that needs to be done? Does normalizing blood glucose eliminate the risks associated with diabetes?
Regarding CVD, such measures involve eliminating the symptoms, and also preventing the occurrence of complications such as abnormal skin changes, and more rarely, thromboembolic events, and as far as possible, slowing down the progressive course of this disease. Does elimination of one or more varicose veins treat varicose patients?
In this perspective, schematically, four therapeutic modalities currently are available: compression therapy, venoactive agents, sclerotherapy, and surgery. Each of these methods is somewhat in competition with the others. Often they are complementary, having more or less the same aims. Therefore, for better medical practice it is useful to evaluate, validate, and determine which of these therapies best achieves the different objectives and, obviously, which one of them is the least complicated and the least costly to achieve the same results.

PRACTICE OF CLINICAL TRIALS

Clinical drug trials in general

Clinical drug trials are defined as all of the strategies, which aim to obtain validated information on medicinal products that can be extrapolated to the general case. Such strategies are based on good clinical methodology[3] and on the rules of Good Practice of Clinical Trials or GCP.[4] Indeed, before undertaking a clinical evaluation, it is essential to have good methodology under penalty of invalidating the results of the trial and reducing years of work to zero, with the economic repercussions this can have.[5]
When such work involves trials in human subjects, quality controls are very strict,[6] ethical considerations are mandatory.[7] Such trials must comply with the Declaration of Helsinki, a document described by the Canadian Medical Association as "the stone tablet of medical research ethics",[8] (a compulsory legal framework exists in France since the adoption of the Huriet-Serusclat Law of December 20, 1988). This legal framework is aimed at protecting all persons who are subjects in biomedical research, and systematically requires that informed consent be obtained and signed by the patient or healthy volunteer.

Based on these obligations, several types of trials are possible:
- *according to the Law*: this differentiates a trial with direct benefit, which makes it possible to observe improvement in a volunteer subject's condition; from a trial with no direct benefit, which enables advances to be made in knowledge of a disease;
- *according to chronology of development*: schematically, trials are divided into three phases:

• phase I trial: conducted in healthy volunteers, its objectives are:

- in the first phase, to evaluate the safety of the new drug (maximum, well-tolerated dose);
- to determine its pharmacokinetic and pharmacodynamic profile;

• phase II trial: corresponds to pilot drug trials conducted in patients for the following reasons:

- to determine the optimum dose (does-effect relationship);
- to evaluate short-term safety and efficacy.

• phase III trial: this involves large-scale clinical drug trials, conducted in comparison with a placebo (if this is ethically acceptable) and/or with a reference therapy (ies).

- Conditions of the investigation: generally, the location where a trial is conducted has little effect except in case of nonuniform distribution of the disorder involved. But, it may involve single-center or "multicenter" studies.

Taking these criteria into account, currently, for a trial to be validated, it is necessary for the trial to be conducted with double-blind design (if ethically acceptable), versus a control group (reference compound or placebo) with randomized allocation of treatments. Rigorous statistical analysis of results must be performed, taking into account protocol violations (eg, subjects lost to follow-up, subjects withdrawn from the trial, for whatever reasons, etc).

Clinical drug trials conducted in chronic venous disease

Histological, biological, and instrumental criteria

Thickness of the venous wall, blood concentrations of proteins of inflammation, the existence of hemorheological disorders, and venous growth factors can be measured in CVD with/without treatment.

Upstream of the symptom, algesiogenic substances, proteins of inflammation, are released, a finding identified by Remacle.[9] Therefore, it appears possible to measure the course of concentrations of these substances (prostaglandins, thromboxane, LTB4 and LTC4: mediators of inflammation synthesized by white blood cells) in CVD in treated patients and untreated patients to determine whether a significant change exists in some of them.

Keeping in mind that these substances are proinflammatory compounds, it is easy to understand why they are at the origin of pain and edema.

It is also possible to measure changes in the venous endothelium,[10] thickness of the dermis,[11] venous stasis,[12] vascularization of the dermis by photoplethysmography,[13] valve closure time,[14] venous wall oxymetry,[15] and blood flow dynamics.[16]

Clinical criteria

We can use Widmer's classification,[17] that of Porter,[18] or the CEAP classification.[19] The latter defines objective and subjective items (Clinical, Etiological, Anatomical and Pathophysiological) but it is not designed to evaluate the severity of CVD. In a second step, a score to measure severity and disability has been proposed.[20] This score is undergoing validation. In our opinion, it appears utilizable in chronic venous insufficiency and not in CVD.

The CEAP Classification is comprised as follows: the existence of telangectasias or reticular veins corresponds to class 1; varices to class 2; edema to class 3; skin changes to class 4; a closed venous ulcer to class 5; and an open venous ulcer to class 6.

Pain and heaviness in the legs are the two symptoms most commonly identified in studies on symptoms of venous disease. In principle, such symptoms are more easily quantifiable, and in addition are the most commonly observed in the population presenting with CVD, which should facilitate the task for statistical interpretation.

The symptom which in particular is of interest is **pain**, quite well-identified by Langeron,[21] Coger,[22] and Thiery.[23] It involves studying such pain of venous origin from its etiopathogenesis in the venous wall to its clinical expression. Once these reliable and quantifiable criteria have been identified, all that need be done is to measure them in CVD with and without treatment.

Pain related to these different manifestations can be evaluated in rating scales, which have been validated, or in visual analogue scales (VAS), ranging from no pain to unbearable pain. They are used in the evaluation of subjective parameters (a 10-cm long straight line demarcated by two marks, on which the subject determines the point corresponding to his or her pain). Patients rate severity of pain on a 10-point scale.

Therefore, reliable criteria exist which make it possible to objectively assess the efficacy of medicinal product on symptoms and, in particular, pain.

One of the difficulties in methodology involving the study of symptoms is that symptoms improve with placebo in at least 30% of cases.[24] Thus, it is necessary to have a large enough sample size for a clinically relevant difference to be significant. However, it should be kept in mind that it is not the subjective aspect of the evaluation, which makes such studies difficult, since currently sufficiently reliable tools are available to quantify symptoms, and in particular, pain.

EVALUATION OF SYMPTOMS OF CHRONIC VENOUS DISEASE AND STUDIED POPULATIONS

A review of the French literature[25] and foreign literature[26] shows that CVD most commonly is expressed by the following symptoms: heaviness, pain, sensation of swelling, restless legs, paresthesias, nighttime cramps, tiredness, and itching.

It should be noted that none of these symptoms is specific for CVD, and is even less pathognomonic. Since it is not possible to know whether these symptoms are of venous origin, it appeared important to seek to characterize these symptoms based on secondary criteria.

CHARACTERISTICS OF SYMPTOMS

Thus, we sought the features which would allow us to assign specific value to these signs. A consensus was found in recognizing that three criteria specific for CVD exist. Two criteria are major ones; the third is a minor one. Variability with position and variability with outdoor temperature are major criteria, while variation with hormonal status in women is a secondary criterion.
The existence of two major criteria is mandatory to confirm that the symptoms are related to CVD, but the absence of these criteria does not rule out the possible venous origin of the symptoms.

Variability with position or physical activity

Generally symptoms occur at the end of the day after remaining in a standing position for a prolonged period, and do not exist or are diminished in the morning, by supine position, or with the legs raised.

Variability with temperature

Symptoms occur or are worsened by warm temperatures in the summertime, hot baths, depilatory treatment with hot wax, and floor-mounted heating systems, and they disappear or are diminished in the winter and with cold temperatures.

Variability with sex hormones

Symptoms vary with time of the menstrual cycle. They can occur during hormonal therapies (estrogen or estrogen-progestin therapies), and disappear when treatment is discontinued. However, it is not for such reasons that recruitment will be limited to female subjects.

Choice of symptoms

Among the many symptoms in the literature generally associated with CVD, we had to select those, which, first, are the most common, and second, the easiest to quantify in terms of evaluation of a venoactive agent in a clinical trial.
For this purpose, we tended to proceed by elimination: symptoms such as sensation of edema or swelling, itching, restless legs, nighttime cramps, paresthesias, or tiredness were either frequently associated with CVD or more difficult to quantify.
The partially arbitrary choice of eliminating these symptoms does not mean that they are less important, but that they appear less appropriate in the setting of a clinical study, in a first phase of study, in any case.
The two symptoms chosen, that are found in all studies,[26-28] are **heaviness** and **aching,** sometimes referred to as "**throbbing**" in the USA.

STUDY POPULATION

Before we continue our review on determination of the means necessary to evaluate symptoms, we sought to precisely identify the type of population that we could study. Since CVD is a disorder with multiple presentations and which progresses without any nosographic continuity, we had to determine whether to conduct clinical studies on the entire population involved or only a part of it.
In this regard, we proceeded by elimination. It did not appear justified to study the effect of venoactive agents in patients with edema (C3) because another Committee was examining this aspect. We also excluded patients classified C4-C6 because we considered it impossible to analyze them for the symptoms chosen (pain and/or heaviness) what aspects were associated with the disease and which resulted from existence of a venous ulcer or lipodermato-sclerosis. This does not mean that such therapies are not effective on symptoms applied at this stage of CVD, but we know that validated treatments (compression, surgery, sclerotherapy) are available for patients classified C4-C6. Therefore, it appeared impossible to precisely analyze improvement in symptoms with venoactive agents.
Lastly, and for the same reasons, we excluded deep venous insufficiency from the studied population. Here too, we think that validated therapies exist not only to treat such patients but also to prevent the complications of deep venous insufficiency.

By proposing to study a venoactive agent in the treatment of CVD outside of involvement of any deep veins, we defined a more restricted context than that covered in the current marketing authorisation (MA) for this class of agents.
In fact, currently venoactive agents are indicated as symptomatic therapy of venous lymphatic insufficiency

(heaviness, pain, restless legs, etc.) According to the level of evidence provided by the MA file, three levels of indications have been differentiated:
- "treatment of symptoms,"
- "improvement of symptoms,"
- "proposed in the symptomatic treatment"

Referring to the CEAP classification, we determined two subgroups to study:
- the first (Group 1) was comprised of symptomatic patients apart from any anatomical lesion and physiological anomaly, ie, Cos, En, An, Pn, n indicating "No venous pathology" according to the latest refinement of the CEAP classification,[29] referred to as "functional insufficiency" with no real justification;
- the second (Group 2) was comprised of patients presenting with hemodynamic disorders of the superficial venous network and possibly perforating and/or gastrocnemius veins, apart from any skin changes and edema with a reflux incriminated in pathophysiological mechanisms, ie, C1S-C2S, As, Pr.

**However, at the plenary session it was agreed that venoactive drugs can have an effect on pain and heaviness in case of lipodermato-sclerosis or deep venous insufficienty and thus it is possible to plan a study proposing their use as adjunctive therapy.*

PROTOCOL FOR AN EVALUATION

Tools

Evaluation of heaviness in the legs

This symptom is a complaint which, in spite of attempts of quantification,[27, 28] is difficult to assign to a numerical value. A consensus was reached in the Working Group to note its presence or its absence. Therefore, to assess the therapeutic benefit of a venoactive agent in a group of patients, the percentage of patients will be evaluated in whom heaviness has completely disappeared.

Evaluation of pain in the legs

Leg pain should be differentiated from heaviness or the sensation of heaviness, which are not really painful in all cases. A consensus exists that states that pain can be quantified on a VAS.
No rating scale has been specifically developed for CVD, but rating scales exist that established in the evaluation of analgesic agents in rheumatology. Their intraindividual sensitivity and reproducibility appear relatively satisfactory.

Evaluation of quality of life

This is a basic tool, which, beyond the symptom(s), allows objective assessment of the impact of the disease on the patient's socio-occupational activity and personal status. "Generic" quality of life protocols or specific protocols for CVD such as the one by Launois[30] can be used.
Two subgroups of patients will be differentiated according to different criteria:
- selection criteria (inclusion and noninclusion criteria);
- evaluation of therapeutic effect (evaluation criteria);
Expected clinical benefit has been chosen as the primary evaluation criterion.

Inclusion criteria

Group 1
Clinical studies should be conducted in symptomatic patients, with no clinical or hemodynamic sign that can be identified with Duplex scanning, who present with symptoms involving both lower limbs (Co, En, An, Pn). The primary symptom chosen in this case is **heaviness of the legs**. It is an inclusion criterion (existence), whatever its severity, or its impact on quality of life.

Group 2
Patients are symptomatic (uni- or bilateral symptoms). Hemodynamic parameters of superficial venous network dysfunction and possibly anomalies of the perforating veins and/or gastrocnemius veins exist that can be identified by Duplex scanning.
In terms of symptoms, inclusion criteria will be the reverse of those in the former group:
- **pain** in the legs is the primary symptom, necessary and sufficient, provided that it is experienced with minimal severity (see above);
- **heaviness** in the legs is optional (its presence or absence will simply be noted).
For the two groups (see § *Characteristics of symptoms*), the need for the two following criteria is necessary to confirm the venous origin of symptoms: pain and/or heaviness; variability with position or physical activity, variability with temperature.

Methodology and duration of trials

In phases II b/III, it can be useful to study the 1-week analgesic effect of the venoactive agent tested, comparing its effect during this period to a pure reference analgesic agent (not specific for CVD).
The following methodologies correspond to phase III trials, with the dose having been determined.

Group 1
Studies should be conducted with double-blind design and strict monotherapy (in particular, analgesics are not allowed), in combination with dietary and lifestyle measures:
- either versus placebo
- or versus a reference product (for example, class I compression hosiery according to French or European standards, to be specified). The existence of a third placebo group in such a case is essential to ensure internal validity of the trial.
The duration of studies will be 2 months.

Group 2
It appears essential to conduct a placebo-controlled comparative study on the effect of the venoactive agent **in addition to a reference therapy** (sclerotherapy or surgery).
If the same trial mixes two types of study populations (sclerotherapy/surgery), treatment allocation (placebo/active drug) should be randomized according to reference therapy (stratification).
Duration of the study will also be 2 months.

Evaluation criteria and extent of clinical benefit

It is essential to define a **single** primary criterion. This will be different depending on group of patients due to the different inclusion criteria and therapeutic effects sought.

Group 1
- *Primary criterion*
  - Intergroup comparison of percentages of patients whose symptoms of leg heaviness **have disappeared**.

It appears reasonable to define that an effective treatment will allow heaviness to disappear in 90% to 95% of patients.

- *Secondary criteria*:
  - Intergroup comparison of leg pain measured by a VAS before and after treatment (+ intermediate measurement??);
  - Intergroup comparison of a quality of life scale before and after treatment.

Group 2
- *Primary criterion*
  - Intergroup comparison of leg pain measured by a VAS before and after treatment.

A difference of about 15% between the active drug and the placebo appears acceptable from the standpoint of methodology.

- *Secondary criteria*:
  - Intergroup comparison of quality of life scale before and after treatment.
  - If heaviness existed at inclusion, an intergroup comparison of percentages of patients whose symptoms of leg heaviness have disappeared is recommended.

CONCLUSION

Although the Working Group was in unanimous agreement in recognizing that patients consider that venoactive agents are effective on symptoms attributed to CVD, it should be noted that currently no protocol with adequate methodology exists, likely to be accepted by all physicians in judging the efficacy of venoactive agents.

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CONSENSUS COMMITTEE N°2
"EFFECTS OF VENOACTIVE AGENTS ON EDEMA IN CHRONIC VENOUS DISEASE"

Edema associated with chronic venous disease is a commonly mentioned clinical sign. Venoactive agents are the most usual "short cut" because of better compliance than with elastic compression. The aim of this meeting was to define the usefulness of this class of agents in management of edema and the methods for assessing their efficacy in chronic venous disease (CVD).

INTRODUCTION

Chronic venous disease (CVD) is a common disorder, and its prevalence in the general adult population is approximately 10%. In this setting, nearly 100 000 patients, ie, 0.1% to 0.2% of the population of France, suffer from venous ulcers. Consequently, venous insufficiency has an appreciable socioeconomic impact.

Unfortunately, in terms of understanding the underlying microcirculatory mechanism, treatment of CVD has not made any significant progress in about a hundred years. The majority of recommendations (in particular venous compression therapy and raising the legs) given to patients with CVD are based on principles from the last century. However, in an appreciable number of such patients, compression therapy alone often is inadequate and unacceptable in the long run.

The place of edema in the natural course of CVD has not been clearly elucidated. Unlike symptoms of CVD, edema is an objective, measurable parameter.

Members of the Committee wish to remind the reader that no study submitted to the French Medicines Agency (AFSSAPS) has been chosen as demonstrating the efficacy of venoactive agents in treatment of edema, in the setting of CVD.

Thus, the Committee met to define, in a first phase, the role of edema as well as the pathophysiological circumstances of its occurrence in the setting of CVD. An update on the latest pathophysiologic findings that promote the etiopathogenesis of edema then was made, since the participants in this meeting feel that it is important to understand what the targets of the proposed therapies are. Then, the Committee discussed the methods of measurement of edema, validated in the literature. Lastly, in light of the data from the literature on the efficacy of venoactive agents on edema, the Committee proposed conducting a study and discussed the primary methodological objectives.

DEFINITION OF EDEMA

Edema is defined as the existence of excess interstitial fluid in the tissues. However, extension of edema is limited by the skin's elastic properties, starting with a certain degree.

Clinical findings differentiate two types of edema:

- pitting edema, defined as a "palpable indentation of the skin following firm pressure applied for ten seconds by the thumb"[1]
- non-pitting edema.

If edema persists for several days, months, or even years, the extracellular matrix becomes altered, preventing healing of the edema. Thus, a considerable number of patients for whom venoactive therapy is offered already present chronic edema with an altered extracellular matrix as a result of lesions induced by the edema.

The sensation of edema is defined as an impression of tautness without the clinical existence of edema. This impression should be considered as a symptom, and not as a clinical sign.

PATHOPHYSIOLOGY

Causes of edema:

• *Positive pressure in the interstitial fluid*

Guyton demonstrated that pressure in the interstitial space is negative compared with atmospheric pressure in normal subjects. If a moderate amount of fluid is transferred from the intravascular compartment to the interstitial compartment with no change in pressure regimens, clinically detectable edema does not exist. On the contrary, a change to positive pressure in the interstitial medium (greater than atmospheric pressure) is responsible for clinically and physically detectable edema. Animal models have demonstrated that edema is clinically detectable, starting with a 30% increase in the volume of the extracellular medium, compared with normal.

• *Absence of protective factors against edema*

Traditionally, the factors, which protect against edema are grouped under three mechanisms which produce dynamic interaction: negative tissue pressure, lymphatic drainage, and constant renewal of tissue proteins. In fact, patients who consult for treatment of edema present with greater or lesser alteration of these different factors.

Specific causes of edema:

• An *increase in capillary pressure*, either by increased pressure or venous obstruction, or by vasodilatation of arterioles. Such abnormal conditions are found in particular in heart failure patients or in those with serious varicose veins;

• A *decrease in plasma protein concentrations* (eg, in severe burn patients, nephrotic syndrome, exudative enteropathy, deficiency syndrome). Interestingly, deficiency syndromes commonly occur in the elderly;

• *Lymphatic obstruction caused by filaria, or lymphatic agenesis*. Edema can also occur in the aftermath of cancer surgery with lymph node dissection or after a stripping procedure of the greater saphenous vein, and to a letter extent in functional lymphatic insufficiency (decreased lymphatic drainage capacity);

• *Increased capillary permeability* is found in venous insufficiency, in severe burn patients, or in case of an allergic reaction.

Importance of the extracellular matrix and of endothelial cells

The interstitial fluid is embedded in the extracellular matrix comprised of peptidoglycans (formerly known as mucopolysaccharides). When excess fluid accumulates in the interstitial fluid, the matrix attracts this fluid. When the matrix has increased by 30% to 50% in volume, the proteoglycans become altered, thus releasing free spaces in the interstitial tissue. As these channels open, they increasingly release space promoting the entry of fluid arising from blood vessels. Thus, edema becomes clinically detectable. The importance of the extracellular matrix in the balance between intra- and extracellular compartments and in trapping water remains to be determined.

In addition, the extracellular matrix plays a part in the nutrition of endothelial cells. At the same time, alterations in the matrix and interstitial nerves produced by chronic edema also are poorly understood.

Some authors have suggested that, in chronic venous disease, adhesion of inflammatory cells to the vascular endothelium may produce activation of the latter, with release of proteolytic enzymes and free radicals in the tissues. Endothelial cells can represent the key item in the etiopathgenesis of edema, and thus become a potential target for future venoactive agents.

MEASUREMENT OF EDEMA AND LEG VOLUME

Volumetric measurement

A previous consensus meeting considered this technique as the reference method in demonstrating and comparing the efficacy of treatments of edema in CVD.[1] This method makes it possible to demonstrate changes in leg volume under different conditions and at different times before and after treatment. Therefore, it is a method that is both reproducible in terms of its results and over time (good outcome).

One of the techniques consists of immersing the leg in a water-filled Plexiglas boot. The volume of water displaced is collected and measured.[2,3] Other methods have been described but they do not allow measurements under physiological conditions such as orthostatism.[4-7] However, in the setting of volumetric measurements, a certain number of parameters such as leg position, time of measurement, phase of menstrual cycle, room temperature, as well as that of the water used should be standardized to validate the results.

Variability of the method in two consecutive measurements, on the same leg, by two different observers was 0.7%. Intraindividual variability was 1.3%. Interindividual variability was 6.2% in normal control patients and 11.2% in patients with venous disease.[3]

Volumetric measurement was used to show that the most painful legs are those which have the greatest tendency to swell up.[8] In addition, volumetric technique has shown that erect posture is responsible for an increase in leg volume, and this is correlated with the degree of venous insufficiency. Similarly, legs swell during long-haul air travel[9] and can decrease in size after venous surgery or after different pharmacological therapies of venous or lymphatic insufficiency are initiated. Advances in study methods have made it possible to show how changes in leg volume are correlated with severity and prognosis of chronic venous disease.

Other methods of evaluation of edema

The Group validated the following methods:

- *measurement of leg circumference;*[10-13]
- *electro-optical volumetric method;*[4,5,7,8,14]
- *digitised measurement of leg volume.*[3,5]

Other methods have been described in the literature: *tomographic method,*[5] *high-resolution magnetic resonance imaging*[5]*, and X-ray absorptiometry.*[5]

ANALYSIS OF STUDIES TESTING VENOACTIVE AGENTS IN LEG EDEMA

Many studies can be found in the literature demonstrating the efficacy of different classes of venoactive agents on edema in CVD. The efficacy of several agents has been demonstrated on symptoms and edema by volumetric methods,[12-19] and on concomitant pathophysiological factors in patients presenting with CVD.[20-24] Thus, the factors promoting edema on which the action of therapies has been demonstrated are as follows:

Venous hypertension and venous tone,[16,25-31] increased capillary permeability,[32-37] and lymphatic drainage.[13,24,38]

Why does enhanced lymphatic flow promote edema?

It is essential to demonstrate that, although venoactive agents decrease edema, it is through their action on pathophysiological factors of edema (such as venous hypertension).

Different factors that promote edema can be measured more or less routinely:

- venous hypertension;
- capillary permeability (by the radiolabelled albumin method);
- lymph flow (by isotopic lymphography).

On the contrary, other factors (for example, macrophage activity, sequestering of water, change in the gel/sol phase) cannot be measured.

Several studies have also demonstrated that after 1 or 2 months' treatment with venoactive agents versus placebo, patients with CVD had an increase in venous tone assessed by plethysmography, and showed regression of clinical signs of edema as well as associated symptoms.[12,20,23,39-41]

The Doppler laser method has also been used in some studies to demonstrate the effects of venoactive agents on the microcirulation, in particular on vascular motricity and arteriovenous reflex.[42]

Effects of venoactive therapies

A certain number of randomized, controlled studies can be found in the literature showing the efficacy of venoactive agents on edema.[6,9,12,15,17,18,37,39-45] However, efficacy criteria on regression of edema, most often assessed by leg circumference in such studies, in addition is not always very representative. Indeed, the problem of these studies is that efficacy is set at a level ranging from 4-mm reduction in circumference to a 40 mL volume. Certainly, this involves a decrease in edema, but a very small one. For example, in a calf with a circumference of 37.08 cm,

a 2-mm reduction in thickness produces a 12.4-mm reduction in circumference (2 π r). More than the reduction in edema, it is possible to speak of a "reduction in the sensation of swelling." These two criteria are not clinically relevant, Therefore, venoactive agents decrease edema, but in a clinically minimally significant manner.

And yet, it is necessary to have studies which show a clinically significant reduction in edema with venoactive treatment. Furthermore, it is important to show how venoactive agents decrease edema, in particular which pathophysiological factors they act on. Indeed, the action of such therapies on venous tone, venous hypertension, increased capillary permeability, and lymphatic drainage can be demonstrated by the previously mentioned methods of investigation.
The conclusion that can be drawn from these results is that it is necessary to carry out a well-conducted study based on scientific principles and approved by an Ethics Committee.
Such a study should be randomized, controlled, versus a placebo group, with double-blind method, and sufficient power to answer the question raised at the stage of disease studied. Inclusion criteria for patients should be clearly defined. A classification system for patients such as the CEAP system (the Committee estimates that, in spite of its shortcomings, it is the best rating scale for CVD), should be used to assign patients to groups with increasing severity, to better define the indications.
Symptoms, signs of the disease and quality of life (rating scale) should be evaluated at the start, in the aftermath and at the end of the study. The hemodynamic evaluation will be assessed by noninvasive methods of investigation, preferably chosen for their ability to best answer the question asked. Efficacy of treatment on edema will be evaluated by a standardized volumetric method (control of room temperature, constant time of day, etc).

Although many venoactive agents have demonstrated their activity on edema, its sole existence is not a therapeutic indication. The main indication for such agents continues to be the symptoms of CVD. Therefore, it is necessary to use composite criteria in which edema and the sensation of swelling will be present and separate.

OBJECTIVES OF FUTURE STUDIES ON USE OF VENOACTIVE AGENTS IN EDEMA OF CVD

Background on the compound

Obviously, it is essential to provide an update on the in vitro efficacy of the compound tested, as well as to collect experimental and pharmacological data, (effective concentrations), clinical findings (indications chosen, results of previous studies, etc).

Level of evidence

a) The objectives of this randomized, double-blind, controlled trial (versus a placebo group and versus another "venous compression" group) must show clinically relevant real efficacy, in the reduction of edema. However, it is up to members of the Committee to determine the level of clinically relevant reduction. Indeed, the Committee was unable to reach a decision on this point, which is essential to validate the results of the study.
b) Furthermore, to give weight to the conclusions, demonstration of the evidence of efficacy of venoactive agents can be based on a dose-effect relationship which to date has never been demonstrated in the literature.
c) Lastly, it is essential to insist on showing that a pathophysiological explanation exists for the effect of the therapies initiated.

Study objectives should be chosen carefully

- Reduction of edema will be assessed clinically and measured in a standardized instrumental manner, as previously reviewed;
- Concomitant symptoms, symptom severity score (CEAP), as well as quality of life scale will also be evaluated;
- Lastly, it will be necessary to define a composite criterion in this study, which will represent an item of weight to demonstrate the efficacy of the therapy. For example, this criterion can be a 50% decrease in edema, as well as a 50% reduction in concomitant pain or heaviness.

Therefore, before conducting such a study, the Committee must resolve several items, which remain to be discussed:
- defining the studied populations as well as study inclusion criteria (usefulness of CEAP);
- what level of reduction in edema (%) represents a significantly relevant reduction?

- to what extent is this decrease in edema (%) correlated with a decrease in symptoms?
- which composite criterion has to be defined to demonstrate efficacy of the treatment?

M. Perrin commented that it was unfortunate that, in the end, members of the Committee did not select a method for measurement of edema, for example, leg circumference or volumetric method.

CONCLUSION

Venoactive agents have demonstrated their efficacy in the reduction of symptoms of edema in the setting of chronic venous disease. However, it should be noted that for the majority of patients treated, edema – unlike symptoms – is the not the primary indication for treatment. Thus, edema is not an indication for treatment with venoactive agents in the absence of symptoms of venous disease.

Therefore, the Committee has proposed conducting a study to demonstrate the efficacy of venoactive agents in patients with isolated edema in the setting of CVD. The different methodological aspects of this study have been discussed and determined by the Committee. However, a few points remain to be set such as the percentage reduction of significantly relevant edema or the composite criteria to be determined.

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Protection of the microcirculation during ischemia/reperfusion is enhanced by micronization of purified flavonoid fraction

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ABSTRACT

This study was designed to evaluate the importance of micronization on the protective effect of micronized purified flavonoid fraction (MPFF) on increases in macromolecular permeability induced by ischemia/reperfusion in the hamster cheek pouch microcirculation. Male hamsters (*Mesocricetus auratus*) were treated orally, twice a day, with vehicle (lactose), MPFF and nonmicronized purified flavonoid fraction (PFF) at 5, 20, 80, and 320 mg/kg per day for 10 consecutive days. On the 11th day, cheek pouches of anesthetized animals were prepared for intravital microscopy. Local ischemia of 30 min was obtained by clamping the neck of the everted pouch and the increase in microvascular permeability was quantified as leakage (leaks) of intravenously injected fluorescein isothiocyanate-labeled dextran. Reperfusion resulted in an immediate but reversible increase in postcapillary leakage. MPFF induced a significant dose-dependent reduction in the increased permeability (control: 115.7 ± 4.1 and 320 mg/kg body weight/day: 19.2 ± 1.9 leaks/cm^2, $P<0.0001$). Nonmicronized PFF was significantly less effective (60.3 ± 1.0 leaks/cm^2, also for the highest dose, $P<0.0001$). In conclusion, micronization significantly enhances the protective effects of MPFF, and this improvement is probably related to the better absorption of the micronized formulation and could explain the superior clinical efficacy shown in previous studies.

Keywords:
micronization, macromolecular permeability, cheek pouch, hamster, micronized purified fraction (MPFF).

INTRODUCTION

Micronized purified flavonoid fraction (MPFF) contains 90% of micronized diosmin (diosmetin-7-rhamnoglucoside) and 10% flavonoids expressed as hesperidin (hesperitin-7-rhamnoglucoside). Clinically it is used to treat chronic venous insufficiency and hemorrhoidal disease, due to its anti-

inflammatory properties.[1,2] The pathophysiology of chronic venous insufficiency seems to involve cyclic periods of ischemia/reperfusion.[3,4] Early reperfusion of the ischemic tissue is essential to stop the progression of cellular injury associated with oxygen and nutrient depletion. However, it has already been demonstrated that reperfusion to an ischemic area produces a complex cascade of pathological events which could potentially lead to the same end result as prolonged hypoxia, which is cellular dysfunction and necrosis. Reactive oxygen species induce the production of proinflammatory agents like platelet-activating factor, leukotriene B_4, and activated complement components. They also modify the expression of adhesion molecules on the surface of leukocytes and endothelial cells (CD11b/CD18, P-selectin and intercellular adhesion molecule-1) and reduce the levels of nitric oxide (NO) due to decrease in NO-synthase in the muscle, NO-synthase being a potent vasodilator and antiadhesive substance.[5] The activated leukocytes migrate into the interstitial tissues, inducing microvascular barrier dysfunction via release of oxidants and hydrolytic enzymes.[6] Flavonoids inhibit the phosphodiesterases involved in cell activation. This effect is mainly produced by the biosynthesis of cytokines that mediate adhesion of circulating leukocytes to sites of injury. Thus, enhanced vascular macromolecular leakage is one of the earliest signs of microvascular dysfunction elicited by ischemia/reperfusion. The increased permeability leads to interstitial edema, which will lead in time to physical compression of capillaries promoting the development of the no-reflow phenomenon.[7,8]

Micronized purified flavonoid fraction (MPFF) has been shown to exert a protective effect on the microvascular barrier function in experimental conditions, such as ischemia/reperfusion,[9-11] oxidative stress,[12] inflammation,[9,13] or venous hypertension.[14]

This study was designed to evaluate the influence of micronization on the protective effects of the purified flavonoid fraction on microvascular barrier disruption. The dose-related effects of the micronized form were compared to those of the nonmicronized form on macromolecular permeability increase induced by ischemia/reperfusion using the hamster cheek pouch.[15]

MATERIAL AND METHODS

Animals

Male golden hamsters (*Mesocricetus auratus*), 7 to 10 weeks old, weighing approximately 100 g, were obtained from Engle Labs., Farmersburg, Indianapolis, USA. The experiments were performed according to protocols approved by the Ethical Committee of the State University of Rio de Janeiro (H36/94). The animals received an appropriate laboratory diet, Nuvital, from Nuvilab, PR, Brazil.

Experimental Groups

There were nine animal groups (n=6, each), MPFF and nonmicronized purified flavonoid fraction (PFF) at different doses and with different vehicles. The drugs were obtained from Servier Laboratories (Gidy, France) and were suspended in 10% lactose solution, before each administration. The vehicle, 10% lactose solution, MPFF and nonmicronized PFF, at doses of 5, 20, 80, and 320 mg/kg body weight/day, were administered by gavage, twice a day, at 8:00 AM and 5:00 PM, for 10 consecutive days. Each animal received 0.2 mL of suspension per 100 g body weight. The investigator was blinded for formulation being administered (micronized or nonmicronized), in order to avoid bias. The last dose was given 30 min before the induction of anesthesia.

Surgical Procedures

Intraperitoneal injection of sodium pentobarbital (Pentobarbital sodique, Sanofi, Paris, France, 60 mg/mL) was used to induce anesthesia, maintained with α-chloralose [1,2-O-(2,2,2-trichloethylidene) α-D-glucofuranose], (Merck, Darmstadt, Germany, 100 mg/kg) administered through the femoral vein. The femoral artery was cannulated to measure arterial pressure. During the experiment, the temperature of the animals was maintained at 37.5° C with a heating pad controlled by a rectal thermistor. A tracheal tube was inserted to facilitate spontaneous breathing. The hamster was placed on a microscope stage similar to that described by Duling[15] and Svensjö and coworkers,[16] modified by Bouskela and Grampp.[17] The cheek pouch was everted and pinned with four to five needles into a circular well, filled with silicone rubber to provide a flat, bottom layer, thus avoiding stretching of the tissue, but preventing shrinkage. In order to produce a single-layer preparation, an incision was made in the upper layer so that a triangular flap could be displaced to one side. The exposed area was dissected at 10-16X magnification under the stereomicroscope, and the fibrous, almost avascular, connective tissue covering the vessels was removed using ophthalmic instruments. The dissected part of the pouch was 125 to 150 µm thick. Pouches with petechial hemorrhages or without blood flow in all parts were discarded.

The superfusion solution was a HEPES-supported HCO^-_3-buffered saline solution; the temperature was maintained at 36.5° C and the superfusion rate was 4 mL/min. The pH was set to 7.40 by bubbling the solutions continuously with 5% CO_2 in 95% N_2.
Thirty minutes after the completion of the preparative procedure, FITC-dextran 150 (Bioflor HB, Uppsala, Sweden) was given at a dose of 25 mg/100 g as an intravenous injection of a 5% solution in 0.9% saline.[16]
Observations were made with a Leitz Ortholux microscope with a x3.5 objective and x10 oculars.
Local ischemia of the cheek pouch was produced with a cuff, made of thin latex tubing, which was mounted around the neck of the everted pouch where it leaves the mouth of the hamster.[18] The intracuff pressure could be rapidly increased by air compression by using a syringe and could be just as rapidly decreased when required. An intracuff pressure of 200-220 mm Hg resulted in a complete arrest of the microvascular blood flow within a few seconds (*Figure 1*).

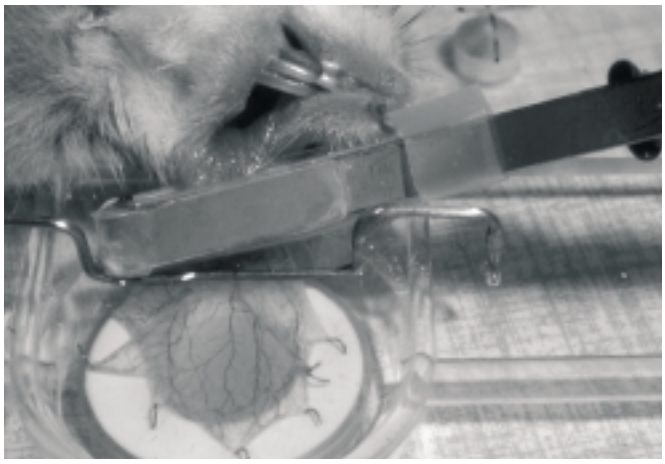

Figure 1. Local ischemia of the cheek pouch. A latex cuff is mounted around the neck of the everted pouch where it leaves the mouth of the hamster.

The fluorescent spots seen at leakage sites could be counted when they reached at least 100 µm size.[9] The leakage was expressed per cm^2. The number of leaks was obtained before, immediately after the ischemic period, and every 10 min thereafter for 1 hour. All hamsters with the prepared area showing spontaneous nonfading leaks or more than 10 fading leaks during the first 30-minute control period, after FITC-dextran was given, were discarded.
The results are presented as mean ± SEM. After a one-way analysis of variance, comparison of each dose of MPFF and nonmicronized PFF with the vehicle-treated group was evaluated using the bilateral Dunnett's test. Comparisons between two groups were performed by contrast and Bonferroni's method was used to compensate for multiple tests.

RESULTS

No significant difference in mean arterial pressure could be detected among the groups treated with vehicle or any of the treatment formulations.
Figure 2 shows an example of microvascular leakage. The fluorescent spots indicating edema are located in the microscope field, mainly at the right side.

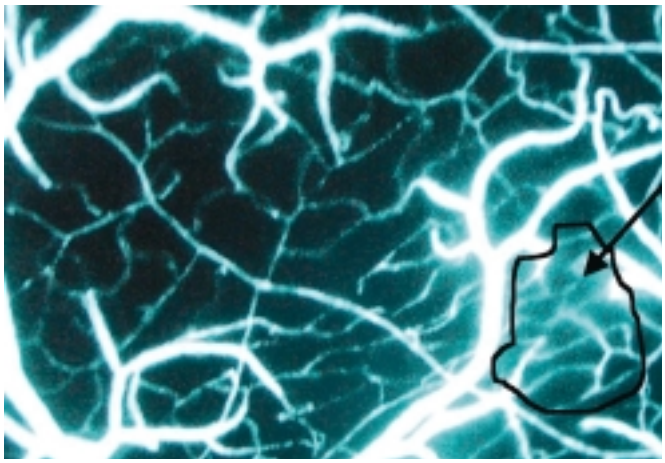

Figure 2. Example of microvascular leakage during reperfusion phase. Black arrow shows white fluorescent spots of FITC-dextran indicating edema.

The maximal numbers of venular leakage sites per cm^2 during reperfusion, after 30 min of total ischemia, in animals treated for 10 days with either vehicle, MPFF, or nonmicronized PFF can be seen in *Figure 3*. Reperfusion resulted in a reversible increase in postcapillary leakage (leaks). In vehicle-treated hamsters, the mean maximal response was 115.7 ± 4.1 leaks/cm^2. Oral treatment with MPFF inhibited, in a dose-dependent fashion, the macromolecular permeability increase induced by ischemia/reperfusion. At the highest dose, 320 mg/kg body weight/day, there was a 83.4% inhibition (19.2 ± 1.9 leaks/cm^2, $P<0.0001$). Oral treatment with the nonmicronized PFF also induced a significant reduction in macromolecular permeability increase induced by ischemia/reperfusion compared with the vehicle-treated group, but to a lesser extent and without a dose-dependent relationship. The maximum decrease in plasma leakage with nonmicronized PFF was obtained at the dose of 80 mg/kg body weight/day, with a 48.8% inhibition

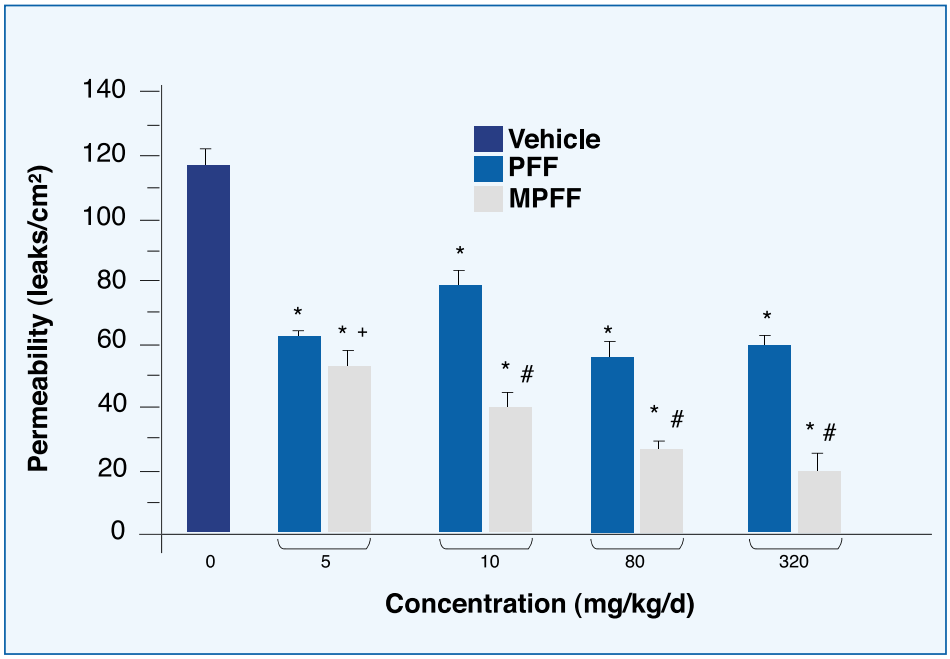

*Figure 3. Mean maximum number of venular leakage sites per cm² during reperfusion after 30 min of total ischemia in the cheek pouch of hamsters treated orally for 10 days with micronized purified flavonoid fraction (MPFF) or nonmicronized PFF (n = 6 animals per group). *P<0.0001 compared with vehicle-treated group and # P<0.0001, + P<0.001 compared with the same dose of nonmicronized PFF.*

(59.3 ± 1.7 leaks/cm², *P*<0.0001). For each dose tested, the mean number of leaks was significantly lower in animals treated with MPFF compared with those treated with the same dose of the nonmicronized form (*P*<0.001).

DISCUSSION

As experimental models of venous disease are rare, the activity of MPFF was investigated using a pharmacological model with microvascular alterations similar to what is observed in chronic venous insufficiency. Several studies have been carried out in models of ischemia/reperfusion,[9,10,11,19] inflammation evoked by edemogenic substances,[13] oxidant challenge,[9,12] venular occlusion,[14] and transcapillary fluid shift after postural changes[20] to evaluate this disease.

Hamster striated muscle preparation has been studied with pretreatment with MPFF for 8 days in a 4-hour ischemia model where the tissue damage was probably prevented by reducing the number of extravasated leukocytes.[21] Two other studies have shown that pretreatment with MPFF reduces the expression of adhesion molecules (ICAM-I) in the rat cremaster exposed to 4 hour ischemia/reperfusion[4] and the spontaneous neutrophil activation and expression of CD 62 L (L-Selectin) after 60 minutes of local venular occlusion followed by a 1-hour reperfusion in the rat mesentery without effect on CD 18 or P-Selectin.[14]

Our results show that MPFF has more effect in reducing microvascular permeability than nonmicronized PFF. Inflammatory reactions could be avoided with less edema formation. Similar results have also been reported in the literature.[22,23] In postischemic reperfusion injury, leukocyte activation and adhesion to the endothelium and an increase in macromolecular permeability are a characteristic pattern. [9,24,25,26]

The pharmacokinetics and metabolism of MPFF could explain the differences between this form and the other one. Johnston and coworkers,[27] using ^{14}C in the position 2 of the intermediate ring of diosmin, the major component of MPFF, demonstrated that micronization significantly improved the absorption of a single dose of 10 mg/kg body weight compared with nonmicronized PFF after 168 h of collection (fecal excreted radioactivity – MPFF 21% and nonmicronized PFF 72%; urinary excreted radioactivity – MPFF 77% and nonmicronized PFF 17%).

Another example of effectiveness of the digestive absorption of MPFF is the double-blind, crossover study in which 12 volunteers used 500 mg tablets containing trace amounts of ^{14}C-diosmin in a single oral dose. The reduction of particle size of ^{14}C-diosmin resulted in a marked increase in the proportion of the dose excreted in the urine.[28] The increased urinary excretion possibly reflects higher absorption of the micronized formulation giving a pharmacokinetics explanation of its better clinical efficacy.

Our results are in accordance with clinical findings in the treatment of chronic venous insufficiency and hemorrhoidal disease,[27,28] where MPFF reduced significantly the symptoms and objective signs of these diseases with equivalent acceptability.[27,28]

Oral treatment with MPFF, at 20 mg/kg body weight/day for 10 consecutive days significantly decreased the macromolecular permeability increase induced by ischemia/reperfusion (103.6 ± 15.4 versus 42.6 ± 9.3 leaks/cm^2, $P<0.01$).[9] The effects of MPFF on microvascular macromolecular permeability increase were confirmed in different models and species.[9-13] Leukocyte adhesion to endothelial cells of postcapillary venules, which plays a major role in the pathogenesis of venous disease,[29] decreased in various experimental models (ischemia/reperfusion, venular occlusion/reperfusion, oxidant challenge), as demonstrated by intravital microscopy or histomorphological analysis.[9,10,12,13,19,22] Some observations suggest leukocyte adhesion may contribute to damage of the microcirculation and, ultimately to the formation of leg ulcers in patients suffering from chronic venous insufficiency.[20]

In conclusion, micronization significantly enhances the protective effects of oral administration of the purified flavonoid fraction on macromolecular permeability increase induced by ischemia/reperfusion in the hamster cheek pouch preparation. Better absorption of the compound can explain its superior clinical efficacy shown in previous studies.

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Apoptosis and cell cycle regulation in the vein wall – new elements related to varicose vein occurrence

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SUMMARY

According to the majority of pathological studies in varicose vein specimens, various structural changes have been documented, including an irregular accumulation of the extracellular matrix within the vein wall and the disruption of the smooth muscle cell (SMC) bundles. Valve injury seems not to be the only factor leading to varicose vein development, and for vein dilation additional factors related to vein wall homeostasis disturbances are needed. Extracellular matrix accumulation within the vein wall related to SMC phenotype transdifferentiation and secretory activity can result in weakening of the vein wall. Among the various stimuli responsible for this process, endothelial cell hypoxia, hemodynamic disturbances, and cytokine (especially growth factors), influences are discussed. In the study, we confirm an increase in TGF-ß1 mRNA expression level and its protein product presence as well as the presence of SMC hypertrophy within the medial vein layer.

Due to the possibility of pathological cell elimination, p53 - related apoptosis is an important mechanism influencing vein wall homeostasis maintenance. In the study we documented that its activation is characteristic of the relatively early stages of varicose vein diseases. In the later course of the disease, the presence of the downregulation of SMC apoptosis within varicose vein media leads to an increase in structural changes related to local extracellular matrix accumulation.

In the proximal saphenous vein segments, despite SMC reduction and the presence of structural changes, there was no increase in programmed cell death activity. The reduction in the SMC population corresponding to cyclin dependent kinase inhibitor (p21) expression suggests a role of cell cycle disturbances that may lead to vein wall architecture destruction and further dilation.

Keywords:
varicose veins - apoptosis - smooth muscle cell - p53 - TGF ß.

INTRODUCTION

Although the mechanism of development of chronic venous insufficiency is becoming more and more understood, the sequence of events is still under discussion.[1,2] Due to developments in angiology and molecular biology, new

factors are constantly being added.[3,4] The prolonged hydrostatic load in the incompetent vein, followed by activation of endothelial cells, leukocytes, macrophages, and mast cells leads to the enhanced production of cytokines, growth factors, and to adhesion molecule expression.[3,4]

According to the "valve failure" theory, descending vein incompetence is related to proximal valve failure.[2] Despite the data documenting the possibility of congenital proximal saphenous vein valve abnormalities, in view of some clinical observations, this theory does not seem to be satisfactory.[1,5,6] The presence of venous reflux or varicosities in the saphenous trunk or its collaterals without proximal valve incompetence (reported in 10% to 30% of cases), unilateral varicose veins despite bilateral saphenous junction incompetence, or the lack of varicose vein dilation in cases of insertion into the arterial high-pressure system (even in cases of valve destruction by valvulotom) suggest an important role for simultaneous (or previous) vein wall injury required for its dilation.[1,5-7]

According to the majority of pathological studies in varicose vein specimens, various structural changes have been documented, including an irregular accumulation of the extracellular matrix within the vein wall and the disruption of the regular pattern of the smooth muscle cell (SMC) bundles.[1,8-10] Moreover, significant quantitative differences in the principal vein wall compounds (SMCs, collagen, and elastin) were also reported, although contradictory results have been described. According to the literature, an increase[1,9,10] or decrease[11,12] in collagen in the varicose vein wall was suggested. Other authors described not only quantitative but also qualitative collagen changes. An imbalance in the collagen III:collagen I ratio, and an increase in collagen type I was documented in an ex situ study concerning vein wall SMCs as well as dermal fibroblasts, suggesting the possibility of a nonlocal but rather systemic character of the disease.[1,3]

Despite quantitative differences, the majority of authors report the presence of significant structural changes destroying the vein wall architecture[1,8,10,11] (*Figure 1*). However, we still do not know whether the valve failure is the beginning or the end of the cascade of these events. Corcos et al describe the widening of the proximal saphenous vein valve annulus with structural changes within the vein wall in the majority of the investigated cases.[2] According to Strucker et al, the complete destruction of the three-layer structure of the vein wall with concomitant thickening of the intima and disruption of the lamina elastica interna concerned 21% of cases.[14] Varicose veins

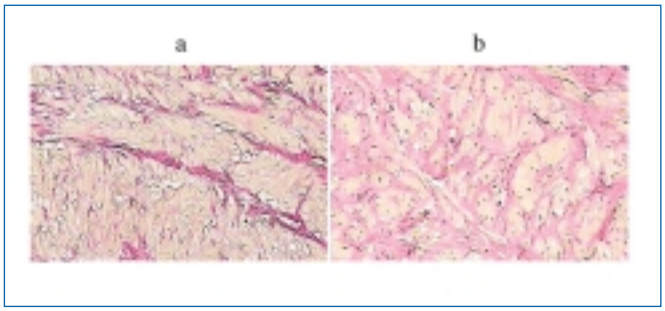

Figure 1. Verhoeff-Van Gieson staining of the normal (a) and incompetent (b) saphenous vein – collagen deposits in the vein media destructing regular vein wall architecture.

can also develop in the presence of competent valves, or can occur below a competent vein segment.[5,6] Moreover, a saccular dilation of the vein can occur as a blowout on the normal vein.[1,8] On the other hand, according to several other reports, in some cases, shortening, thickening, or disruption of the incompetent valve leaflets are described.[5,15,16]

VARICOSE VEINS - THE ROLE OF VEIN WALL REMODELING

According to recent findings, the initial phase of the vein remodeling process can be related to endothelial cell activation.[3] Shear stress disturbances, intraluminal hypertension, hypoxia of the endothelial cells, as well as ischemia of the vein medial layer (due to vasa vasorum compression) result in inflammatory reaction mediator and growth factor production.[3,17] The endothelial cell activation and the release of cytokines induce the process that leads to intimal thickening and vein structure remodeling.[2-4,8,19] In the mechanism leading to the vein wall dysplastic changes, its structure disorganization and intimal thickening, the possibility of SMC proliferation and migration into the vein intimal layer is taken into consideration.[2-4,8,19] Another proposed mechanism can be related to the presence of SMC phenotype transdifferentiation and their secretory activity.[2,3,8,20] The change in SMC phenotype from a contractile into a secretory one can result in local extracellular matrix production and its further accumulation.[20-22] SMCs are also responsible for production of metaloproteinases (MMPs), and their tissue inhibitors (TIMPs), that is, for important mechanisms controlling extracellular matrix turnover in the vessel wall.[23] According to the data reported by Badier-Commander, higher TIMP:MMP ratio facilitates an accumulation of fibrous tissue within the vein wall.[24]

The role of some other factors has also been suggested, such as neutrophil adhesion and activation or presence of monocyte and mast cell infiltrations.[2,18,23]
Besides the influence of cellular elements, the role of growth factors has also been investigated. Hollingsworth documented altered VEGF and VEGF receptor transcription in the initial stage of the disease with femoral junction incompetence.[25] Michiels suggested the role of the release of ßFGF and PGF-α2 from activated endothelial cells in ischemic conditions.[4] In a paper by Badier-Commander the presence of ßFGF and TGF-ß1 in the hypertrophic varicose vein segments was documented, whereas the lack of the increased PDGF activation (responsible for SMC migration and proliferation) was reported.[20]

VENOUS TONE - SMC ROLE IN THE VEIN WALL HEMOSTASIS MAINTENANCE

SMCs, being a part of the local contractile units, are responsible for active venous tone maintenance. The passive venous tone is related to vessel wall structure and to the mechanical properties of collagen and elastin fibers. Contradictory reports concerning SMC amounts within varicose vein walls can be found. According to some authors, the SMC content in the vein wall can be reduced.[1,26] Others have reported unchanged or increased SMC amounts within the vein wall.[12,17] These differences could probably be related to the unhomogenicity of the investigated material, often containing hypertrophic or atrophic varicose vein segments. In our study, concerning the patients in relatively early stages of the disease with femoral junction incompetence and crural varicose veins (without dilation of femoral segment of the saphenous vein), the reduction in the SMC amount within the vein media was confirmed in both proximal and distal vein segments (with simultaneous thickening of the intimal layer related to SMC and extracellular matrix accumulation).[27]
As was previously mentioned, SMCs are one of the most important factors controlling not only venous tone but also vein wall homeostasis.[2,8,20,21,27] Their role in extracellular matrix and growth factor production has been described in many models of other vascular diseases.[21,22]
The possibility of phenotypic transformation of the SMC into the secretory type within the vein wall was also discussed. Jurkova documented the presence of altered collagen containing SMCs within varicose vein intima.[28] Porto described the presence of structurally different subtypes of SMC in the injured vein wall, explaining these differences by SMC phenotype transdifferentiation.[29] In the paper by Kockx the presence of hypertophic modified SMCs surrounded by extracellular matrix deposits was reported.[30] SMC hypertrophy in varicose vein specimens was also documented by Wali and Knaapen.[3,31] These observations were also confirmed in our study. Morphometric analysis of the longitudinal transsections of the vein wall specimens confirmed the local decrease in medial layer cell nucleus density in the areas qualified as SMCs, suggesting the presence of cell hypertrophy within the medial layer of incompetent veins.[27,32]
The mechanism of the SMC phenotype change in the arterial wall has been meticulously investigated in injured arteries or veins implanted into the arterial system.[21,22] Although some factors had previously been suggested, the mechanism of the proposed phenotypic turnover remains unclear within the wall of the incompetent veins, especially in the presence of hypertrophic or atrophic segments within the same vein.[2,4,20] Among various stimuli responsible for SMC dedifferentiation, hypoxia, hemodynamic disturbances, and cytokine (especially growth factors), influences were discussed.[2,4] In our study, we confirm an increase in transforming growth factor-ß1 (TGF-ß1) mRNA expression level and its protein product presence within the wall of varicose veins, especially in the medial and intimal layer (*Figure 2*). TGF-ß1 is one of the multipotential cytokines regulating cell proliferation, differentiation, and apoptosis.[33-36] The final effect of TGF-ß1 activity depends on the type of the cell, its surroundings, and presence of other cytokines.[33-36]

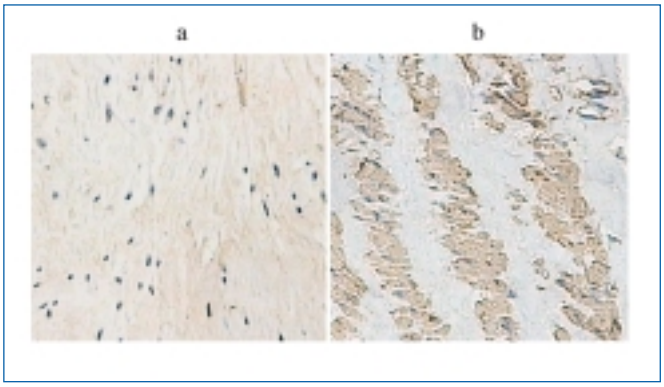

Figure 2. TGF-ß1 immunostaining – normal vein (a) and incompetent (b) saphenous vein.

According to the literature, TGF-ß1 can be one of the factors responsible for SMCs dedifferentiation into secretory type.[36,37] This cytokine also stimulates the cascade of events resulting in secretory fibroblast activity in

trophic skin changes in patients with chronic venous insufficiency, and is probably one of the factors responsible for the local vein remodeling process connected with an increased extracellular matrix production within the vein wall.[2,4,20,38] Beside the stimulation of collagen and fibronectin production by altered SMCs, TGF-ß1 also induces expression of TIMP and PAI-1 - two important inhibitors of extracellular matrix-degrading enzyme.[38]
Taking into account the role of membrane TGF-ß receptor complex activation and the method of its signal transduction, we documented the presence of increased immunoreactivity of TGF-ß receptor type I, as well as an increased transcription level of TGF-ß receptor-regulated intracellular factors - SMADs (mad related proteins) (*Figure 3*). According to the literature, SMAD proteins are responsible for TGF-ß1 signal transduction into the nucleus.[39] According to our study, the ratio of mRNA expression level of TGF-ß1 receptor-regulated SMAD 2 to the inhibitory SMAD 7 was higher in varicose vein specimens than in the normal control veins. These data confirm the previous findings reported by Bujan documenting an increase in TGF-ß immunorectivity in the wall of varicose veins, as well as the results of Badier-Commander and coworkers, suggesting the role of TGF-ß1 and bFGF in the vein wall remodeling process.[20,40] The coincidence of intimal hyperplasia and expression of TGF-ß1 in saphenous veins harvested for coronary artery surgery was also documented.[41]

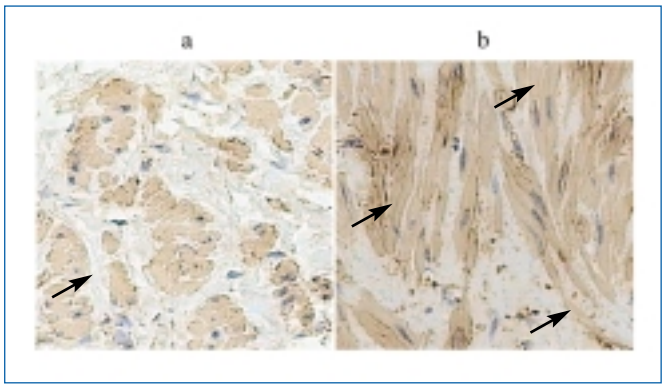

Figure 3. TGF-ß receptor type I immunostaining in the normal (a) and incompetent (b) veins.

PROGRAMMED CELL DEATH (APOPTOSIS) IN VASCULAR REMODELING

Another important factor in embryonal, neonatal, and postnatal vessel remodeling is the programmed cell death (PCD).[42,43] This method of cell elimination is one of the most important mechanisms responsible for vessel wall structure changes. Apoptosis can occur as a normal physiological process, which controls development and tissue homeostasis.[42-44] PCD and its disturbances can be also involved in many pathological events such as cardiovascular diseases or malignancy.[43,44] In the cardiovascular system a number of studies confirmed the role of PCD in vessels that remodel postnatally.[42,43] Apoptosis can also be present in some chronic vascular conditions such as aortic aneurysm, atherosclerotic or restenotic lesions.[43] PCD plays an important role in the removal of unwanted cells; however in some conditions properly functioning cells are also eliminated via apoptotic death.
Up till now, there have been very few studies performed describing the relationship between apoptotic cell death and varicose vein occurrence.[19,45,46] Theoretically, an increase in PCD within the vein wall (especially SMC apoptosis), could lead to the elimination of this important component of the vein wall. Successive vein structure destruction as well as the decrease in venous tone could result in the vein wall weakening and its further dilation.
According to the very few previously performed studies, contradictory results were reported. In the paper published in 1999 by Bujan and coworkers, a relatively high apoptotic cell rate was reported, concerning 41% to 97% of cells.[19] According to Ascher, in the varicose vein wall very few apoptotic cells were reported, and down-regulation of the PCD within the dilated vein should be rather suspected.[45,46]
Verifying the previously published results by the means of TUNEL (Tdt mediated dUTP nick end labeling) method, as well as by an assessment of apoptosis-regulating genes mRNA and protein expression (FAS, p53, BAX, BCL-2), we did not confirm the hypothesis suggesting the role of the apoptosis activation as a principal mechanism leading to the reduction in smooth muscle cell number within the vein wall.[27] Very low indexes (from 0.017 to 0.12) of apoptotic cells were reported within intima, media, and adventitia of both incompetent and healthy control veins. The apoptotic cells were present in the intima in 45% of the specimens only (versus 38% for the control veins). For the media and adventitia the respective values were 93.2% (versus 93.7% in control) and 82,5% (versus 73% in control) (*Figure 4*). Concerning patients' age (two groups of patients were evaluated – younger and older than 50 years), in the young patient group an increase in apoptotic index within the media of the incompetent crural saphenous vein was reported. In the older patients, as well as in the prox-

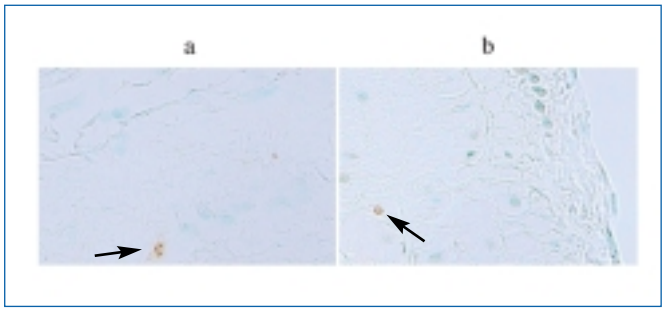

Figure 4. Apoptotic cells within the media of incompetent saphenous vein (TUNEL–Tdt mediated dUTP nick end labeling – in situ study).

imal saphenous vein segments, there were no differences in the apoptotic index values. Concerning the character of the apoptotic cells, the presence of apoptotic SMCs in the intima and media, as well as apoptotic SMCs and macrophages within vein adventita was documented.

THE ROLE OF PROGRAMMED CELL DEATH IN VARICOSE VEIN WALL REMODELING: APOPTOSIS - REGULATING PATHWAYS

Various factors influencing the activation of PCD were discovered.[43,47] Among many pathways leading to apoptotic cell death activation, two were most commonly investigated: the "death receptor" pathway (related to the presence of so called "death receptors" of the TNF receptor family), and the "mitochondrial" pathway (related to the activation of BCL-2 protein family members and controlled by p53 transcriptional factor activity) (*Figure 5*).[43,44,47,48] All of them activate the cascade of intracellular apoptosis executory enzyme - caspases, however, in the initial stage of the apoptotic changes, a balance between pro- and antiapoptotic signaling regulates cell viability.[47,48]

According to our study, in young patients with saphenous vein incompetence and shorter disease duration an increase in p53 activity (mRNA expression level and p53 - positive cell number in the immunostaining in the vein media) in the distal (crural) saphenous vein segment was present.[27] In these specimens higher (proapoptotic) BAX to (antiapoptotic) BCL-2 mRNA expression ratio was also found, suggesting the role of "mitochondrial" pathway activation in this group. These findings correlated with a statistically significant increase in the media apoptotic cell index in this group. In the proximal vein segments, as well as in older subjects, despite previously mentioned structural changes related to extracellular matrix accumulation, the lack of enhanced apoptotic activity was reported. Simultaneously, in the older population with consequently longer disease duration, the total SMC

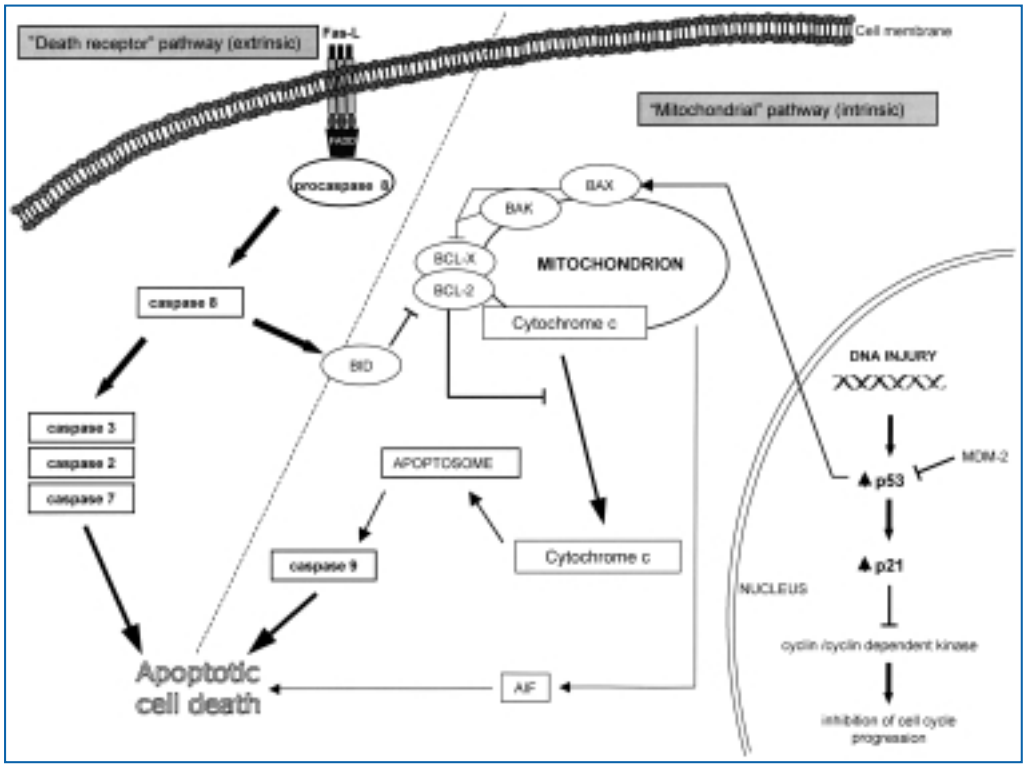

Figure 5. Apoptosis activation pathways ("mitochondrial" and "death receptor"pathways) BAX, BAK, BCL-2, BCL-X, BID – members of BCL-2 protein familiy, AIF – Apoptosis Inducing Factor, Fas-L – ligand of the Fas death receptor, FADD – Fas Associated Death Domain.

amount within the vein media of incompetent veins was bigger than in young subjects, although in the morphometric analysis. In both groups the presence of SMC hypertrophy (in the morphometric analysis) the vein media was reported that suggest the accumulation of hypertrophic, secretory SMCs along the disease course.
According to our findings, activation of PCD seems to be related to the relatively early stages of varicose vein disease.[27] In the further course of the disease, the down-regulation of p53-dependent apoptotic cell death within the media of the incompetent veins influences the further structural change increase due to hypertrophic SMC accumulation and secretory activity. Of course, apoptotic elimination could also concern normal cells surrounded by accumulated extracellular matrix. However, we did not document enhanced apoptotic activity in the specimens, where extracellular matrix totally disrupted SMC bundles separating several SMCs. There was also no correlation between apoptotic cell death and FAS death, receptor mRNA expression or its protein presence, although the presence of other apoptotic pathways should also be taken into account. The lack of apoptosis upregulation within varicose vein media as well as the role of mitochondrial pathway of the programmed cell death activation was also confirmed in the study published recently (2005) by Ducasse and coworkers.[49]
As we could not confirm any differences concerning apoptotic activity in the proximal incompetent saphenous vein segments, where the reduction in SMC amount within the vein media was also observed, we looked for other mechanisms that could control in SMC population within the vein wall.[32]

According to many authors, during varicose vein development the thickening of the vein intima occurs due to extracellular matrix accumulation that results in the course of intimal and medial SMC transdifferentiation, proliferation, and migration into the subendothelial layer.[2,20,28,30] On the other hand, in some previous studies conflicting data concerning proliferating activity within the varicose vein wall were reported, especially if we take into consideration the possibility of the presence of atrophic (with complete lack of cellular elements) and hypertrophic segments within the same dilated vein. An increase in the number of the proliferating cells (assessed by the means of anti-PCNA immunostainings) was suggested in the paper published by Bujan.[19] In another study (Bader-Commander and coworkers) using anti-PCNA and anti-Ki67 immunostainings, the lack of an increased rate of proliferating cells was reported.[20]

The role of disturbances of the molecular regulation of cell cycle within the varicose vein wall was for the first time investigated by Papas the means of retinoblastoma protein (Rb) assessment.[50] Phosphorylation of Rb induces release of transcriptional factors (E2F) that activate the genes required for the cell cycle progression. The authors suggested a role of Rb not only in cell cycle control, but also in the dedifferentiation and phenotype change within the vein wall.[50] Asher and coworkers assessing cyclin D1 expression (the molecule playing an important role in the cell cycle arrest mediation via p53 dependent pathway), suggest the deregulation of the cell cycle progression in varicose veins.[45]

In our study, an expression of another important molecule p21 was investigated.[27,32] Protein product of p21 (Waf/Cip1) gene was previously identified as a cyclin-dependent kinase inhibitor controlling cell proliferation due to inhibition of the cell cycle progression from phase G1 to phase S.[51] P21 is also a downstream regulator of the p53 tumor suppressor gene which can control not only apoptosis but also the cell cycle.[32,52] The negative regulation of the cell growth by p53 activity is related to p21 induction.[52] In both control and incompetent veins, very low levels of p21 immunopositivity was present.[32] However, in the harvested proximal saphenous vein segments p21 mRNA expression levels as well as the number of p21-positive cells within the vein media were significantly higher than these of the control veins. This corresponded to the decrease in the SMC amount within the vein media of the proximal long saphenous vein, although previously described morphological changes, related to the local extracellular matrix accumulation and vein structure disorganisation, were present also in these vein segments.

The lack of the enhanced apoptotic activity in the region of the proximal valve can suggest the role of cell cycle regulation disturbances or SMC transdifferentiation but not apoptosis in the vein wall destructive changes leading to this valve incompetence. In particular, if the presence of unchanged, in pathological examination, proximal valve cusps could be found in some patients in whom an incompetence of proximal valve was clinically and sonographically confirmed (with simultaneously microscopically documented vein wall remodeling in the region of valve annulus).

As there is still a lack of consensus as to what is the proper sequence of the events in the initial phase of the disease (primary valve incompetence or primary vein

wall injury), the local differences of the reported results (between proximal and distal vein segments) could also be related to the hemodynamic conditions along the dilating incompetent vein.[1,26,27] The role of the previously discussed factors should be also taken into account (eg, TGF-ß). According to the literature there are some reports describing the possibility of TGF ß1 related and p53-depenedent or independent induction of p21, which is one of the antiapoptotic mechanisms by which this multipotential cytokine (TGF-ß1) can control SMC viability.[53,54] In both distal and proximal incompetent saphenous vein segments the presence of TGF-ß1 expression was confirmed, although, as previously mentioned, the role of other cytokines produced by activated endothelial cells, smooth muscle cells, or cells of inflammatory reaction should be evaluated.

CONCLUSIONS

Despite many papers suggesting the role of valve dysfunction in vein incompetence, valve injury seems not to be the only factor leading to varicose vein development. For vein dilation additional factors related to vein wall homeostasis disturbances are needed, such as SMC transdifferentiation. One of the important mechanisms influencing vein wall homeostasis maintenance is p53-related apoptosis; however, its activation is characteristic to the relatively early stages of the diseases. Further dowregulation of SMC apoptosis within vein media leads to an increase in structural changes and vein wall weakening related to extracellular matrix accumulation.

The reduction of the SMC population corresponding to the p21 expression in the proximal saphenous vein segments suggests the role of the cell cycle disturbances that may lead to vein wall architecture destruction. However, further studies examining the role of other cell cycle-related molecules are needed. As was previously mentioned we still do not know what is the sequence of the events in the vein and valve injury process, although, according to the performed study, some new data can be introduced into varicose vein theory. The necessity of the presence of not only valve incompetence but also the molecular defect of the vein wall homeostatic mechanism can be an important argument explaining the progressive character of vein incompetence in patients with varicose veins. This observation can provide new arguments for discussion of the character of chronic venous insufficiency, which has to be evaluated not as a single vein problem but more as a systemic disease.

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Fax: +420 485 312 466

E-Mail: jan.marusiak@nemlib.cz
Website: www.nemlib.cz

Xth CONVENCION DE CIRUJANOS VASCUALRES DE HABLA HISPANA

This congress will be held in Las Palmas de Gran Canaria, from October 12-14, 2005.

President: Vicente Cabrera Morán
Organizing secretariat:
Apartadod e Correos 24314
08080 Barcelona, Spain

Tel: +34 (0)93 863 05 31
Fax: +34 (0)93 863 05 57

E-mail: cvhh@uriach.com
mailto:cvhh@uriach.com

XIVth CONGRESS OF THE EUROPEAN ACADEMY OF DERMATOLOGY AND VENEREOLOGY (EADV) Skin and sexual health: the challenge for Europe

This congress will be held in London (UK) from October 12 to 16, 2005.

• *For further information, please contact:*

Congress President: Prof Martin Black

EADV Congress Secretariat
4 Fitzroy Square
London WIT 5HQ, UK

Fax: +44 20 7383 0266

E-mail: eadv@bad.org.uk

LYMPHO 2005

This congress will be held in Praha (Czech Republic) from October 14 to 15, 2005.

• *For further information, please contact:*

Congress President:
MUDr Zuzana Navrátilová, PhD

Dermatologická a flebologická ambulance
Jamborova 22
615 00 Brno, Czech Republic

Tel: +420 548 531 690
Fax: +420 548 531 690

E-mail: zuz.navratilova@seznam.cz
Website: www.lympho.cz

XXXth SCIENTIFIC MEETING OF THE GEL (GROUPEMENT EUROPEEN DE LYMPHOLOGIE)

This congress will be held in Brussels (Belgium) from October 16 to 17, 2005.

• *For further information, please contact:*

Congress President: M. Ohkuma

Fabienne WILPUTTE – JP BELGRADO
Department of Vascular Diseases
CUB – Hôpital Erasme
Route de Lennik, 808
1070 Brussels, Belgium

Fax: +32 2 555 35 36

E-mail: Fabienne.Wilputte@ulb.ac.be

CONGRESSO NAZIONALE GIUV

This congress will be held in Milano (Italy) from October 20 to 22, 2005.

• *For further information, please contact:*

Congress President: Prof F. Annoni

Daniele Righi
Viale Mamiani, 24
50137 Firenze, Italy

Tel: +39 (06) 372 9466
Fax: +39 (06) 370 0541

E-mail: segreteria@gccongress.it / segretaria@sidv.net
Web site: www.sidv.net

XIXth ANNUAL CONGRESS OF THE AMERICAN COLLEGE OF PHLEBOLOGY

This congress will be held in San Francisco (USA) from November 10-13, 2005.

• *For further information, please contact:*

President: Neil S. Sadick

ACP Headquarters
100 Webster Street, Suite 101
Oakland, California 94607-3724, USA

Tel: +1 510 834 6500
Fax: +1 510 832 7300

E-mail: acp@amsinc.org

IXth CONGRESSO NAZIONALE COLLEGIO ITALIANO DI FLEBOLOGIA - CIF

This congress will be held in Fermo (Italy) from November 10 to 13, 2005.

• *For further information, please contact:*

Congress President: Prof G. Genovese

D.ssa Andreina Mancini
G.C. CONGRESSI Srl
Via P. Borsieri, 12
00195 Roma, Italy

Tel: +39 (06) 37.29.466
Fax: +39 (06) 37.35.23.37

E-mail: segreteria@gccongressi.it

XIIth ANNUAL NATIONAL CONFERENCE OF VASCULAR SOCIETY OF INDIA (VSICON 2005)

This congress will be held in Kerala (India) from November 10 to 13, 2005.

• *For further information, please contact:*

Dr M. Unnikrishnan
Sree Chitra Tirunal Institute for Medical Sciences & Technology, Medical College
Maharani Sethu Parvathy Bai Surgical Block
Chamber no 3710, 7th floor
Trivandrum
Kerala 695011, India

Tel: +91 471-2524463 / 471-2443152
Fax: +91 471-2524463

E-mail: mail@vsicon2005.com

IXth CONGRESSO NAZIONALE CIF

This congress will be held in Fermo (Italy) from November 11 to 13, 2005.

• *For further information, please contact:*

Congress President: Prof G. Genovese

GC Congressi - Roma

Tel: +39 (06) 3729466
Fax: +39 (06) 3700541

E-Mail: segreteria@gccongress.it
Web site: www.flebologia.unisi.it

XXVIIth CONGRESSO NAZIONALE DELLA SOCIETA' ITALIANA DI ANGIOLOGIA E PATOLOGIA VASCOLARE (SIAPAV)

This congress will be held in Rome, (Italy) from November 16 to 19, 2005.

• *For further information, please contact:*

Congress President: Prof. C. Allegra

Dssa Andreina Mancini
G.C. CONGRESSI Srl
Via P. Borsieri, 12
00195 Roma, Italy

Tel: +39 (06) 37 29 466
Fax: +39 (06) 37 35 23 37

E-mail: segreteria@gccongress.it
Web site: www.siapav.it

VIth ASIAN CHAPTER CONGRESS OF THE INTERNATIONAL UNION OF ANGIOLOGY "UPDATE MANAGEMENT OF VASCULAR DISEASE"

This congress will be held in Bangkok (Thailand) from November 18 to 20, 2005.

• *For further information, please contact:*

Congress President: Prof Novo S.

Chairman organizing committee: Sopon Jirasiritham, MD

Tel: +662 20 11 576
+662 20 11 315
+662 20 11 527
Fax: +662 20 11 316

E-mail: rasja@mahidol.ac.th

XXIInd WORLD CONGRESS OF THE INTERNATIONAL UNION OF ANGIOLOGY

This congress will be held in Lisbon (Portugal) from June 24 to 28, 2006.

• *For further information, please contact:*

Congress President:
Prof José Fernandes e Fernandes

Organizing secretariat:
AISC & MGR – AIM Group
Via Adelaide Ristori, 38
00197 Rome, Italy

Tel: +39 06 809681
Fax: +39 06 8088491

E-mail: iua2006@aimgroup.it

VIIth ANNUAL MEETING OF THE EUROPEAN VENOUS FORUM

This congress will be held in London (UK) from June 30 to July 2, 2006.

• *For further information, please contact:*

President: Prof Alun H. Davies

Anne Taft, Executive Secretary
EVF, Beaumont Associates
PO Box 172
Greenford, Middx, UB6 9ZN, UK

Tel: +44 20 8575 7044
Fax: +44 20 8575 7044

E-mail: evenousforum@aol.com

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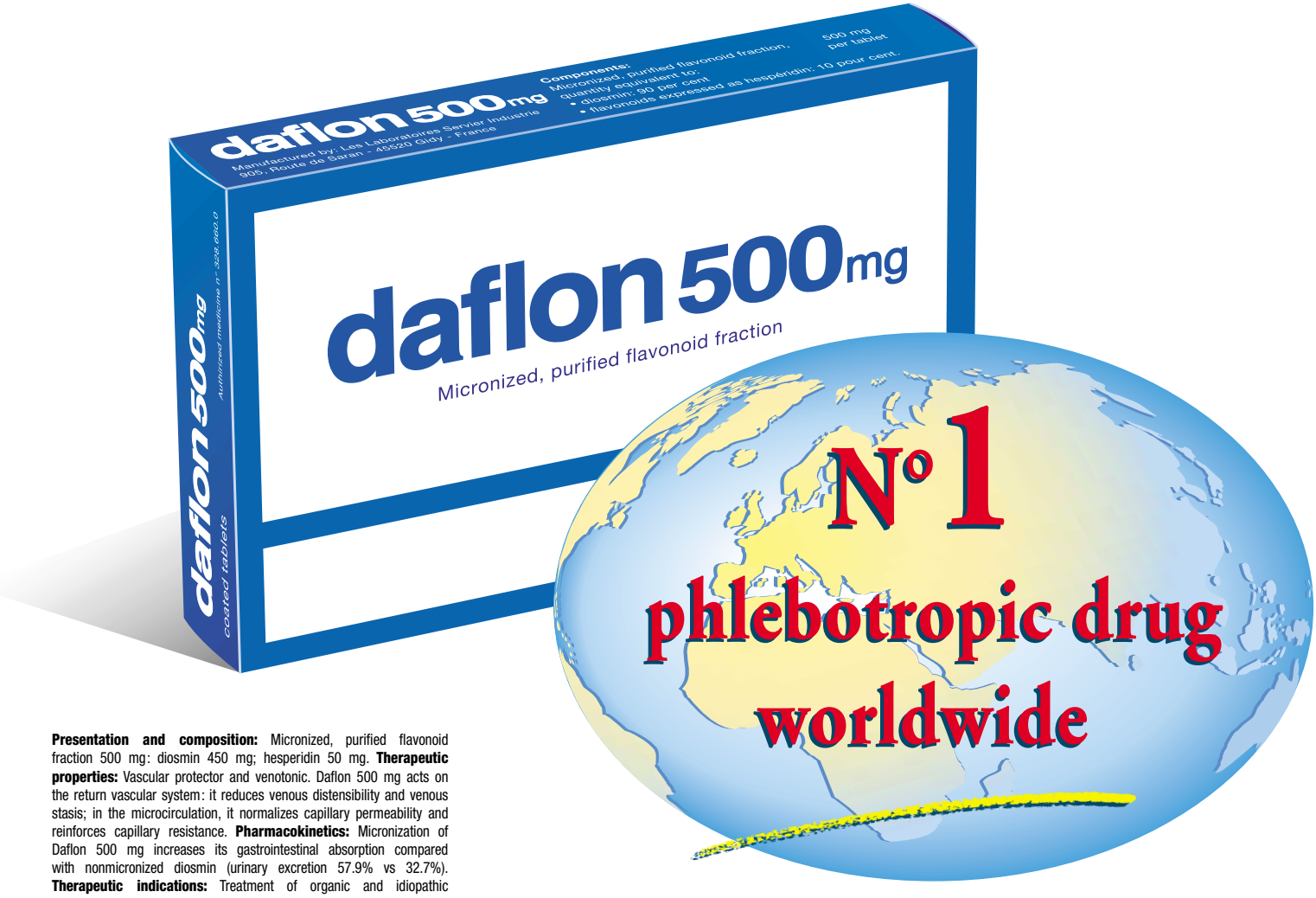

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Chronic venous insufficiency

2 tablets daily

Hemorrhoidal disease

up to 6 tablets daily

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