Refluxing venous valves: from pathophysiology to treatment

“Chance favors only the prepared mind”

Pasteur
Refluxing venous valves: from pathophysiology to treatment

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Although the anatomy of the cardiovascular system was depicted perfectly by Vesalius in his *De Humanis Corporis Fabrica* in 1534, the concept of “circulation” did not exist at the time; heart and vessel function were being explained by Galen’s theory of the “four humors.” Great advances in the description of the venous circulation took place between the mid-16th century and early 17th century. Venous valves were first mentioned in 1544 by the Spanish anatomist Ludovicus Vassaeus in *De Anatomen Corporis Humani Tabulae Quator.* Six years later in 1550, Sylvius Ambianus described the presence of valves in the veins of the lower limbs, and, in 1559, Andrea Cesalpino in *De Re Anatomica* clearly described the function of the valves and the centripetal direction of blood flow into the veins. Hieronymus Fabricius ab Aquapendente published an extensive treatise on venous valves entitled *De Venarum Ostiolis* in 1603. In his treatise, Fabricius not only meticulously described venous valve anatomy and topography of the whole venous system, but also, more importantly, a maneuver to locate venous valves in superficial veins. This maneuver led his student, William Harvey, to explain the circulation of blood in *De Motu Cordis* (1628). The only question Harvey was unable to answer was how blood passes from the arteries to the veins.

The term “blood circulation” was borrowed from Andrea Cesalpino, who coined it in 1571 after he described the centripetal direction of blood flow in the veins and hypothesized the existence of capillaries (*vasa in capillamenta risoluta*). The anatomy of the circulatory system was only completely defined in 1661, when Marcello Malpighi demonstrated in *De Pulmonibus* the existence of capillaries, arterioles, and venules. According to Fabricius, the predominant belief at the time was that venules were avaluated. Nearly four centuries after Vassaeus’s description of venous valves, Canadian pathologist Nicholas Popoff demonstrated the existence of microscopic venous valves (MVVs) in the skin of human digits, in 1934. MVVs were then found in the venules of vascular territories with unfavorable venous hemodynamics, where they fragment the hydrostatic column at the microcirculatory level. The exact role of MVVs in the pathophysiology of venous diseases still needs to be comprehensively evaluated, but Van Rij and colleagues demonstrated in 2011 that MVV incompetence can occur in small superficial veins in the absence of reflux in the saphenous axis and tributaries. This could explain signs and symptoms of venous insufficiency in legs with effective valvular function in the greater veins. In 1998, Aharinejad and colleagues described the treatment of recalcitrant venous ulcers with a free fasciocutaneous flap taken from areas of the skin rich in MVVs. Unfortunately, technical difficulties in evaluating the morphological and functional characteristics of MVVs make this field of investigation very challenging.
The role of venous valves was clearly described by Cesalpino in 1559: “...certain membranes placed at the openings of the vessels prevent the blood from returning...” Then it was postulated that venous valves should fragment the hydrostatic column. This has been the predominant belief since 2012, when Fedor Lurie and colleagues demonstrated experimentally that venous valves not only block reflux, but also play an important role in increasing the efficiency of venous return by creating a helical flow because of their respective angulation.

How do venous valves work? It is generally thought that reverse flow is necessary for venous valve closure. In his studies of the heart, Leonardo da Vinci predicted that the blood stream should divide when passing valves; he found that half the aortic blood flow reverses into the aortic sinus, generating a vortex, while the other half follows the path of the aorta. Vortical flow at the level of the sinus was confirmed experimentally by Carrier in 1922, but it was only much later in 2002 and 2003 that Lurie and colleagues demonstrated the complexity of flow dynamics at the site of venous valves as well as the mechanism of venous valve closure10,11—just as Leonardo had predicted, thanks to a glass model of the aortic sinus. Venous valve closure coincides with the decrease of flow velocity and the ballooning of the sinus. This is one of the major bioengineering concepts underlying the construction of artificial valves.

Abnormalities of this sophisticated mechanism provoke blood reflux, the most frequent abnormality in legs with venous insufficiency. Tommaso Rima was the first to identify the pathophysiological role of venous valve failure in 1806, followed by Brodie in 1846. Both of them had no idea about the causes of venous valve failure.

Bardeleben in 188012 suggested that involutive phenomena were responsible for the progressive reduction in the number of venous valves, and Klotz in 188713 demonstrated that the number of incompetent venous valves increases in venous disease. Powell in 195114 proposed that venous valve function was affected by age, and senile changes in venous valves were effectively demonstrated by Saphir in 1953: “...starting after the age of 30, venous valves undergo a progressive increase in collagen fibers...”15 However, senile changes do not necessarily imply venous valve dysfunction with significant reflux, with the exception of the “partial valves” described by Marinov in 1973.16

Venous insufficiency can also be caused by agenesis of venous valves, which is very rare. Agenesis was first reported in 1941 by Josephus Luke.17 Avalvulia syndrome was clearly defined by Lodin in 1958.18

Besides congenital and primary venous valve failure, there is also secondary venous valve failure, which was first reported in 1937 by Edwards and Edwards.19 They described the destruction of valves following venous thrombosis and related it to the onset of both deep vein insufficiency and varicose veins. Between these two antithetic conditions (physiologic involution and postthrombotic venous valve degeneration), the inflammatory theory of venous valve destruction was introduced in the late 1990s. Ono and colleagues demonstrated that venous hypertension can lead to a chronic inflammatory condition of venous valve leaflets characterized by monocyte infiltration.20

All these contributions, however, did not solve the dilemma of whether valve insufficiency or wall dilation occurs first. Is vein dilation due to or responsible for venous valve failure? In fact, primary structural changes of the valves may make them “leaky,” with progressive reflux causing secondary changes in the vein wall with dilation. Alternatively, or concurrently, the valves may become incompetent secondary to structural abnormalities and focal dilation of vein wall segments near the valve junctions. Accordingly, venous valve reflux ensues as an epiphenomenon of primary vein dilation. In 2008, Raffetto and Khall evaluated the expression of matrix metalloproteinases, which influence the metabolism of extracellular matrix proteins as well as the function of the endothelium and smooth muscle, determining changes in venous constriction/relaxation properties.21 Their observations suggest that vein wall dilation appears to precede valve dysfunction related to metalloproteinase activation, superimposed inflammation, and fibrosis.

Once the predominant role of venous valves in the pathophysiology of venous disease was recognized, how could their efficiency evaluated? In 1603, Fabricius ab Aquapendente involuntarily illustrated the test to evaluate valve competence, but unfortunately the test was not adopted by surgeons who followed him. It was only two centuries later, in 1806, that Tommaso Rima proposed a specific test to evaluate venous valve competence of the saphenous system. This test was then refined by Sir Benjamin Brodie (1846) and Trendeleburg (1890) and remained in use until the introduction of ultrasound in vascular diagnostics. The first clinical experiments in venous ultrasonography were done by Sigel in 196722 and Sumner in 1968.23 The method for detecting venous valve insufficiency was described in detail by Folsé and Alexandre in 1970,24 after the introduction of directional Doppler instruments; this was followed six years later by the first report of venous echotomography of deep vein thrombosis.25 The next milestone in venous valve ultrasound semiotics was in 2002, when Labropoulos and colleagues26 furnished the parameters to evaluate venous valve incompetence by duplex scanning (reflux duration greater than 500 ms in superficial and calf deep veins, and 1000 ms in the femoropopliteal axis).

**SELECTED ABBREVIATIONS AND ACRONYMS**

| MVV | microscopic venous valve |
Despite these technical improvements, semiological differences still remain to be clearly defined between the numerous maneuvers proposed for eliciting venous valve reflux (Valsalva, distal compression/release, and dynamic tests).

Once it was demonstrated that venous valve incompetence was the cause of reflux, the next obvious, clinically relevant question was: how do you treat venous valve failure?

The first to propose a “treatment” for incompetent valves was Astley Paston Cooper, a pupil of John Hunter, who in 1824 affirmed that limb compression by bandaging allowed the venous valves to regain their competence. The only alternative was to ligate the vein. No further suggestions to treat incompetent venous valves appeared until the middle of the 20th century, when Eisemann and Malette created valve-like structures,27 Warren and Thayer proposed saphenopopliteal bypass,28 and Palma and Esperon put forward vein transplantation.29 After these first experimental procedures, it was finally in 1968 that Kistner popularized a technique to restore venous valve function by direct surgery and Psathakis proposed a “substitute valve.”30 Surprisingly, all the techniques for restoring venous competence proposed in the subsequent fifty years were based on the principles of these pioneers.

More recently, over the past 30 years, multiple methods have been developed to surgically restore valvar function, but only a few of them have been applied in humans. Half a century after Eisemann, Corcos reconstructed a monocuspid popliteal vein by intimal flap,31 whereas Maleti and Lugli proposed a technique to safely create an antireflux mechanism in both postthrombotic limbs and in veins with valve agenesia.32

However, the majority of these studies await confirmation by other investigators over extended periods of time. Guidelines or consensus documents that establish which patients should be considered for venous valve surgery as well the criteria for evaluating clinical results are eagerly awaited.

References

Keywords: venous valve; venous circulation; microscopic venous valve; valve dysfunction; history; venous insufficiency.

Venous valves: gateway to the circulation – Caggati
Vésale a parfaitement décrit l’anatomie du système cardio-vasculaire en 1534 dans son *De Humanis Corporis Fabrica*, mais le concept de « circulation » n’existait pas à cette époque. La théorie de Galien dite des « quatre humeurs » expliquait les fonctions cardiaque et vasculaire. Entre le milieu du XVIᵉ siècle et le début du XVIIᵉ, la description de la circulation veineuse fait de grands progrès. En 1544, l’anatomiste espagnol Louis Vassé (Ludovicus Vassaeus) mentionne pour la première fois la notion de valvules veineuses dans son livre *De Anatomen Corporis Humani tabulae Quator*. Six ans plus tard en 1550, Sylvius Ambianus décrit la présence de valvules dans les veines des membres inférieurs et, en 1559, Andrea Cesalpino décrit clairement dans l’ouvrage *De Re Anatomica* la fonction des valvules et la direction centripète du flux sanguin dans les veines. En 1603, Hieronymus Fabricius ab Aquapendente publie un important traité sur les valvules veineuses intitulé *De Venarum Ostiolis*. Dans ce traité, Fabricius décrit méticuleusement non seulement l’anatomie des valvules veineuses et la topographie de tout le système veineux, mais aussi surtout une manœuvre pour localiser les valvules veineuses dans les veines superficielles. Cette manœuvre conduit son élève, William Harvey, à expliquer la circulation sanguine dans *De Motu Cordis* (1628). La seule question à laquelle Harvey ne peut pas répondre est « Comment le sang passe-t-il des artères aux veines ? ».

neux réfractaires à l’aide d’un lambeau libre fasciocutané pris sur des zones cutanées riches en VVM. Malheureusement, des difficultés techniques dans l’évaluation des caractéristiques morphologiques et fonctionnelles des VVM rendent les recherches dans ce domaine très difficiles.

Cesalpino a clairement décrit le rôle des valvules veineuses en 1559 : « …certaines membranes placées aux ouvertures des vaisseaux empêchent le sang de refuer… » L’hypothèse qui a suivi est celle de la fragmentation de la colonne hydrostatique par les valvules veineuses. Cette hypothèse prédomine depuis 2012, lorsque Fedor Lurie et al. démontrent expérimentalement que non seulement les valvules veineuses bloquent le reflux, mais aussi qu’elles jouent un rôle important dans l’augmentation de l’efficacité du retour veineux en créant un courant héliocidal grâce à leur angulation respective.

Comment fonctionnent les valvules veineuses ? Il est généralement supposé que la fermeture des valvules nécessite un flux inversé. Dans ses études sur le cœur, Léonard de Vinci a pressenti que la circulation sanguine se divisait en passant les valvules : il a constaté que la moitié du flux sanguin aortique s’inverse dans le sinus aortique générant un tourbillon, tandis que l’autre moitié suit la voie de l’aorte. La présence d’un flux tourbillonnant au niveau du sinus est confirmée expérimentalement par Carrier en 1922. C’est seulement beaucoup plus tard, en 2002 et 2003, que Lurie et al. démontrent la complexité de la dynamique du flux au niveau des valvules veineuses ainsi que le mécanisme de la fermeture des valvules veineuses, grâce à un modèle en verre du sinus aortique, exactement comme Léonard de Vinci l’avait prévu. La fermeture de la valvule veineuse coïncide avec la diminution de la vitesse d’écoulement et l’expansion du sinus. C’est l’un des principaux concepts de bio-ingénierie à la base de la construction des valvules artificielles.

Des anomalies de ce mécanisme sophistiqué provoquent un reflux sanguin, fréquemment retrouvé dans les jambes ayant une insuffisance veineuse. En 1806, Tommaso Rima est le premier à identifier le rôle physiopathologique de l’insuffisance valvulaire veineuse, suivi en 1846 par Brodie. Ni l’un ni l’autre ne connaît les causes de l’insuffisance valvulaire veineuse.


En 1937 Edwards et Edwards sont les premiers à observer une insuffisance valvulaire veineuse secondaire en plus de l’insuffisance valvulaire congénitale et primaire. Ils décrivent la destruction des valvules suite à une thrombose veineuse et la rattachent à la survenue d’une insuffisance veineuse profonde et à des varices.

Entre ces deux pathologies contradictoires (involution physiologique et dégénération valvulaire post-thrombotique), la théorie inflammatoire de la destruction des valvules veineuses est introduite à la fin des années 1990. Ono et al. démontrent que l’hypertension veineuse peut conduire à une pathologie inflammatoire chronique des feuillots valvulaires veineux caractérisées par une infiltration de monocytes.

Toutes ces suppositions, cependant, ne répondent pas à la question de savoir qui de l’insuffisance valvulaire ou de la dilatation pariétale apparaît en premier. La dilatation veineuse est-elle due à, ou responsable de l’insuffisance valvulaire veineuse ?

En fait, des modifications structurelles primaires des valvules peuvent diminuer leur étanchéité, un reflux progressif provoquant des modifications secondaires avec dilatation dans la paroi veineuse. De façon alternée ou simultanée, les valvules deviennent incontinentes à la suite d’anomalies structurelles et d’une dilatation locale des segments pariétaux veineux près de la jonction valvulaire. Le reflux valvulaire veineux en résulte donc comme un épiphénomène de la dilatation veineuse primitive. En 2008, Raffetto et Khalil évaluent l’expression des métalloprotéinases de la matrice, qui influent sur la métabolisme des protéines extracellulaires de celle-ci ainsi que sur la fonction de l’endothélium et du muscle lisse, déterminant des modifications des propriétés veineuses de constrictions/relaxation. Leurs observations suggèrent que la dilatation de la paroi veineuse précède la dysfonction valvulaire liée à l’activation de la métalloprotéinase, à l’inflammation qui s’y rajoute et à la fibrose.

Une fois reconnu le rôle prédominant des valvules veineuses dans la physiopathologie de l’insuffisance veineuse, comment évaluer leur efficacité ? En 1803, Fabricius ab Aquapendente illustre involontairement le test pour évaluer l’incontinence valvulaire, qui n’a malheureusement pas été adopté par les chirurgiens qui lui ont succédé. Ce n’est que deux siècles plus tard, en 1860, que Tommaso Rima propose un test spécifique d’évaluation de la continence valvulaire veineuse du système saphène. Ce test est ensuite amélioré par Sir Benjamin Brodie (184) et Trendeleburg (1890) et reste utilisé jusqu’à l’arrivée des ultrasons dans le diagnostic vasculaire. Sigel en

Malgré ces améliorations techniques, des différences sémiologiques restent à définir clairement entre les nombreuses manoeuvres proposées pour rechercher un reflux valvulaire veineux (manœuvre de Valsalva, compression/relâchement distal et tests dynamiques).

L’incontinence valvulaire veineuse une fois démontrée comme la cause du reflux, la question évidente, cliniquement pertinente est : comment traiter l’insuffisance valvulaire veineuse ?

Astley Paston Cooper, un élève de John Hunter qui, en 1824, affirme que la compression de la jambe par bandage permet de redonner leur continence aux valvules veineuses, est le premier à proposer un « traitement » des valvules incontinentes. La seule alternative est de ligaturer la veine. Aucune autre suggestion pour traiter les valvules veineuses incontinentes n’apparaît jusqu’au milieu du XXe siècle, lorsque Eisemann et Malette créent des dispositifs imitant les valvules, Warren et Thayer proposent un pontage saphéno-poplitée et Palma et Esperon proposent une transplantation veineuse. Après ces premières procédures expérimentales, c’est finalement en 1968 que Kristner vulgarise une méthode pour restaurer la fonction valvulaire veineuse par chirurgie directe et que Pethakis propose un « substitut de valvule ».

Plus récemment, ces 30 dernières années, de nombreuses méthodes ont été développées pour la restauration chirurgicale de la fonction valvulaire, mais seules quelques-unes sont appliquées chez l’homme. Cinquante ans après Eisemann, Corcos reconstruit une valvule poplitée monocuspide grâce à un clapet intimal, tandis que Maleti et Lugli proposent une technique pour créer de façon sécurisée un mécanisme antireflux dans les jambes et les veines post-thrombotiques ayant une agénésie valvulaire. Cependant, la plupart de ces études attendent d’être confirmées par d’autres chercheurs sur une période prolongée. Nous attendons donc impatiemment les recommandations et consensus définissant les patients à l’indication de chirurgie valvulaire ainsi que les critères d’évaluation des résultats cliniques.
Sequential treatment has the objective of extracting the system from the attractor basin in order to stabilize it. Sequential treatment starts with inhibiting endothelial-leukocyte activation and reducing venous distention. Next, it progresses through to controlling transversal, pumping reflux and ends with improving longitudinal, gravitational reflux. The use of any single treatment has proven to be insufficient to interrupt disease progress due to multiple pathogenic mechanisms.

The significant impact of lower limb venous lesions upon a patient’s quality of life explains why knowing about the mechanisms of chronic venous disease is of such value. With the impressive evolution in investigative methods, we now have the opportunity to explore new pathophysiological concepts that have been postulated. The latest imaging investigations—endoscopy and ultrasound—have distinct roles. Endoscopy is currently an invasive procedure used for research purposes, but with great potential for use in minimally invasive endovenous surgery. Information obtained with this investigation method—regarding endovenous morphology, valvular morphology and dynamics, and pathological changes relating to these—is genuinely new and has led to the introduction of new venous concepts, such as valvular segments, commissural slits, commissural reflux channels, etc. Ultrasound examination, a noninvasive method, provides exceptional data about venous anatomy (eg, valve position and dynamics) and transvalvular hemodynamics (reflux and turbulence). Ultrasound procedures can show reflux gates in the deep venous system, venous reflux eccentricity or axiality, and the length and duration of reflux. In addition, the effectiveness of treatments can be determined with this investigative technique. The importance of ultrasound is illustrated by the fact that a phlebological intervention cannot be performed without this examination. Endoscopy and ultrasound for the morphological and dynamic evaluation of the venous system are indisputable means of advancing the development of phlebology and of helping make the most appropriate treatment decisions for patients with venous lesions of the lower limb.

Before addressing the main topic of the paper, it is necessary to clarify some essential notions and concepts for an accurate understanding of vein structure and function under normal and pathological circumstances. Venous circulation is a subsystem of the circulatory system. Its functionality develops in three phases: stability, oscillatory, and chaotic. As paradoxical as it may seem, the chaotic phase contributes to the adjustment of flow rhythms under variable stress conditions. By exploiting chaotic oscillations with a low energy intake, flow rate is brought back to the dynamic stability phase, after passing through the oscillatory phase.

Just as the body, as a dynamic system, extracts negentropy from the surrounding environment in order to amplify its internal structural stability, the venous system in the same way uses deterministic chaos as a reservoir of potentiality. It is important
that the ratios of the durations of the above mentioned phases stay in balance; the dynamic stability phase needs to be prevalent, as the oscillation phase is transitional in nature. Changes in the structure of the valvular segment lead to the alteration of venous flow patterns, which amplifies parietal-valvular structure changes, both locally and within the whole venous system.

The older the venous disease, the greater the extent of venous remodeling. Multisequential, accurate, and early treatment provides the best results and impedes the progress of chronic venous disease of the lower limb towards later stages associated with disability. The pathogenic cycle starts with local hemodynamic phenomena (stasis and temporary increase in venous pressure at the transversal, pumping reflux gates), progresses with morphological changes of the valvular segments and venous walls, and finishes with longitudinal, gravitational reflux. This pathogenic cycle is a strange attractor (chaos theory), and it is proof of dynamic system destabilization of venous flow.

Sequential treatment has the objective of extracting the venous system from the attractor basin in order to stabilize it. Sequential treatment starts with inhibiting endothelial-leukocyte activation (eg, by using Detralex, flavonoids, etc) and reducing venous distention (compression). Next, it progresses through to controlling transversal, pumping reflux (femoral-saphenous, popliteal-saphenous, via the perforating veins) and ends with improving longitudinal, gravitational reflux (via ablations and sclerosis of saphenous trunks using various methods and technologies). The use of any single treatment has proven to be insufficient to interrupt disease progress due to multiple pathogenic mechanisms. Valvular insertion ring. This is a dense, muscular collagenous structure; at this level, the diameter is smaller than the rest of venous section. It represents the point at which blood enters the valvule. Its position may be studied by ultrasound exam, while endoscopy may visualize the ring as a circular threshold in the venous lumen, during the open valvule stage.

Valvular defile. The valvular defile is limited by the cusp sides and has the shape of a flat cone, with the base diameter parallel to the skin surface. Its length is determined by the valve size. The open-section area is approximately elliptic in shape, with obvious angulation towards the commissures. This caliper constraint (at least 1/3 of the venous lumen) induces an increase in venous flow speed and a consequent drop in static pressure on the luminal side of the cusps, which is transmitted across the cusps into the sinus.

Valvules may be considered flow accelerators. The increase in dynamic pressure in the valvular channel encourages the flow of blood from collateral veins and perforating veins (the Venturi effect). Valvules also have the role of flow stabilizers; in the narrow section of the valvule, laminar flow is partially restored. Downstream valvular flow can be easily highlighted via ultrasound (“flow line clusters” — linear ordering of red blood cells). The simultaneous drop in static pressure within the sinus leads to the attraction of blood that exerts force on the cusps and impedes their lining to the venous wall (the intermediary position of cusps during flux as revealed by echo-Doppler examination).

Valvular sinus formation is obviously determined by hemodynamics, and not gravity. In the superior vena cava, the valvular sinuses are open towards the heart. The jugular sinus is very well represented and easy to picture. In decathletes and marathon runners, the narrow valvular section is longer due to valvular hypertrophy that occurs as a result of adaptation to high dynamic pressure (high shear stress).

The exit orifice of the valvular defile corresponds to a closed curved line tracing the free side of the cusps found in different cross-sectional areas. Upstream valvular flow can be described as a jet produced by constriction down the free side of the cusps. Flow speed is increased by constriction at the valvular gap and also by other mechanisms, eg, vis a tergo (force driving the venous return of peripheral blood) and efficient dynamics of the musculovenous pumps. The increase in flow speed is followed by an additional reduction in the cross-section of flow (via a drop in pressure on the luminal side of the cusps). Soon after exiting the valvular narrowing, the flow lines compress, the respective flow cross-section being minimized (“constriction index” in fluid mechanics). Subsequently, the area of action of the flow widens via aspiration into the surrounding fluid (the efflux phenomenon).

At the frontier of the laminar flow that leaves the valve into the surrounding blood, inertial resistance appears that inverts the marginal flow vectors, resulting in vortices towards the venous wall that lead to the emergence of a “dead water” area. Flow in this area has a circular motion that increases in line with the speed of efflux. Intrasinus rotational flow represents a stabilizing influence for the sinus endothelium of the cusps and, at the same time, a protective, antigavitational hemodynamic phenomenon for the cusp-parietal channel, where the commissural clefts are under low pressure.
and their terminal marginal “icicles” amplify the valvular efflux phenomenon and protect the valvular sinuses. In the closed position with the cusps in contact, the gravitational and stress pressure vectors of the valves are redirected towards the sinus wall.

The valvular sinus is the space limited by the parietal sides of the cusp, cusp-parietal angle, and venous wall. It is shaped like a bulb, with the maximal diameter above the cusp’s insertion ring. The valvular sinus is filled with blood, which, contrary to general opinion, does not stagnate, but flows in a circular pattern. This circular flow prevents blood stasis and the concentration of procoagulant factors, possibly to counterbalance the high expression of C-reactive protein and thrombomodulin receptors. Moreover, it provides shear stress at a physiological level necessary for the trophicity and stability of sinus endothelial cells and of the glycocalyx film in the sinus. The ultrasound sections in natural contrast highlight turbulence dynamics in the valvular sinus. The presence of blood in the valvular sinuses allows the cusps to maintain an “on hold” position, allowing prompt valve closure at flow reversion.

Our endoscopic observations established that there is a dynamic pattern of valve opening and closing in normal valves: opening has an eccentric, commissural debut, which continues along the venous axis; retrograde flow causes closure, which follows the same order as opening, but in reverse.

The open valve, with its diaphragm-like particularity, firstly amplifies movement resistance and secondarily contributes to the increase in inertial resistance of the system. In the closed position, it exerts a clack valve effect on subvalvular flow, with consequences on flow rate. At the time of complete closure of the valvule, under the action of hydraulic (hemodynamic) shock, a Windkessel-like phenomenon is produced.

Turbulence resulting from the reversal of flow is produced both on the cardial side of the valve and under the valve, with mainly hemodynamic consequences (stasis and turbulence), but also thermodynamic ones that have not yet been clarified (energy conversion, heat transfer to the venous wall, possibly the inhibition of \( \alpha_2 \) adrenal receptors). Without the existence of ultrasound, all these phenomena would not be known.

The above description is also valid when the valvular system is placed in a horizontal position. In the case of valves in the saphenous and perforator veins, the valves are approximately positioned in the sagittal plane, perpendicular to the axis of flow. In the first case, deterioration of the valvular component leads to longitudinal, gravitational reflux. In the second case, reflux is transverse, generated by the increase in pressure within the “venous room,” which is closed and driven by the abdominal pump. This is an argument for describing the two distinct types of reflux in terms of direction and mechanism: (i) a longitudinal, gravitational reflux; and (ii) a transverse, pumping reflux. Gravitational reflux takes place preferentially in the superficial venous system (due to particularities of tissue texture and composition) and also in the deep venous system, when valves are insufficient or destroyed. Pumping reflux transfers blood from the deep venous system to the superficial venous system (Figure 1).

Acquired venous pathology emerges due to structural changes in the valvular segment components, followed by the progressive perturbation of venous reversion. The morphological processes are, most frequently, degenerative ones, which explains the high incidence of primary varicosity in adults and the elderly.

Primary inflammatory changes are less common in terms of incidence, but their severity is greater and they have important functional consequences, seriously affecting patients’ lifestyle. Postthrombotic syndrome, which principally affects the deep veins of the lower limbs, progresses in an oscillatory manner, with recurrences and no permanent disappearance of inflammatory phenomena.

Venous endoscopy

Venous endoscopy, a recent method of exploring the vascular system, has provided genuinely new information about endovenous morphology, the valvular system, valvular dynamics, and transvalvular blood flow. Even though venous endoscopy does not yet benefit from dedicated equipment, it has real potential for promoting minimally invasive endovenous procedures in deep, extended thromboses in primary valvular insufficiency. This invasive method, which has only been used in research so far, has introduced new concepts of functional anatomy and pathology, eg, the valvular segment, commissural reflux cleft, commissural reflux channel, endophlebitis, etc.

Following endoscopic observations, we proposed a pathogenic classification of valvular lesions:
Functional valvular lesions (type I)

Functional valvular lesions are dysplastic and determined by progressive prolonged increases of retrograde venous pressure. Obviously, the valvular segment suffers most because of the mechanic effect of blood stasis during prolonged standing. However, the involvement of inherited, hormonal, nutritional, and behavioral (eg, fashion) factors cannot be excluded from the pathogenic picture. There are two distinct types of functional valvular lesion:

Commissural reflux slit (type Ia)
This appears as a small commissural opening of the valve. Upon standing, static and dynamic pressures are directed along the valve slopes toward the cuspid-parietal groove. The venous cross-section geometry changes from an ellipsoid to cylindrical shape. Consequently, circumferential movement of cusp insertion generates a tennis racket–shaped commissural slit with its tail directed toward the venous axis, through which a progressively larger volume of blood drains back transvalvularly. The valvular commissures are the valvular feature most at risk from damage from a progressive and prolonged increase in venous pressure.

Commissural reflux channel (type Ib)
The commissural reflux channel (Figure 2) represents an obvious increase in the reflux cleft. It is delimited by the valve commissure, the sinus wall, the cranial surface of the valvular insertion ring, and axial side of the cusps. Refluent blood is often projected from the threshold ring toward the opposite venous wall, leading to asymmetrical development of venous enlargement under the valve.

The difference between these two subtypes is morphological and clearly correlates with reflux volume.

Traumatic organic valve lesions (type II)

- Valvular ruptures
Valvular ruptures may occur with large, sudden stress, which leads to a significant increase in short-term retrograde venous pressure on the cardial side of the valves. The following types can be distinguished:

- Commissural ruptures: clefts and tears (type IIa)
These ruptures flutter in the flow of moving liquid. Depending on the severity of the lesion, ultrasound imaging may reveal eccentric transvalvular reflux, which is more clearly identifiable in transverse cross-sections although this can sometimes be identified in longitudinal cross-sections as well.

- Cusp insertion lesions: linear perforations close to the cusparietal insertion (type IIb), without any hemodynamic consequences
Valvular ruptures are not purely traumatic in origin, with leukocyte infiltration on the cusp cranial side and stimulation of endothelial cell apoptosis also determining breaches in their structure. Nevertheless, trauma is the most important factor, which explains why ruptures occur in the valve insertion or nearby, where the strength of the dynamic reflux force is greatest. In these cases, lesion direction is perpendicular to the direction of reflux.

Valvular ruptures, which are most frequently located in the commissures or, more rarely, at the insertion base, lead to valvular failure. The presence of ruptures at this level is an indicator of high hemodynamic stress at the cusps. In line with other endoscopic observations, inflammatory alterations of the valves were not particularly apparent, maybe due to the absence of vascularization at the cuspal level (bradytrophic structure).

Inflammatory organic lesions (type III)
The inflammatory structural remodeling of valves has only been observed in the context of a more extended involvement of the venous wall, with changes in caliber, shape, and parietal stiffness. However, there are some who believe that inflammatory valvular damage is of a primitive and solitary type, arguing that there is a highly significant increase in the expression of adherence molecules and subendothelial traces of monocytes on the cardial side of valves.

Valvular vestiges (type IV)
Here, we have included the valvular debris that appears as a consequence of the dysplastic process and/or iterative traumas caused by the increase in intrathoracic abdominal pressure. They are most often mistaken for endothelial folds and fringes. The sinuses are missing and the venous tracts are almost entirely tubulated.

In postthrombotic syndrome, the valvular segment and extrasegmentary venous wall are polymorphic lesions, in various stages of development. Lesion severity, established en-
dososcopically, is greater in distal segments, where venous hypertension and stasis are permanent, with consequences in neighboring tissues (extravenous hypertension, intratissue hypertension). In seriously affected veins, valvular segment vestiges can hardly be recognized.

At the confluence of the important tributaries of large saphenous vein, I have sometimes seen pearl-colored polyps. Their aspect is similar to that of a champagne cork blocking the collateral vein ostium, and they impede the passage of the endoscope. It is possible that valvular polyps might have developed on an adherent thrombus that has subsequently undergone fibrous remodeling and endothelialization. Their accidental discovery is connected with the introduction of endoscopy.

Ultrasound

Ultrasound is a modern method for noninvasively investigating the venous system. Ultrasonography is capable of high-lighting changes in the valvular segment, venous trajectory (sinuositites, elongations), and diameter (venodilation) as well as the hemodynamic consequences—reflux and turbulence—of these changes. As mentioned before, the valvular segment represents an essential “piece” of the venous system in order for proper venous function to occur. With natural contrast, in B mode, under physiological conditions, ultrasonography highlights the Windkessel-like phenomenon in the inguinal “venous junction,” with valvular continence, both in the great saphenous vein junction and in the femoral (superficial) vein, situated immediately under the confluence with the deep femoral vein. When intra-abdominal pressure increases—eg, during the Valsalva maneuver—the retrograde wave determines the rise in pressure in the upstream valvular segment. The special morphology and positioning of the cusps transfer maximum pressure into the valvular sinus. The cusps, found in an intermediary position, are medially pushed; they come into contact on their axial side and close firmly, at the same time as plateau descent. Both the closed-valvule stage and the open-valvule stage are oscillatory, occurring in synchronization with flux-induced oscillations. Sometimes, one can visualize the transvalvular retrograde shock wave transfer through the plateau, as subvalvular turbulence.

Venous diameter measured in the sinus clearly increases, with the venous wall elastically expanding and storing the kinetic energy of the reflux wave blocked by the continent valves. When flow restarts, the potential energy transfers to the flow, which increases in speed, and the diameter goes back to its original size before the Valsalva maneuver.

Ultrasoundography can be performed in various ways (B mode, color duplex, power-Doppler, spectral-Doppler) to investigate the following:

- **Functional hemodynamic events.** These events include axial reflux, transvalvular reflux, reflux/flux collision, and reflux turbulence at a transvalvular level. With color duplex ultrasound imaging, in a transverse cross-section, eccentric transvalvular reflux may be easily highlighted.

- **Structural changes.** These changes can include venous diameter, loss of the valvular sinus, and “fractured,” amputated, inverted, “frozen,” or floppy valves (Figure 3). We systematically explored the Cockett perforators and all the insufficient perforators. In the inguinal “venous junction,” we frequently found an interesting hemodynamic phenomenon in patients.
with old, voluminous varices in the great saphenous vein region: the transfer of blood from the deep femoral vein directly into the saphenous vein junction (Figure 4). The saphenous junctions are highly distensible (Ø >20 mm) and offer favorable conditions for the transfer of venous blood during the Valsalva maneuver directly from the deep femoral vein, situated posteromedially, into the anteromedial saphenous vein junction. The saphenous junction acts as a “reception area” as a result of its remarkable capacity to distend, its compliance to thermic stress being twelve times higher (358%) than that of the common femoral vein (29%).

Postoperative functional results. Ultrasonographically, the postoperative success of therapeutic procedures—eg, valve continence, “fixed splint” (Figure 5), and relapses—can be checked.

Obtaining ultrasound evidence for both cusps is not always possible under similar resolution conditions due to the variability of their position in distinct planes. Most commonly, with venous section enlargement, “polychromatic” reflux (turbulence) is highly suggestive of valvular failure. Such an image, even in only some sections, is evidence of valve insufficiency.

Conclusion
Venous endoscopy and ultrasound are relatively recent and highly effective procedures for the morphological and dynamic evaluation of the venous system. They undoubtedly represent a means of developing knowledge in the medical specialty of phlebology, with practical importance for accurate diagnosis. Through the information these techniques provide, they help provide important evidence for helping clinicians make the most adequate therapeutic decisions. Venous endoscopy is likely to become a major procedure in minimally invasive endoluminal therapy, with the continuing technological development of endoscopes and adequate instruments. Ultrasound is a complementary procedure and the ideal posttreatment method for evaluating the results of venous interventions.

References

Figure 4. Complex longitudinal and transverse reflux in the inguinal “venous junction” during the Valsalva maneuver. With dynamic imaging, one observes that deep femoral venous flow runs through the femoral vein to the saphenous junction, from the beginning to the end of the Valsalva maneuver.

Figure 5. A synthetic patch (indicated by the two arrows) wound around the femoral vein acts as a “fixed splint” that maintains valvar continence.

Abbreviations: DFV, deep femoral vein; FV, femoral vein; GSV, great saphenous vein.
Keywords: venous endoscopy; ultrasound; valvular segment

Observations endoscopiques et ultrasonographiques des lésions des valves veineuses

Les mécanismes de la maladie veineuse chronique permettent de comprendre l’impact significatif des lésions veineuses des membres inférieurs sur la qualité de vie des patients. À ce jour, les méthodes de recherche ayant évolué de façon impressionnante, de nouveaux concepts physiopathologiques sont exigés et encouragés. Une place toute particulière est accordée à l’échographie et à l’endoscopie, acquisitions les plus récentes de l’imagerie médicale. L’endoscopie est une procédure invasive, actuellement réservée à la recherche, mais dotée de perspectives intéressantes en chirurgie endoveineuse mini-invasive. Les informations sur la morphologie endoveineuse, la dynamique et la morphologie valvulaires et leurs modifications pathologiques sont substantielles et ont rendu possible l’introduction de nouveaux concepts comme le segment valvulaire, la ferite commissurale, le canal commissural de reflux, etc. L’échographie, méthode non invasive, fournit des données exceptionnelles sur l’anatomie veineuse : la dynamique et la position valvulaires, l’hémodynamique transvalvulaire, le reflux et les turbulences. Grâce à différentes procédures échographiques, les « barrières empêchant le reflux » [NDT : les valves] issues du système veineux profond, l’excentricité ou l’axialité du reflux veineux, ainsi que son étendue et sa durée peuvent être détectées. Il n’est pas possible actuellement de réaliser une intervention phlébologique sans échographie, qui permet aussi d’objectiver l’efficacité des traitements. Ces méthodes d’imagerie morphologiques et dynamiques du système veineux sont des moyens irrefutables pour le développement de la phlébologie, aidant aussi à la mise en œuvre des décisions thérapeutiques les plus adaptées.
The vast majority of patients with chronic venous disease (CVD) have reflux in the superficial veins, with the saphenous veins and their tributaries having the highest prevalence. This is true for all CEAP classes from 2 to 6. Variable patterns of reflux have been described in the great saphenous vein and small saphenous vein. The location and extent of reflux in the saphenous veins has been associated with severity of CVD.

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Venous reflux location and clinical severity of CVD: a complex link – Labropoulos

In the last 20 years, a number of articles have reported a correlation between venous reflux location and clinical severity in patients with chronic venous disease (CVD). Work from epidemiological, cross-sectional, and prospective cohort studies has identified important issues regarding this association. Overall, there is a connection between reflux location and severity of CVD, but the strength of this association has not been determined, as many other factors play an important role. Some pertinent factors include amount of reflux, efficiency of the calf-muscle pump, duration of CVD, lifestyle, obesity, physical activity, and biological responses of tissues in the affected areas. Furthermore, there is no clear distinction between symptoms arising from CVD and those arising from other causes that may coexist with or be separate from CVD. The nature of what the patient feels has not been studied in depth, because of the complexity of a patient’s neuropsychological reflections on limb symptoms and venous function. Reflecting the complexity of the correlation between CVD severity and reflux location, owing to factors such as those mentioned above, are the following examples: a patient with CVD class 1 who has local pain and tingling over the reticular veins, and a patient with CVD class 2 who is asymptomatic and has great saphenous vein (GSV) reflux from groin to ankle. Today, research on this important issue should focus more on deriving more precise correlations, taking into account contributing factors.

Medicographia. 2016;38:148-154 (see French abstract on page 154)
male patient, for whom the disease has been apparent for 7 years without any other comorbidity. She presented with some feelings of heaviness and mild aches that are mostly felt at the end of the day. In the second example, the patient has the same age, sex, and duration of disease, but she has a fixed ankle joint. She presented with aches, a burning sensation, and skin damage (CEAP [Clinical-Etiology-Anatomy-Pathophysiology] clinical class C4b). In the third example, the patient has the same characteristics as the first patient, but the disease has been present for over 20 years. She has a small ulcer in the medial malleolus and extensive skin discoloration. As seen from these examples, reflux by itself often is not the only determinant for the development of CVD signs and symptoms. Furthermore, there are other factors that influence the duration and velocity of reflux, as seen in Table II. In the above patient examples, the first patient had a reflux duration varying from 2.2 seconds to 3.8 seconds with a GSV diameter ranging from 3.8 mm to 7.9 mm. The second patient had a similar reflux range, but the GSV diameter ranged from 4.3 mm to 9.2 mm. The third patient had a reflux duration from 3.4 seconds to >8 seconds and a GSV diameter ranging from 4.8 mm to 13.5 mm. Unfortunately, in published papers, although some of these variables are mentioned, data are not provided, and the comparison among patients is clearly unequal and inappropriate. Even more fascinating is the fact that patients with class C1 disease may have more symptoms or symptoms of higher intensity than those with class C2 or more advanced CVD. The reason for this is unknown. This article focuses on patients with primary CVD; thus, patients with previous thrombosis, malformations, and venous obstruction have been excluded from the analysis.

Table I. Factors contributing to the clinical severity of chronic venous disease.

<table>
<thead>
<tr>
<th>Affected vein</th>
<th>Location</th>
<th>Extent</th>
<th>Diameter</th>
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<tbody>
<tr>
<td>Venous wall and surrounding tissue compliance</td>
<td>Location</td>
<td>Size</td>
<td></td>
</tr>
<tr>
<td>Capacitor (Draining area)</td>
<td>Position</td>
<td>Time of the day</td>
<td>Time of the month</td>
</tr>
</tbody>
</table>

Table II. Factors affecting duration and velocity of reflux.

Superficial veins
Reflux in the superficial veins is the most common pathology in patients with primary CVD. It is most often found in the saphenous veins, but reflux in nonsaphenous veins can be found in about 10% of CVD patients.

Saphenous veins
The vast majority of patients with CVD have reflux in the superficial veins, with the saphenous veins and their tributaries having the highest prevalence. This statement is true for all CEAP clinical classes from 2 to 6. Variable patterns of reflux have been described in the GSV and small saphenous vein (SSV). The location and extent of reflux in the saphenous veins has been associated with severity of CVD. This was first shown in 1994. Usually, segmental saphenous reflux is asymptomatic or is associated with mild symptoms. In most symptomatic patients, the saphenous reflux extends below the knee. Many studies, including those having patients with skin damage, have stressed the importance of distal vein reflux. This has been shown for both GSV and SSV. In fact, reflux in both veins together or separately can be responsible for venous ulceration. The hemodynamic importance of reflux is higher when the SFJ is involved. However, this is true when both the saphenous trunk and the SFJ are involved, as there are many patients with SFJ reflux where the saphenous trunk is competent.

In order to better illustrate this subject, Figures 1 and 2 (page 150) and Figure 3 (page 151) show images based on patients from our center. We chose patients with bilateral lower extremity varicose veins in order to compare the extent of disease with the signs and symptoms of the patients. Figure 1 depicts a female patient with bilateral GSV reflux. Only the left

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**SELECTED ABBREVIATIONS AND ACRONYMS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>CVD</td>
<td>Chronic venous disease</td>
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<tr>
<td>GSV</td>
<td>Great saphenous vein</td>
</tr>
<tr>
<td>SFJ</td>
<td>Saphenofemoral junction</td>
</tr>
<tr>
<td>SSV</td>
<td>Small saphenous vein</td>
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</tbody>
</table>
limb is asymptomatic. This is probably because the left limb has larger refluxing veins and somewhat more extensive reflux, which is also significantly more prolonged. Furthermore, the disease duration is twice as long in the left limb. The patient started developing symptoms 4 years ago, which is 11 years after the first varicose veins appeared in her calf. Figure 2 illustrates a male patient with varicose veins in both lower extremities. He has a leg ulcer in the right limb and no symptoms in the left. The right limb has longer disease duration, larger veins, and far more prolonged and extensive reflux. Both patients make a good case for the proposed association, with regard to extent of reflux and vein characteristics, as well as the CVD duration. However, this is not the case in the third patient. Figure 3 demonstrates a female patient with larger veins, more reflux, and longer CVD duration in the right limb than in the left. However, it is the left limb that is more symptomatic. In this case, this finding is most likely due to restricted mobility of the left limb that has resulted in an inefficient muscle pump; her right lower limb is used more to work around the house and support herself.

In patients with saphenous vein incompetence, the presence and severity of CVD symptoms appear to correlate with the location and extent of reflux. However, this is not always the case, and more importantly, the strength of this association is undetermined.

**Non-saphenous veins**

Reflux in nonsaphenous veins is found in about 10% of CVD patients, and this prevalence increases to 20% in patients who present with recurrent varicose veins. Most of these patients are females with two or more pregnancies. CVD signs and symptoms of class C1 to class C3 disease are found in about 90% of these patients, but 10% present with skin damage. Common locations of reflux in these veins are the perineum, vulva, inner thigh, buttck, posterolateral thigh, lower posterior thigh, popliteal fossa, knee, and lateral to posterior calf. Reflux can be found in these veins only or in combination with the saphenous and the pelvic veins. There is high association with reflux in the pelvic veins. The extent and location of nonsaphenous vein reflux is associated with the pres-
Venous reflux location and clinical severity of CVD: a complex link – Labropoulos

From Pathophysiology to Treatment

Perforator veins

The role of reflux in the perforator veins is not clear. The number of incompetent perforator veins and their diameter is higher in patients with skin damage. Patients with ulceration have often been associated with having perforator veins in the ulcer area, but never in isolation. \(10,17,18\) It has also been shown that in patients with primary venous disease, the perforator veins develop reflux through the connections with incompetent superficial veins. \(27\) It is possible that perforator veins may worsen the local hemodynamics and may contribute to the development of symptoms. Some evidence exists for their impact on healing of the ulcer after being treated. \(28\) However, this information comes from poorly designed retrospective studies, and it is far from robust. \(29\) Further work is needed to elucidate the role of perforator veins in development of CVD signs and symptoms.

Deep veins

The deep veins are most often involved in the development of CVD signs and symptoms in post-thrombotic limbs. Primary reflux in deep veins alone is rare. Segmental reflux has been found in deep veins in the presence of saphenous reflux involving the junctions. Such deep venous reflux is correct-

| Figure 3. Female patient, aged 61 years, with bilateral varicose veins. |
| She has three children, and her mother had varicose veins. She has some aching and itching in the right limb, whereas she has more pain, swelling, heaviness, and some skin discoloration in the left limb. She has undergone no procedures for the veins and no other procedures. She takes aspirin and medication for hyperlipidemia. Her body mass index is 26.4, and she has no other complaints. Duplex ultrasound demonstrated great saphenous vein (GSV) reflux in the right limb from the saphenofemoral junction (SFJ) to the calf and in a few of its tributaries. The duration of disease is >20 years; the vein diameters range from 3.2 mm to 8.4 mm, and the reflux duration range is from 2.2 seconds to 4.7 seconds. The left limb has segmental GSV reflux, and one tributary is incompetent. The disease duration in this limb is about 10 years. The vein diameters range from 2.3 mm to 4.9 mm, and the reflux duration range is from 1.3 seconds to 2.7 seconds. She has restricted mobility in the left limb due to degenerative knee disease. Abbreviations: Based on CEAP classification of chronic venous disease: As, anatomy classification (superficial veins); C1-5, clinical classification (small and large varicose veins, asymptomatic); C1-5s, clinical classification (small and large varicose veins, edema, skin changes without ulceration; symptomatic); En, etiology classification (primary); Pr, pathophysiology (reflux). |

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| Figure 5. Female patient, aged 61 years, with bilateral varicose veins. |
| She has four children and had uncomplicated pregnancies. Both her parents had varicose veins. She has no medical issues and has had no previous surgery. She has symptoms in the right limb only, which become worse at the end of the day. She feels pain and heaviness in the vulvar area and along the varicose veins. She has a cluster of varicosities in the vulvar area that extend from her pelvis to the medial malleolus. At the end of the day, she feels a burning sensation, and the pain and heaviness are worse. In the left limb that was asymptomatic, she has small varicosities and reticular veins in the lateral and posterolateral thigh. The saphenous veins are normal in both extremities. In this case, the symptoms have a good association with the location and extent of venous reflux. The second patient, in Figure 5, has varicose veins in both limbs, without symptoms. The right limb has reflux in the accessory saphenous vein and in a thigh tributary. The left limb has reflux in the vein of the popliteal fossa and no saphenous reflux. Many patients with varicose veins may have no symptoms. This patient came to our clinical seeking treatment for cosmesis. Abbreviations: Based on CEAP classification of chronic venous disease: As, anatomy classification (superficial veins); C1-5, clinical classification (small and large varicose veins, asymptomatic); C1-5s, clinical classification (small and large varicose veins, edema; symptomatic); En, etiology classification (primary); Pr, pathophysiology (reflux). |
ed after the treatment of the superficial veins.\textsuperscript{29-31} In a recent Cochrane review, four studies performed deep vein reconstruction to alleviate CVD signs and symptoms; however, in all four interventions, superficial veins were done as well.\textsuperscript{32} Therefore, as these patients also had superficial vein disease, the symptoms cannot be attributed to deep vein reflux alone.

Absence of venous valves, known as avalvulia, is an autosomal-dominant anomaly that leads to severe venous reflux. Patients with this condition develop severe orthostatic edema in the lower extremities and present with large varicose veins and skin damage.\textsuperscript{33} In a review paper on deep vein anomalies, only four reports were found, and each had few cases.\textsuperscript{34} In the last 25 years, I have examined two such patients—one 12-year-old female and one 17-year-old male. Both presented with pitting edema, significant pain on prolonged standing, and ulceration. This is a very rare anomaly, and the extent of the disease correlates with the severity of the presentation.

Combined patterns of reflux

Patients with complex patterns of reflux are more likely to be symptomatic. Reflux in superficial, perforator, and deep veins is reported to be more common in patients with skin damage.\textsuperscript{11,13,15,35-40} These studies reported the combined patterns of reflux by including post-thrombotic limbs, so the effect of primary venous reflux is not clear. The addition of deep vein reflux in the absence of previous thrombosis needs to be studied separately. Furthermore, as stated above, deep vein reflux in the absence of previous thrombosis is not as prolonged as in patients that have had a previous thrombosis. The presence of such deep vein reflux may reflect a more advanced superficial vein disease and should be evaluated in future prospective studies.

**Figure 6.** Demonstrates bilateral varicose veins and swelling. Both limbs look similar and have an almost identical pattern of reflux, with >15 years of CVD duration. The common femoral veins have reflux at the SFJ in both limbs and the GSV from this level to the upper calf, with varicosities extending to the lower calf. A dilated and incompetent perforator vein has reflux at the medial mid-calf in both legs. Which limb is worse? Do both feel the same? The patient has aches and a sense of heaviness in both limbs, but it is worse in the right limb. No obstruction was found in the iliac veins and inferior vena cava. The patient has no oth-
er medical problems. So, what determines the difference in symptom intensity? Even more interesting is what we see in the next patient, in Figure 7. This patient has skin damage and more extensive GSV reflux from the SFJ to the ankle. He has two perforator veins at the mid- and lower calf that are incompetent. The veins are larger in diameter and duration of reflux is longer than that in the previous patient, and CVD has been present for more than 20 years. So, the clinical class of this patient’s disease correlates better with the extent of reflux than in the patient shown in Figure 6; however, this patient is asymptomatic.

Patients with deep vein reflux in ovarian and pelvic veins may present with abdominal and pelvic symptoms and often with symptoms in the lower extremities. Pelvic reflux is often seen to extend to the lower extremities, with a variable presentation ranging from asymptomatic to limb ulceration. Although studies have been published indicating these patterns of reflux, there are no data on clinical correlation in the lower limbs. One study on patients with nonsaphenous vein reflux indicated that about 10% had skin damage, but even in that study a correlation with pelvic reflux was not investigated.23

Current insights
In most patients, the distribution and extent of primary reflux correlates with the presence and intensity of the symptoms. Overall, the strength of this correlation has not been determined. Significant insights have come from the Bonn Vein study, both on such correlation and on CVD progression. It is worth mentioning the neuropsychological reflections that may associate limb symptoms with venous reflux, as recently reported.41 This matter is far too complex for our current understanding and deserves in-depth research.

The factors affecting CVD clinical presentation, shown in Tables I and II, further indicate the complexity of such correlations. Several other issues contributing to the complexity have been raised, such as lifestyle and the difficulty in separating other types of symptoms, eg, musculoskeletal or neurological symptoms, from those arising from CVD.42,43 It is naïve to associate venous reflux location alone with the severity of CVD without taking into account all other factors discussed. Clearly, more work is needed in multiple directions in order to better understand the relationship between symptom presence and intensity in patients with CVD.

References


Keywords: CEAP classification; chronic venous disease; clinical severity; venous reflux

Localisation du reflux veineux et corrélation avec la sévérité clinique dans l’insuffisance veineuse chronique primaire

Ces 20 dernières années, un certain nombre d’articles ont fait état d’une corrélation entre la localisation du reflux veineux et la sévérité clinique chez des patients atteints d’insuffisance veineuse chronique (IVC). Des études de cohorte épidémiologiques, transversales et prospectives ont toutefois soulévé d’importantes questions concernant cette association. Globalement, la localisation du reflux et la sévérité de l’IVC sont liées, mais la force de cette association n’a pu être déterminée car de nombreux autres facteurs sont impliqués, tels l’importance du reflux, l’efficacité de la pompe musculaire du mollet, la durée de l’IVC, le mode de vie, l’obésité, l’activité physique et les réponses biologiques des tissus dans les zones atteintes. De plus, il n’y a pas de différentiation claire entre les symptômes dus à l’IVC et ceux dus à d’autres causes: ils peuvent coexister avec l’IVC ou en être indépendants. La nature du ressenti du patient n’a pas été étudiée en profondeur en raison des considérations neuropsychologiques complexes liant les symptômes et la fonction veineuse. Les exemples suivants, si on les compare, reflètent la complexité de la corrélation entre la sévérité de l’IVC et la localisation du reflux ayant pour cause les facteurs susmentionnés: un patient ayant une IVC de classe 1 et souffrant d’une douleur locale et de picotements au niveau des veines réticulaires, et un patient ayant une IVC de classe 2 avec un reflux de la veine grande saphène (VGS) de l’aïne jusqu’à la cheville et qui est asymptomatique. Aujourd’hui, la recherche sur cette question importante devrait s’intéresser davantage à tenir d’établir des corrélation plus précises en tenant compte de tous les facteurs entrant en jeu.
A recent cross-sectional study on heredity and venous disorders has provided us with a vast amount of data concerning chronic venous disorders (CVDs) across a wide range of clinical stages of CVD and ages. These data have deeply modified our knowledge of CVD. Indeed, it has shown that the evolution of this disease in patients with no direct inherited condition and in those with inherited CVD is different. Differences between paternal and maternal transmission have been identified as well. With inherited CVD, the impact of the disease is aggravated at all ages. The hereditary weakening of the venous system is almost certainly linked to deregulation of parietal and valvular renewal, even though the genetic factor has not yet been formally identified. This matrix metalloproteinase–induced impairment of the renewal mechanism explains the premature weakening of the veins of our patients, who eventually suffer from venous hypertension, in this case “secondary” and linked to venous stasis and inflammation. The roles of flavonoids in regulating matrix metalloproteinase levels and activity help explain their beneficial effect in reducing pathophysiological mechanisms. The hereditary factor is a major criterion in daily practice and can help define the risk profile of each of our patients. These new data have profoundly challenged our assumptions about the therapeutic management of CVD, the advice we give patients, and the way we follow up the disease.

The influence of heredity in venous disease

Although the influence of heredity has been considered clinically obvious in venous diseases for a long time, it has only been scientifically documented since the beginning of the 1990s, thanks in particular to the work of Cornu-Thenard et al. However, despite the carrying out of other epidemiological and genomic studies since, the mode of transmission of the disease remains relatively poorly researched and incompletely determined.

In 2010, an international study—the Vein Consult Program—was carried out to evaluate the prevalence of venous disease in all patients over 18 years of age who consulted their general practitioner. As part of this study, over a 2-day period 1040 French general practitioners described the clinical condition of their patients’ venous circulation according to the CEAP (Clinical–Etiological–Anatomical–Pathophysiological) classification system and recorded, among other things, their patients’ direct parental venous background irrespective of sex or reason for the consultation. Data in the

Parietal and valvular venous insufficiency: the influence of heredity

by V. Crébassa, France
21319 completed medical files (25.9% male, 74.1% female) thus included a hereditary perspective of venous disease. The prevalence of venous disease was 58.8% in the entire population; 38.2% in those with no direct parental background, 67.0% in those with a unilateral paternal background, 71.3% in those with a unilateral maternal background, and 79.2% in those with a bilateral background (P<0.0001). In men, the prevalence of venous disease was 38.9%; 22.5% in men without parental background, 58.1% in men with a unilateral paternal background, 52.6% in men with a unilateral maternal background, and 71.5% in men with a bilateral background (P<0.0001). Venous disease was more prevalent in women; it was present in 46.8% of those without a parental background of venous disease, 70.8% in women with a unilateral paternal background, 75.1% in women with a unilateral maternal background, and 81.5% in women with a bilateral background (P<0.0001).

Nearly half of men (46.0%) had a parental history of venous disease: a quarter of men (26.8%) had a unilateral history of maternal venous disease, 10.7% a bilateral history, and 8.5% a unilateral paternal history. Nearly two thirds of women (63.5%) had a parental history of venous disease: nearly half (45.8%) had a unilateral maternal history, 10.7% a bilateral parental history, and 7.0% a unilateral paternal history.

The risk of venous disease increased significantly with a family history of venous disease: in patients with unilateral paternal heredity (odds ratio [OR], 3.2; 95% CI, 2.8-3.6), those with unilateral maternal heredity (OR, 3.4; 95% CI, 3.2-3.7), and those with bilateral heredity (OR, 5.6; 95% CI, 5.0-6.2), compared with patients with no familial history of venous disease. In comparison to men without a family history of venous disease, the OR of paternal heredity in men was 4.8 (95% CI, 3.9-5.8; P<0.0001), the OR of maternal heredity in men was 3.8 (95% CI, 3.3-4.4; P<0.0001), and the OR of bilateral heredity in men was 8.6 (7.1-10.5; P<0.0001), all in favor of the occurrence of venous disease (Figure 1). The same comparisons in women also found significant ratios in favor of heredity of venous disease: the OR in women with paternal heredity was 2.8 (95% CI, 2.4-3.2), the OR in women with maternal heredity was 3.4 (95% CI, 3.2-3.7), and the OR in women with bilateral heredity was 5.0 (95% CI, 4.4-5.7). The influence of heredity on venous disease appears to be stronger in men than in women, regardless of the source of heredity, ie, maternal, paternal, or both (Figure 1).

A logistic regression model adjusted for age and sex showed that heredity was an independent predictor of venous disease. Similar analyses were also carried out in men and women, separately; in women, by introducing sex-specific risk factors into the model, ie, number of children, oral contraception, menopause, and hormone replacement therapy. These analyses confirmed the finding that heredity was an independent predictor of venous disease. For women, having children increases the risk of venous disease.
The risk of venous disease increases with age. In fact, age-based analyses showed that the prevalence of venous disease increased with age, irrespective of family history of venous disease \( (P<0.0001) \) (Figure 2).\(^8\) Whatever the age group, venous disease was significantly more prevalent in patients with either a unilateral or a bilateral family background of venous disease compared with patients with no family history of venous disease \( (P<0.0001) \). Regardless of the grade of severity of chronic venous disease, the prevalence of chronic venous disorders (CVDs) has been shown to be higher in patients with a family history of venous disease than in those without (Figure 3). The impact of heredity is further enhanced if it is bilateral in nature and if the patient develops CVD when young.

Among recent studies that take age, sex, and information on heredity into account, reference must be made to the study by Laurikka et al\(^3\) in 6874 patients with or without mild venous disease (varicose veins). The risk of varicose veins increased in those with a family background of varicose veins (OR, 4.9). The study was, however, limited by its specific focus on varicose veins (C2) rather than the whole spectrum of venous insufficiency. Nevertheless, the OR identified was globally of the same order as that observed in a recent cross-sectional study on heredity and venous disorders.\(^8\) Further confirmation of the role of heredity in venous disease comes from the San Diego Population Study, in which a family history of venous disease was found to be a sex-independent risk factor for moderate or severe venous disease.\(^2\) We can conclude by saying the following: (i) the hereditary transmission of CVDs is as much paternal as maternal; and (ii) in patients with a family history of venous disease, CVD is much more frequent at all ages, CVD occurs earlier (with its prevalence practically tripling in those 20 to 25 years of age), and at any given age the severity of CVD is likely to be greater than in patients with no family history of venous disease.

**Venous degeneration and its consequences**

Just like bone, whose turnover depends on the equilibrium between destruction by osteoclasts and reconstruction by osteoblasts, the integrity of vein walls and venous valves depends on the renewal of elastin (type III collagen). This elastin, produced continually by smooth muscle cells, is essential to venous parietal and valvular architecture and function.\(^9-11\) If the deterioration of elastin is quicker than its renewal, the architecture of the vein wall changes and the vein loses its elasticity, the capacity of a vein to return to its original form. The vein dilates and consequent stasis, excessive venous pressure, and inflammation contribute to further deterioration of the vein.

Deterioration of elastin by type 3 metalloproteinase

These modifications are due to an increase in the precursor of matrix metalloproteinase 3, type 3 pro–matrix metalloproteinase (pro-MMP-3), which is produced in excess quantities by smooth muscle cells in patients with varicose veins. This deregulation has also been found in the dermis of patients suffering from CVDs.\(^12,13\) An increase in pro-MMP-3 leads to a decrease in the production of elastin and an increase in that of type I collagen, via a decrease in the negative retrocontrol that elastin exerts on the production of type I collagen (Figure 4).

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**Selected Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>CVD</th>
<th>chronic venous disorders</th>
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<tr>
<td>CEAP</td>
<td>Clinical–Etiological–Anatomical–Pathophysiological</td>
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<tr>
<td>pro-MMP-3</td>
<td>type 3 pro–matrix metalloproteinase</td>
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As a result of these changes in composition, the integrity of the venous system alters rapidly and prematurely due to imperfect renewal of elastin fibers within the valves and venous walls of patients suffering from CVDs. The structure of vessels deteriorates before varicose veins appear, which may explain why C0 patients feel pain—caused by the stimulation of C fibers due to inflammation—even though neither varicose veins nor telangiectasias are visible.14

This impaired renewal, caused by the overproduction of pro-MMP-3, may be triggered more easily in prevaricose veins, under the influence of activators, than in ordinary veins.13 Therefore, this hereditary architectural defect reveals itself more quickly under the influence of aggravating and triggering factors, causing CVDs to occur earlier and develop more quickly.

Finally, epigenetic triggers and sensitizing factors that are present in women and nonexistent in men, eg, female hormones (?), may have a role in the manifestation of CVD, as paternal transmission of CVD is more significant than maternal transmission yet the disease is more present in women than men.15 Clinically, this fundamental research shows that we should be targeting both hereditary factors and aggravating factors in order to break the vicious circle of venous wall and valve degradation, which leads to venous hypertension, stasis, and inflammation (Figure 5).

The harmfulness of excess MMP-3 can be effectively decreased by using flavonoids.16-23 Like nano-oligosaccharide factor, which is applied on bandages in the treatment of ulcers, flavonoids block the action of metalloproteinase. We can decrease the pressure of a varicose vein through the vein wall not only by exerting counterpressure using compression devices, but also by restoring the toxicity of the vein by direct action on parietal muscle cells. These two therapeutic targets explain the results of one study14 that showed a synergistic pain relief by associating compression with venotonics.

The Satisfy survey demonstrated a clear reduction in microsclerotic skin complications (OR 2.5) in patients treated with flavonoids, without doubt by the same mechanism: restoration of vein tonicity.25 Of course, many other insights exist and have been summarized.26

**Daily consequences of venous disease in medical practice**

Venous disease involves structural pathology of the vein walls and the valves. Its occurrence is the result of heredity,16 whose predominantly maternal roots1 are now being questioned, and of risk factors favoring its appearance. Some of these risk factors cannot be modified, such as sex and hormones, but others, such as obesity, inactivity, exposure to heat, and prolonged standing or sitting, can.27-31 Besides acting on these modifiable risk factors, which doctors and pharmacists should advise patients to do in order to prevent venous disease, more attention should be paid to ensuring early management of CVD.32-35 Too often, and this is particularly true in men,33 patients fail to seek advice or consult their doctor before the development of venous disease is already relatively advanced.

We need to define a risk profile for each of our patients before considering any therapeutic treatment because we know that venous disease encompasses several types of risk: evolving risk (from diverse trophic disorders to ulcers); inherent risks linked to acute complications (from varicose hemorrhages to thrombosis); and risks of chronic complications (postthrombotic syndrome). However, the treatment of varicose veins also carries risks. Apart from the inherent risks of each treatment, we must, as doctors, protect the integrity of the venous system of our patients by preserving the venous axes, sometimes even those that are flowing back.

We thus wanted to establish a scalability score for venous disease, based on sex, risk factors, and clinical condition, that determined the probability of aggravation of CVD for a patient in future decades. Following on from the evaluation of venous age with VeinScore,35,36 VeinRisk aimed to raise awareness among patients about their CVD and to emphasize the necessity of managing modifiable risk factors to prevent aggravation. Another aim was to help establish therapeutic strategies especially adapted to the risk profile of each patient.
This calculation was based on the analysis of an international database of 124,335 people carried out in the same manner as the Vein Consult Program quoted at the beginning of this article, but in 24 countries with the help of more than 6,000 practitioners who recorded not only established or suspected risk factors, but also the symptoms of each patient. Statistical analysis using sex and risk factor data determined the evolution of CVD on the basis of CEAP classification in different 10-year age categories: 30 to 40 years, 40 to 50 years, 50 to 60 years, and over 60 years.

It is essential to signal that we worked on the different clinical components of the CEAP classification system in order to be able to describe the frequency of the different signs presented (varicosities, varicose veins, edemas, trophic disorders, and healed or open ulcers) rather than the global CEAP classification, as the latter, for example, does not take into account the presence or absence of edema and varicose veins when the patient is C4. For this reason, the sum of the different percentages can exceed 100%.

Of the risk factors presented by patients, 57% had a family background of venous disease. A fifth (20%) were obese and over half (61.5%) were physically inactive (seated or upright for more than 8 hours per day). Each patient had on average 2±1.5 children. More than three quarters (77.4%) felt at least one symptom that may have been related to venous insufficiency: heavy legs, night cramps, leg pain, itching, feeling of swelling, or tingling and burning sensations. Clinical examination of the legs showed that 40.6% had telangiectasia (C1), 34.8% had varicose veins (C2), 24.9% had edema (C3), 14.0% had trophic disorders (pigmentation, eczema, white atrophy) (C4), 7.3% had a healed venous ulcer (C5), and 4.3% had an open venous ulcer (C6).

Statistical analyses showed that the risk factors that played a major role in the evolution of the disease were: a family background of chronic venous diseases; and pregnancy (OR of 1.42 for the first birth, 1.37 for the second, and 1.72 for the third). Other risk factors included: obesity; and physical inactivity, in a broad sense (standing and/or remaining seated for long periods of time).

For instance, according to VeinRisk calculations, an obese female patient between 40 and 50 years of age has a 23% risk of having varicose veins (C2), which rises to 29% if she is also inactive and to 32% risk if she has had more than one child. An obese male patient between 40 and 50 years of age has a 13% risk of having varicose veins (C2); with increasing age, this rises to 21% between 50 and 60 years and to 30% after 60 years. If this male patient is also inactive, the corresponding values increase to 18%, 27%, and 35%. The important impact of heredity on the risks of venous disorders in both women and men should also be noted. If heredity is a factor, the risk of varicose veins in a man over 60 years of age who is obese and inactive increases to 54%, while in a woman with the equivalent characteristics, the risk of varicose veins is 66%. Consequently, in patients for whom heredity is a risk factor, we must be more attentive, manage their disease earlier, and correct different modifiable risk factors to reduce aggravating effects and halt the development of CVD.

It is, of course, in these preventive situations that people have the greatest difficulties in adhering to treatment. The use of VeinScore to determine venous age and now VeinRisk can help persuade patients to use these treatments by showing them their “venous futures” if they don’t take the appropriate steps required to reduce the risks. For instance, an application for smartphones that calculates the daily time spent seated or standing (Hopplivein) can help patients reduce sedentariness by proposing intermittent physical activity (walking or sport) throughout the day.

Of course, advice concerning how to reduce modifiable risks is welcome, but the hereditary fragility of vein wall and valves is unmodifiable. As such, there is little logic in making patients feel guilty about relapses or the development of venous disease. It would be preferable to explain hereditary chronic fragility and to give advice about avoiding triggers or aggravating elements, such as gaining weight during pregnancy.

The preeminent role of heredity as a risk factor relativizes all healthy living advice. Indeed, heredity is a major risk factor not only in terms of prevalence of the disease (and this for all the age groups studied), but also in terms of early development (2.6 times more CVD between 20 and 35 years of age) or seriousness (at all stages of CVD in the same age group with heredity, the frequency of CVD increases).

VeinRisk is already an important decision-making tool. For example, using VeinRisk we would not treat in the same way a 37-year-old female patient with a 6 mm preterminal saphenous incontinence, who is overweight, has a sedentary lifestyle, and paternal and maternal heredity as the same patient without either the hereditary or coronary risk factors; the speed of treatment and follow-up would be different. We hope that VeinRisk will also become a practical, awareness-raising tool that will promote communication between doctors, pharmacists, and patients. Our therapeutic reflections must, from now on, be enriched and guided by the additional information about benefit-risk that VeinRisk and its scalable probabilities provides.

In the same way, the follow-up for patients at high risk of CVD should be at shorter intervals than for other patients, be it only to refresh advice about diet or to monitor clinical signs that patients get used to. There is a 7-year delay in managing medical treatment for venous disorders in France, and over two thirds (70%) of patients suffering from a chronic venous disease in France are not treated.
Physicians should take care not to oversimplify the impact of heredity in CVD. Though important, treating varicose veins, stage C2 of the 7 clinical stages of the CEAP classification, cannot adequately summarize the management of a chronic hereditary venous disease. Furthermore, the end stage of this disease is not systematically an ulcer or a thrombosis.

Each patient’s risk profile and the evolution of the disease according to the risk factors are different and should be included in our decisional arguments at the same time as the clinical, anatomic, and hemodynamic evaluation. This will allow us to better link a patient’s risk profile with his clinical condition, which in turn may allow us to consider an alternative approach that leads to the conservation of a vein despite certain refluxes said to be pathological.

Conclusion

The determination of venous age can make patients aware of their risks in CVD and allows early medical care, if necessary. The evolving risk factors of patients must be weighed against the risks inherent to CVD (its development and complications) and therapy and other risks (autologous bypass, occupational traumatic risks, etc). Knowledge of direct parental hereditary background of venous disease is fundamentally important for setting up a more active CVD prevention plan featuring earlier treatment initiation and shorter follow-up intervals to limit complications. Fundamental research on hereditary factors in CVD will undoubtedly bring us answers. These answers will most probably be genetic in nature, but there will be epigenetic ones, too, that will elucidate the triggering and aggravating factors of CVD.

References

INFLUENCE DE L’HÉRÉDITÉ SUR L’INSUFFISANCE VEINEUSE PARIÉTALE ET VALVULAIRE

Une récente étude croisée sur l’hérédité et les troubles veineux nous communique une quantité importante de données sur la maladie veineuse chronique (MVC), quel que soit le stade clinique de la MVC ou l’âge des patients étudiés. Ces données ont profondément modifié notre connaissance de la MVC. En effet, l’évolution de cette maladie chez les patients qui ont ou pas de pathologie héréditaire directe est différente, la transmission paternelle et maternelle identifiée étant également différente. Dans le cas d’une MVC héréditaire, les conséquences de la maladie sont aggravées à tous les âges. La fragilisation héréditaire du système veineux est sans aucun doute liée à un trouble du renouvellement pariétal et valvulaire, même si le facteur génétique n’a pas encore été formellement identifié. Ce mécanisme explique la fragilisation prématurée des veines de nos patients, qui souffriront finalement d’hypertension liée à la stase veineuse et à l’inflammation, dans ce cas « secondaire » et déjà bien identifiée. Ces mécanismes physiopathologiques nous permettent d’expliquer le rôle bénéfique de régulation des flavonoïdes. Le facteur héréditaire est devenu un critère majeur en pratique quotidienne et peut aider à définir le profil de risque de chacun de nos patients. Ces nouvelles données ont profondément bousculé nos hypothèses sur la prise en charge thérapeutique de la MVC, les conseils que nous donnons à nos patients et la façon dont nous suivons la maladie.

Keywords: cardiovascular disease; genetic; heredity; matrix metalloproteinase; risk factors
Hypoxia, inflammation, and the occurrence of venous reflux and its interaction with aging

by J. Buján, M. A. Ortega, C. Mesa-Ciller, F. Sainz, and J. Leal, Spain

The inflammatory process plays an important role in the aging process and vein wall failure (dilation). The distribution of inflammatory cells suggests that dysfunction of the microvascular endothelium is the primary effect of aging, and valve alterations are related to venous insufficiency. Aging and venous insufficiency occur in a parallel, overlapping course, but the aging process may be accelerated in chronic venous insufficiency...

The failure of vein wall competence in chronic venous insufficiency (CVI) lacks a clear etiology, despite scientific and technological advances. Multiple factors contribute to its development, including vein wall failure or stretching, valve failure, or valvular agenesis, which are key factors that promote venous reflux.1,2 The measurement of venous reflux assesses the degree of venous involvement in the lower limbs. Clear evidence for a correlation of the severity of reflux with vein wall damage is lacking, which impedes the implementation of any therapeutic measure.

We have previously reported that aging is one of the many insults that affect the morphology and function of the venous wall and contribute to venous failure.3 Venous insufficiency—with or without reflux—dilates the vein wall, which initiates compensatory changes in the structure, including initial hypertrophy in areas that eventually fail, and results in a final fibrosclerotic process that is characteristic of varicose vein walls.4 Inflammation and ischemia induce, encourage, and sustain these alterations, leading to remodeling of the cytoarchitecture of the vein wall that manifests as functional incompetence and is evaluated as venous reflux (Figure 1).

The inflammatory process plays an important role in the aging process and vein wall failure (dilation). The distribution of inflammatory cells5 suggests that dysfunction of the microvascular endothelium is the primary effect of aging, and valve alterations...
Hypoxia and inflammation in venous reflux interactions with aging – Buján and others

CVI induces changes in venous flow return, which may increase venous filling, intraluminal pressure, venous stasis, and relative hypoxia, and these changes are associated with increased oxidative metabolites and reactive oxygen species, which are damaging to the venous wall. Nitric oxide is a potent molecular messenger for the regulation of vascular tone and mediation of the inflammatory cascade. Nitric oxide increases the functional activation of monocytes and macrophages and activates matrix metalloproteinases (MMPs). MMP2 (gelatinase A) and MMP9 (gelatinase B)—which degrade elastin and collagen gelatinases—and inhibitors of tissue inhibitor of metalloproteinases 1 and 2 (TIMP-1 and TIMP-2 inhibitors) are the most studied enzymes in venous disease. MMP2 and MMP9 are produced in vascular and inflammatory cells. MMPs play an important role in the synthesis and degradation of the extracellular matrix under physiological and pathological conditions, and any alterations in this balance may lead to a degradation of the matrix with degenerative and structural changes in the venous wall. MMPs also alter muscle and endothelial cells in the absence of severe changes in the extracellular matrix.

The level of hypoxia caused by venous hypertension triggers molecular pathways that are involved in the cellular response to the lack of oxygen (O2), such as hypoxia-inducible factor (HIF) transcription factors, which are members of the basic helix-loop-helix (bHLH)-PER-ARNT-SIM (PAS) family. HIF is formed by α and β subunits. Three isoforms of the α subunit have been identified, HIF-1α, HIF-2α, and HIF-3α. The function of HIF-3α is the least characterized. HIF-2α and HIF-1α exhibit a 48% homology and have similar biochemical structures. These factors play a similar role in the induction of gene expression during the hypoxic response, and expression depends on tissue type. Research suggests that the stabilization of HIF-2α requires less severe hypoxia than HIF-1α, but the mechanisms are not known. HIF-2α may be a first-line response to moderate or less severe declines in O2. The involvement of interleukin 6 (IL-6) in the regulation of cell metabolism was also noted recently. A hypoxic environment produces a significant increase in IL-6 expression, which leads to a positive feedback of the inflammatory response.

CD206 was observed to play a role in the inflammatory response after IL-6 activation. CD206 is a protein receptor for cysteine in various tissues, and it exhibits a relationship with the functional activity of lectin. This activation plays an essential role in cellular homeostasis as a turning point for the resolution of inflammation. This protein is a marker for the activity of alternatively activated (M2) macrophages, which exhibit a reparative role in chronic inflammation.

Our study investigated the relationship between hypoxia or inflammation—which induce changes in the venous wall—and the grade of clinical reflux and its interaction with aging.

**Patients and methods**

The study was performed using 30 vein samples from patients undergoing lower-extremity saphenectomy. Patients were divided into three study groups according to the diagnosis of the presence or absence of reflux and the severity grade. Patients were further classified by age—younger vs older (ie, under or over 50 years, respectively). Reflux studies were performed using noninvasive Doppler (7.5-10 MHz color Doppler ultrasonography), which allows adequate exploration of superficial and deep venous systems using the appropriate maneuvers. Patients were classified as having no apparent clinical reflux, moderate vein reflux, or severe vein reflux, as follows:

(i) No apparent clinical reflux (NR): Duration of venous reflux (DVR) <0.5 seconds (n=10). Six patients were under 49 years of age (range 33-42 years) and four were aged 50 years or more (range 52-68 years).

(ii) Moderate vein reflux (MR): DVR between 0.5-2.0 seconds (n=10). Six patients were under 49 years of age (range 33-42 years) and four were aged 50 years or more (range 52-68 years).

(iii) Severe vein reflux (SR): DVR >2.0 seconds (n=10). Four patients were under 49 years of age (22-48 years) and six were over 50 (55-73 years).

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<th><strong>SELECTED ABBREVIATIONS AND ACRONYMS</strong></th>
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<tr>
<td>CVI</td>
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<td>DVR</td>
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Figure 1. Representation of the processes that occur during venous remodeling.
Procedures were followed in accordance with institutional guidelines and conform to the standards set by the latest revision of the Declaration of Helsinki.

**Immunochemistry and polymerase chain reaction studies**

We used the avidin-biotin complex (ABC) method with alkaline phosphatase as a tracer for immunohistochemical detection of antigens of interest. Samples were washed and balanced in phosphate-buffered saline (PBS), and nonspecific binding sites were blocked for 45 minutes at room temperature in a blocking solution (10% fetal bovine serum [FBS], 1% bovine serum albumin [BSA], and 0.05% Tween 20 in PBS). Samples were incubated with the following primary antibodies overnight at 4°C: rabbit monoclonal anti-human-HIF-1α (1:800) (Abcam, Cambridge, UK), rabbit monoclonal anti-human-HIF-2α (1:2000) (Abcam, Cambridge, UK), mouse monoclonal anti-human-MMP2 (1:25) (Neomarkers, Fremont, California, USA), rabbit monoclonal anti-human-MMP9 (1:100) (Abcam, Cambridge, UK), and mouse monoclonal anti-human-CD206 (1:1000) in blocking solution. Samples were incubated with the appropriate secondary antibodies bound to biotin for 1.5 hours at room temperature: biotinylated anti-rabbit immunoglobulin G (IgG) (1:1000) (Sigma, St. Louis, Missouri, USA) and biotinylated anti-mouse IgG (1:300) (Sigma, St. Louis, Missouri, USA) in PBS. Samples were incubated with avidin-conjugated alkaline phosphatase (ExtrAvidin–Alkaline Phosphatase, Sigma-Aldrich, St. Louis, Missouri, USA) for 1 hour at room temperature at a 1:200 dilution in PBS. A chromogenic substrate was used for color development under a microscope. A Zeiss Axiophot light microscope equipped with an AxioCam HRc (Carl Zeiss, Oberkochen, Germany) digital camera was used for observations.

Complementary DNA (cDNA) was produced using reverse transcriptase polymerase chain reaction (RT-PCR), and the amount of cDNA for the following genes was quantified in each sample of interest: IL-6, MMP2, and MMP9. The results were normalized using the constitutively expressed gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Specific primers for all genes studied were designed de novo using the online applications Primer-BLAST and AutoDimer. RNA extraction was performed according to the method of guanidine isothiocyanate-phenol-chloroform extraction of Chomczynski and Sacchi.

Quantitative PCR (qPCR) was performed in a StepOnePlus System (Applied Biosystems-Life Technologies, Massachusetts, USA) using the relative standard curve method. A volume of 5 µL of each sample was diluted

Table 1. Immunohistochemical detection of hypoxia-inducible factor (HIF)-1α and HIF-2α in patients with no apparent reflux, those with moderate reflux, and those with severe reflux. Results show the percentage of patients staining positively, according to age group.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NR</th>
<th>MR</th>
<th>SR</th>
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<tr>
<td>HIF-1α</td>
<td>&lt;50 (%)</td>
<td>&gt;50 (%)</td>
<td>&lt;50 (%)</td>
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<tr>
<td>Total (%)</td>
<td>100</td>
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<tr>
<td>&gt;50 (%)</td>
<td>20</td>
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Abbreviations: <50, under 50 years of age; >50, over 50 years of age; HIF, hypoxia-inducible factor; MR, moderate vein reflux; NR, no apparent reflux; SR, severe vein reflux.

Figure 2. Immunohistochemical images showing the detection of HIF-1α in patients with no apparent reflux, those with moderate reflux, and those with severe reflux, by age group.

Abbreviations: <50, under 50 years of age; >50, over 50 years of age; HIF, hypoxia-inducible factor; MR, moderate vein reflux; NR, no apparent reflux; SR, severe vein reflux.
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1:20 in nuclease-free water, mixed with 10 μL of iQ SYBR Green Supermix (Bio-Rad Laboratories, California, USA), 1 μL of forward primer (IL-6, MMP2, or MMP9; sequences available on request), 1 μL of reverse primer (IL-6, MMP2, or MMP9; sequences available on request) and 3 μL of deoxyribonuclease (DNase)- and ribonuclease (RNase)-free water in a 96-well plate (MicroAmp, Applied Biosystems-Life Technologies, Massachusetts, USA), for a total reaction volume of 20 μL. We used Microsoft Office Excel 2010 to analyze the obtained data.

Results

*Hypoxia markers*

- HIF-1α: Immunohistochemical analysis showed positive staining for HIF-1α in all patients studied, regardless of reflux grade (Table I, Figure 2). However, in the NR and MR groups, HIF-1α staining intensity was greater in the younger patients (aged <50 years) than in the older (aged >50 years). The inverse was observed in the SR group, with staining intensity greater in older patients.

- HIF-2α: Statistically significant differences between the study groups were observed. Only 20% of patients in the NR group showed positive staining for HIF-2α, whereas 100% of patients in the MR group and 40% of patients in the SR group showed staining (Table I, Figure 3). We observed less HIF-2α staining in patients older than 50 years than in younger patients in the NR and MR groups, a pattern that is reversed in patients with severe vein reflux. The age difference between the younger and older groups also became more significant in the SR group.

*Inflammatory markers*

- MMP2: messenger RNA (mRNA) levels for this marker were clearly different across population profiles according to age and grade of vein reflux. The younger population (<50 years) with moderate reflux (MR) exhibited values 4 times greater than the NR group and 1.5 times greater than the SR group (Figure 4A). MMP2 mRNA levels in the older patients in the NR and SR groups were similar, but were statistically significantly different from levels in older patients in the MR group. Immunohistochemistry revealed a global immunostaining score of 60% (including both age groups together) for MMP2 protein expression in the NR group, 20% in the MR group, and 80% in the SR group (Figure 5, page 166). Using PCR analysis of cDNA, we observed a similar global mRNA expression regardless of reflux severity (Figure 4D).

Figure 3. Immunohistochemical images showing the detection of HIF-2α in patient groups with higher values of positive expression.

Abbreviations: <50, under 50 years of age; >50, over 50 years of age; HIF, hypoxia-inducible factor; MR, moderate vein reflux; NR, no apparent reflux; SR, severe vein reflux.

Figure 4. (A) Messenger RNA (mRNA) levels of MMP2, (B) MMP9, and (C) IL-6, quantified using quantitative real-time polymerase chain reaction (qRT-PCR) in patients with no apparent reflux, those with moderate reflux, and those with severe reflux, according to age group. (D) Comparisons of different mRNA levels of inflammatory markers for the three general groups of patients quantified using qRT-PCR.

Abbreviations: <50, under 50 years of age; >50, over 50 years of age; IL-6, interleukin 6; MMP, matrix metalloproteinase; MR, moderate vein reflux; NR, no apparent reflux; SR, severe vein reflux.
◆ MMP9: Protein expression was evident (positive staining) in 60% and 100% of the studied patients, according to reflux severity (Figure 5). Gene expression of this marker depended on age. Lower levels of MMP9 protein and mRNA were observed in the younger patients than in the older patients in the NR and SR groups (Figures 4B and 5). Significant differences were observed in mRNA levels of the SR group between the younger and older patient groups (Figure 4B). Overall, higher values correlated with the degree of reflux (Figure 4D).

◆ IL-6: This marker exhibited a direct relationship with age and the grade of vein reflux. Higher values were observed in the SR group. Very low levels were observed in the NR group in the younger population. The presence of this cytokine was higher in the older patients in the NR and SR groups, with the difference reaching significance in the latter (Figure 4C).

A comparison of the overall mRNA expression profiles revealed a clear tendency of an increase in the three inflammatory markers with the degree of vein reflux in our studied population (Figure 4D).

◆ CD206: Immunohistochemical results of CD206 expression, which is involved in M2 macrophage-like activity and other cell types, revealed an interesting pattern with positive staining in smooth muscle cells. A very characteristic patchy pattern of vein wall remodeling was observed. Comparisons by age and grade of reflux revealed significant differences in expression in smooth muscle cells during remodeling, which was more pronounced in the NR group of the young population than in the aged group of the SR population (Figure 6).

**Discussion**

In this study, patients were classified according to degree of reflux—no apparent clinical reflux, moderate reflux, and severe reflux—which we measured using Doppler ultrasound. Greater reflux severity may correspond to the increasing age of the vein wall. We investigated the role of this blood flow alteration in inducing slowing of venous return and stasis, which promote the development of hypoxia. The evaluation of two markers of the initial state of acute hypoxia—transcription factors HIF-1α and HIF-2α—improved assessment of damage status. We observed the presence of HIF-1α in all patients studied, which reveals a significant state of hypoxia in the venous walls. This hypoxia appeared in the young population—vein stasis triggered a greater degree of hypoxia in young people than older people—and was more marked in the initial stages of venous insufficiency where reflux was not detectable or was moderate. However, hypoxia clearly increased with severe reflux in the patients aged over 50 years.

Our results are in general agreement with authors who observed a significant increase in the expression of the HIF pathway in patients with varicose pathology. The dysregulation of this pathway results in increased angiogenic factors and a significant increase in HIF-1α in the muscle layers of diseased vessels has been described. This expression was related to the increased presence of BCL2 (B-cell/CLL lymphoma 2 protein) on the endothelium of the vessel, which leads to an inhibition of apoptosis and increased dilation of the vein wall.
We estimated overall expression of HIF-2α to be lower than HIF-1α, on the basis of immunohistochemical staining observed. We found that in the young patients, HIF-2α expression is lower in those with a severe degree of reflux than in those without severe pathology. Our results are generally consistent with Lim et al., who found that in varicose veins, HIF-1α and HIF-2α expression was elevated and inversely proportional to the physiological process of hypoxia.

The presence of HIF-1α and HIF-2α overexpression may be a marker of a hypoxic environment at the beginning of the pathological process in young patients. HIF-1α and HIF-2α reach peak expression during moderate reflux and have lower expression when the hypoxic environment becomes severe. HIF factors are short-lived, and ubiquitination inhibits HIF expression and prevents translocation to the nucleus. Notably, our study demonstrated that MR is a turning point for adaptation to hypoxia.

The process of cell division is dependent on the availability of O2, but an inhibition of cell proliferation is not always observed in hypoxic situations. Therefore, various O2-independent mechanisms may exist. In this context, HIF-1α, in contrast to HIF-2α, plays a role as a cell attenuator of autonomous proliferation. The amount of O2 is sufficient to produce cellular proliferation in situations of moderate hypoxia; HIF-2α is easily activated because it does not depend on the involvement of many more molecules in this process. An increased expression of HIF-1α is observed in severe hypoxia, which slows cell proliferation. These changes are compatible with the hypertrophy-atrophy sequences that occur during the remodeling process of the insufficient venous wall.

IL-6 significantly correlated with increasing age in the NR and SR groups. These results concur with previous studies that related this increase with the development of chronic inflammation of the vascular wall. Notably, expression of IL-6 mRNA in the MR group did not differ between age groups in our study.

One of the important roles of IL-6 involves immunomodulation via the macrophage activation pathway and tumor necrosis factor α (TNF-α). Deng et al. suggested an immunomodulatory role of IL-6 on CD206 in this context. The CD206 activity, characteristic of macrophages, was inversely related to the functional capacity of IL-6, perhaps due to differences in the timing of each protein’s period of activity. That we found CD206 expressed in the smooth muscle cells of the vein wall is not surprising, based on the characteristics of this dimeric cysteine-lectin receptor, which may induce differentiation of mesenchymal cells into smooth muscle cells. The patchy expression pattern of this protein is consistent with observations for previously studied proteins, such as elastin and fibrillin-1. Cells expressing fibrillin-1 and tropoelastin mRNA have been shown to exhibit a patchy disorganized pattern, particularly in the proximal varicose segments of patients under 50 years of age. In that study, enhanced elastase activity was reported in control and varicose samples from older subjects. Varicose vein samples showed greater expression of latent-TGF-β-binding protein 2 (LTBP-2) and TGF-β expression.

The increased MMP2 protein expression in young patients with venous insufficiency corroborated previous work by our group on varicose veins and age. MMP9 expression is expected to increase with age and disease, and our results agree with previous studies. These results support an acute remodeling of the cytoarchitecture of vein walls in young people, which is partially regulated via late MMP2- and MMP9-induced remodeling.

These processes are part of the characteristic events that occur during the final wall remodeling after failure. Our results suggest that the initial moments of vein wall failure may occur in patients even when no reflux is evident and that the events of inflammation-hypoxia-remodeling are triggered in young people and then continue to evolve into a more severe condition over time.

Therefore, factors involved in the process of venous insufficiency are present early, from the first moments of the disease in young patients without clinical symptoms of vein reflux. A treatment plan that is similar to that used in older patients should be implemented in young patients in order to prevent the symptomatology that arises with severe reflux, as immunohistochemical and gene-expression findings reveal a similar pattern in physiological responsiveness during the process of inflammation-hypoxia remodeling. Older patients exhibited a high level of expression of inflammation markers in this study, regardless of the degree of reflux.

Our findings indicate a clear relationship between aging and an inflammation-hypoxia-remodeling process, a process that is irreversible if therapeutic action is not taken.

Acknowledgments. This work was supported by grants from the National Institute of Health Carlos III (FIS-P113/01513).

References
HYP oxie, inflammatio n, survenue d’un r eflux veineux et son in teraction avec le vieillissement

Le reflux veineux est un signe clinique associé à une incapacité de contrôler le retour du flux veineux. L’insuffisance veineuse chronique évolue avec le temps. Différents facteurs, comme l’hypoxie et l’inflammation, peuvent modifier l’architecture cellulaire des parois veineuses, la sévérité du reflux et ses interactions avec le vieillissement. La présence de marqueurs de l’hypoxie, HIF-1α et HIF-2α, et de marqueurs de l’inflammation, IL-6, MMP2, MMP9 et CD206, montre que la sévérité du reflux est liée à l’âge du patient chez les personnes jeunes (moins de 50 ans) et que le reflux clinique est lié à l’hypoxie et à l’inflammation chez les patients plus âgés (50 ans et plus). Nos résultats démontrent aussi que les marqueurs de l’hypoxie et de l’inflammation augmentent dans les parois veineuses de patients sans reflux clinique apparent, ce qui indique que des modifications histopathologiques surviennent avant l’apparition du reflux veineux. La présence de HIF-2α, un marqueur précoce de l’hypoxie, est observée dans la population jeune alors même que le reflux clinique n’est pas apparent. Les marqueurs de l’inflammation sont étroitement liés à la sévérité du reflux veineux chez les sujets plus âgés plus.

Keywords: hypoxia; inflammation; remodeling; venous insufficiency
The prevalence of chronic venous disease in the adult population has been reported to be as high as 60%, particularly in developed countries, and in one third of cases the deep venous system is affected. Chronic deep venous insufficiency has an important clinical and financial impact on society. Primary deep venous insufficiency, which is related to venous valve dysfunction without evidence of previous deep venous thrombosis, leads to venous hypertension. Deep reflux may be associated with superficial and perforating insufficiency. Conservative management includes compression therapy, while ablative procedures aimed at eliminating superficial and perforating reflux remain the first-line treatment. Surgical reconstruction of the deep vein system is reserved for cases with severe, intractable symptoms and signs, such as recurrent ulceration, lipodermatosclerosis, and severe edema and pain that compromise a patient’s quality of life. Reconstruction involves procedures aimed at in situ repair of the valve or valve transplantation. Attempts have been made to use prosthetic venous valves, which have been evaluated in animal models and clinical studies. The results are controversial, as so far most of the valves have not performed as expected. However, with the evolution of endovascular techniques, recent attempts have been made using implantable valves, which are expected to dominate treatment strategy in the near future. In the meantime, the design of the ideal valve is still awaited.

The latest guidelines of the American Venous Forum refer only to surgical repair of primary deep vein valve incompetence, with valve reconstruction recommended (grade 1, level A) in primary valvular incompetence after less invasive therapies have failed. However, the procedures involved are very demanding, the results are not good, and availability restricted to only a few specialized centers. With the development of novel endovascular technologies, patients with primary chronic deep venous insufficiency are expected to benefit.

Replacement venous valve prosthesis in primary chronic venous disease: is it likely to be developed in the future?

by A. D. Giannoukas, K. Spanos, A. E. Giannakopoulos, Greece

Primary chronic venous disease imposes an important socioeconomic burden on the adult population, especially in developed countries. Primary chronic deep venous insufficiency (CDVI) affects one third of these patients. Over a long-term period, this condition may lead to varicose veins, chronic venous ulcers, and persistent, related pathological conditions. Because of this, various reconstructions of the deep vein system have been developed and evaluated not only in animal models, but in clinical studies too. Since the description of the first successful repair of a deep vein by Kistner et al and the Straub Clinic team, the introduction of prosthetic valves via an open surgical approach has broadened this field. Prosthetic valves implanted using an endovenous approach have recently been evaluated as a treatment option in CDVI, with the advent of endovascular techniques in venous disease. In the near future, patients with CDVI are expected to benefit from minimally invasive procedures. In the first part of this article, we will review currently available prosthetic vein valves used in deep venous surgery and see how they were evaluated in animal models and clinical studies. The second...
part of the article will be devoted to describing implantable valves in primary CDVI, their indications, and the difficulties in designing and developing them.

**Surgical reconstruction with prosthetic valves**
Prosthetic venous valves provide an alternative option to CDVI treatment, when conservative treatment has failed and symptoms remain persistent. Since the mid-1960s, several potential off-the-shelf implantable valves have been tested as a substitute for autogenous venous valves. Prosthetic valves have been designed with single, double, or triple cusps leaflets from allografts, xenografts, or synthetic material attached to a carrier or a frame. However, most of these efforts yielded poor results in animal studies and have thus not been pursued further in clinical trials.

**Animal studies**
In 1988, Taheri et al implanted a center-hinged bileaflet valve constructed from platinum or a pyrolite carbon-covered titanium frame into the femoral vein of dogs. Prior to implantation, the valves were tested for fatigue and wear for 5 months, with only one valve developing a crack next to a cusp hinge. Additionally, flow and hydrodynamic pressure studies demonstrated that regurgitation was almost 48%. However, after 2 years of follow-up, it was reported that all the implanted valves had growths of dense intima hyperplasia that rendered them nonfunctional.

Although, the long-term results of this study were poor, one could argue that there was promising evidence that modification could extend the life of the valves sufficiently to be clinically useful.

An early clinical experiment with a cryopreserved decellularized allograft, when used as a conduit for arteriovenous fistula (AVF), was shown to be promising, inciting very little antigen response, as determined by panel reactive antibody. Thus a decellularized venous valve allograft could minimize the immunologic burden of donor cells. Additionally, this material has also been used in heart valve replacement, where it demonstrated a similar lack of antigen response and acceptable valve function.

However, in 2003, when Teebken et al designed a prosthetic valve using a decellularized allogenic ovine vein and subsequently implanted this valve into the external jugular vein of sheep, the results were disappointing. Twenty four grafts were used without any anticoagulation, and all of them were completely occluded after 6 weeks.

Even though this, the only existing animal study using decellularized venous valve allograft, had a negative outcome, this method still seems to hold promise. The method has been used successfully in other vascular beds, and further studies examining its use with anticoagulation are needed.

Burkhart et al evaluated the patency rate and hemodynamic behavior of a cryopreserved allograft venous valve in dogs in 1997. In this study, the dog recipients had pre-established lower limb venous insufficiency and were transplanted with cryopreserved veins containing valve allografts that were matched with dog erythrocyte antigen. The whole process was aided by the construction of a distal AVF. After subsequent ligation of this high-flow distal AVF that had been functioning for 3 to 6 weeks, all the transplants remained patent and competent for 3 more weeks, after which the recipients were sacrificed for histological examination. The histological results showed that endothelial cells were identified on the luminal surface only, and there was no presence of thrombus even in the cusp sinuses.

**Clinical studies**
These promising results triggered the initiative for a phase 1 multicenter feasibility study. During the first 6 months of follow-up, the primary patency and competency rates were 67% and 56%, respectively, indicating that a low-grade rejection phenomenon might be affecting the function of the valves. In 2003, Neglen et al reported the long-term results of this clinical study: after 2 years of follow-up, the percentage of cryovalves that remained both patent and competent was 27%. The authors concluded that cryovalve insertion was associated with high morbidity, high occlusion rate, poor cumulative midterm rate of patent graft with competent valve, and poor clinical results. The procedure should not be used as a primary technique for valve reconstruction, and it is questionable whether it is useful even in patients in whom autologous reconstruction techniques have been exhausted. However, if cryovalves are to become a viable alternative for valve repair, improved cryopreservation techniques, immunologic modifications, or better matching must be achieved.

A cryopreserved valve allograft (cryovalve), available from CryoLife Inc (Kennesaw, Georgia, USA), can remain competent with up to 125 mm Hg of retrograde pressure. However, there are two important issues to address for optimal competency outcome with the cryovalve: firstly, at the time of implantation, a primary valvuloplasty would be necessary; and, secondly, someone has to manage the potential issue of rejection with the valve substitute. Since patients suffering from primary CDVI are not critically ill, immunosuppression should be minimal so it is well-tolerated and complication-free. There are no protocols for the administration of immunosuppression after cryovalve implant for CDVI treatment.

### Selected Abbreviations and Acronyms

<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AVF</td>
<td>arteriovenous fistula</td>
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<td>BVV</td>
<td>bioprosthetic venous valves</td>
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<td>CDVI</td>
<td>chronic deep venous insufficiency</td>
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<td>EVTS</td>
<td>endovenous valve transfer stents</td>
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Implantable endovenous valves

In 1981, Charles Dotter was the first person to perform a transcatheter delivery of prosthetic venous valve for the treatment of venous reflux. This study triggered many attempts to develop various valves for percutaneous implantation in the following decades. Uflacker et al inserted a percutaneously delivered, stent-based, membrane-like venous valve into the inferior vena cava of a pig. This artificial monocusp venous valve, which consisted of a thin polyether urethane membrane inside a single body Z-stent, could be inserted percutaneously through a 10-Fr sheath (Figure 1). However, the valve only functioned for a week, and after that a partial thrombus appeared inside the valve cusps. A later development was the bovine glutaraldehyde-preserved, valve-containing jugular vein segment, which was also percutaneously delivered in a pig model. This segment was sutured inside a self-expanding nitinol stent (Boston Scientific, Boston, MA, USA), which could be introduced through an 18-Fr sheath (Figure 2). Although the xenografts were patent and competent in the surviving animals after 2 weeks of follow-up, the implant itself caused complications due to a foreign body reaction.

One of the glutaraldehyde-preserved bovine prosthetic valves was included in a clinical trial and assessed by de Borst et al. This was a percutaneously delivered bovine jugular valve-bearing venous xenograft sutured inside a memory coded nitinol frame (VenPro Inc, Irvine, CA, USA) (Figure 3). When phase 1 of the trial was completed, the prosthesis was improved; the bovine jugular venous valve was replaced by pericardial tissue, with a heparin coating, and the nitinol stent’s radial stiffness was increased. Currently, phase 2 of the clinical trial is awaiting launch in Europe and in the US.

Pavcnik et al have been heavily involved in the technological development of prosthetic venous valves. They have developed three generations of bioprosthetic venous valves (BVVs) and assessed their performance and their characteristics in animals; their bioprosthesis is currently part of clinical trials (Figure 4, page 172). The BVVs consist of a small leaflet of porcine small intestinal submucosa (Cook Biotech, West Lafayette, IN, USA) attached to different types of square stent frames.

Porcine submucosa is a relatively acellular, nonimmunogenic, biodegradable, xenogenic, collagen-based extracellular matrix material that provides a temporary scaffold for cellular colonization. For their experimental animal work, they used an ovine jugular vein due to anatomical and functional similarities of the sheep jugular vein to the human femoral vein.
The first-generation prosthesis was developed using nitinol or stainless steel wires for the frames and led to promising patency rates in animals. It was demonstrated at six months that patency and competency rates were 88% for the implanted valves, with host cell integration into the submucosa collagen matrix. In 2003, in the first clinical use, three patients with severe deep chronic valvular insufficiency who received anticoagulation pre- and postoperatively were treated with a square stent, submucosa BVV in the safety part of the trial, with a mixed outcome. Based on these previous studies, improved second-generation prostheses were developed to eliminate occasional tilting of the original BVV. The new valve was a sturdier version of the device described previously, and researchers had to assess several stent frame modifications to improve valve positioning during placement and to ensure its centering in the vein without tilting. The new BVV was composed of two overlapping frames made from nitinol (Figure 4), which provided four extra points at which the device could make contact with the inner wall of the vessel. Two sizes of BVV were tested in veins with diameters of 10 mm to 12 mm and 12 mm to 14 mm. The results were even better than those previously described; venograms at 6 weeks follow-up demonstrated no reflux in most (92.3%) of the valves. Third-generation valves were designed to prevent potential contact of the free leaflet portions with the inner venous wall and to ensure continued leaflet coaptation, even with their possible shortening. This time, the frame of the valve was made from nitinol tubing, which was cut by a laser device and again had four barbs for valve stabilization. The round geometry of the BVV allowed the leaflets to coapt and improved blood flow in the larger pockets of the valve, which prevented potential thrombus formation. In addition, two gold markers were placed on the nitinol frame to ensure precise anatomical orientation during valve deployment. The desired spatial orientation was achieved after deployment for all the valves, and all remained stable and completely functional on venography after implantation over a 5-week follow-up. On gross examination, the remodeled submucosa leaflets were free from the vein wall except at their distal parts, where the submucosa was thickened and attached to the vein. In an one-year clinical feasibility study, the third-generation BWVs had no migration and a 73% patency rate, but none remained competent.

Another group used a self-expanding stent (Wallstent, Schneider Inc., USA) with an autograft valve-bearing segment of vein secured within; overexpansion was utilized to hold the device in place. In this animal study, after one week of follow-up, residual nonocclusive thrombus was found attached to the exposed stent struts on the downstream end of the valve-stent in all animals, despite the universal use of anticoagulation in the first week post implantation. At six weeks, a manual strip test showed that all valves were patent and most of them competent. Overall findings suggested that less exposed metal would be better; however, no clinical paper has been published on the valve-stent design yet.

Following the development of novel endovascular technology, a balloon-expandable stent with a glutaraldehyde xenograft valve mounted within the stent was used for the treatment of primary CDVI. In this animal study, the implanted valves performed quite poorly; occlusion of all inferior vena cava implants at two months’ follow-up was demonstrated, with collateral circulation present. It was not clear whether the presence of xenograft material or metal or the trauma from balloon expansion to either the recipient vein wall or the donor valve was responsible for the poor results. It is possible that each factor could have to some degree contributed to these poor outcomes, and this particular valve-stent arrangement is unlikely to be the focus of investigation in the near future.

In addition, another implantable bioprosthesis valve was developed and evaluated using endothelial progenitor outgrowth cells isolated from whole bovine blood, as a source of in vitro autologous seeding for submucosal endothelialization. When examined by immunofluorescent staining, the endothelial monolayer remained intact after loading and delivery and...
when subjected to flow in the in vitro loop. After histological examination of the valves subjected to the ex vivo shunt loop, retention of the endothelial monolayer was revealed. According to this study, endothelial progenitor outgrowth cells seem to be a promising cell source for autologous endothelialization of bioprosthetic valves for the treatment of CDVI. However, clinical studies should be undertaken to clarify outcomes in the near future.

In the most recent study, autologous endovenous valve transfer stents (EVTSs) were evaluated in animals; a stent was placed around a native vein with a functional valve. The EVTSs (9 × 24 mm, including barbs) were developed by AllVascular (St Leonards, NSW, Australia) and the introducing system was produced by Cook (Bloomington, IN, USA). For optimal wall fixation, the fine barbs (3 mm in length) penetrated not only the donor’s wall, but also impinged on the recipient wall. In addition, the body of the stent had a zigzag pattern, which created high frictional resistance between the external surface of the stent and the recipient vein wall. A 2-mm expansion oversizing was considered optimal in the assessment of the recipient diameter. The length of the EVTSs were 20 mm to avoid tilting within the recipient vein and to minimize the possibility of endoleak. At harvest, all the transferred valves were competent, with no evidence of thrombosis, tilting, endoleak, or migration. Following these promising results, a clinical study in four patients was undertaken. Four males with recalcitrant ulcers (with a mean age of 22 years) had axillary veins transferred to the popliteal vein and were followed up. All of the ulcers improved and 50% healed completely; furthermore, all the valves were competent and patent.

**Optimal design and development**

Primary CDVI is a health problem that is underestimated and that has increased the socioeconomic burden of our era. It is a fact that the current treatment modalities are unsatisfactory, with poor outcomes despite clear indications. According to the guidelines of the American Venous Forum on surgical repair of deep vein valve incompetence, valve reconstruction is recommended in primary valvular incompetence after less invasive therapies have failed. Most of these procedures require considerable surgical skill and experience and, furthermore, these operations are not commonly performed and are associated with significant morbidity and mortality because of the risk of venous thromboembolism.

A venous valve delivered remotely with minimally invasive treatment (percutaneously) may remove the difficulties associated with open surgery. As previously mentioned, many animal studies and a few clinical ones have been undertaken to assess the feasibility and efficacy of prosthetic valves in primary CDVI. It has been suggested that the optimal prosthetic valve should be made from either synthetic or biological material attached to the frame of a stent that comes in a variety of diameters, with a proper fixation system to avoid migration. The venous connections to the stent would need to be fluid sealed to prevent endoleak, and the stent itself should have a minimal blood interface to minimize thrombogenicity as the barbs would be in contact with blood. Additionally, the device should be designed with a small-diameter profile for the system of introduction to be less traumatic and more feasible. The prosthetic valve should be durable, stable, and non-thrombogenic and should function properly under a diverse range of physiological conditions. Another review has stated specific design characteristics that future venous valve implants should have such as:

- Biocompatible materials
- Collapsible to a diameter <2 mm (6 French)
- Stent length <50 mm; a functional valve expandable to 20 mm
- Hydrodynamic performance comparable to predicate devices in the functional expanded range of 10 mm to 20 mm
- No migration under transvalvular pressure differences up to 200 mm Hg (in the functional expanded range)
- In vitro accelerated life prediction >200 megacycles (equivalent to 5 years).

**Summary**

In conclusion, we do not currently have an optimal endovalve available. The latest guidelines of the American Venous Forum refer only to surgical repair of primary deep vein valve incompetence, with valve reconstruction recommended (grade 1, level A) in primary valvular incompetence after less invasive therapies have failed. However, the procedures involved are very demanding, the results are not good, and availability restricted to only a few specialized centers. With the development of novel endovascular technologies, patients with primary CDVI are expected to benefit, but we still lack an effective endovalve and we do not know if the indications for its use will be the same as those for surgical repair.

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References

L’UTILISATION DES PROTHÈSES VALVULAIRES VA-T-ELLE SE DÉVELOPPER?

La prévalence de l’insuffisance veineuse chez les adultes est de 60 %, en particulier dans les pays développés. Dans un tiers des cas, le système veineux profond est atteint et cela a un impact clinique et financier sans thrombose veineuse préalable et conduit à une hypertension veineuse. Le reflux profond peut être associé à une insuffisance superficielle et à une insuffisance des perforantes. La prise en charge conservatrice réservée aux patients présentant des signes et symptômes sévères, intraitables, comme les ulcères récidivants, la lipodermatosclérose, et en cas d’œdème et de douleur sévères compromettant la qualité de vie du patient. La reconstruction chirurgicale comprend des techniques dont le but est la réparation en site des valvules ou la transplantation valvulaire. Des prothèses valvulaires veineuses ont été testées dans des modèles animaux et des études cliniques. Cependant, l’utilisation de ces prothèses reste controversée car elles n’ont pas donné les résultats escomptés pour le moment. L’évolution des techniques endovasculaires a récemment permis de tenter l’implantation valvulaire, une technique qui est amenée à dominer la stratégie thérapeutique dans un futur proche. Toutefois, la valve idéale n’a pas encore vu le jour.

Keywords: prosthetic venous valve; primary chronic deep venous insufficiency; endovenous; animal study; clinical study
Assessing compliance with nonoperative treatments of chronic venous disorders: the VEIN Act Program in Romania

by D. E. Branisteanu, Romania

Compliance with compression therapy differed according to sex, age, BMI, and symptom intensity. Most women preferred mild compression (37% of women vs 20% of men), while men preferred moderate and strong compression (80% of men vs 63% of women).... In young people, noncompliance was related to the perceived unattractiveness or discomfort of wearing stockings, while older patients and those with advanced CVD (CEAP classes C3-C6) complained of having difficulty in putting the hosiery on.”

Compliance with nonoperative treatments for chronic venous disorders (CVDs) has been poorly studied. The Romanian arm of the VEIN Act Program (VEnous disorders maNagement and evaluaTion of Chronic venous disease treatment effecTiveness program), an international, observational, prospective, multicenter study, assessed compliance with and the effects of nonsurgical CVD treatments (lifestyle advice, venoactive drugs and compression therapy) in symptomatic patients. Adult patients complaining of venous pain and/or signs and seeking treatment for a CVD underwent a leg examination during ordinary consultations. Following diagnostic confirmation of CVD, the patient’s clinical presentation and history, symptoms, and prescribed nonsurgical treatment(s) were noted. Compliance with treatment, treatment effect, and patient satisfaction were assessed in a follow-up consultation. Nearly two-thirds (64%) of 2444 Romanian CVD outpatients (1816 female, 608 male) were in CEAP stages C3 to C6, and very few presented with symptoms only (C0s; 3%) or mild signs (C1; 16%). Nearly all patients were prescribed a treatment (99.6%); 42% a combination of lifestyle advice, venoactive drugs, and compression therapy; and 42% venoactive drugs and advice. At the follow-up visit, 99%, 92%, and 30% of patients reported being compliant with venoactive drug prescription, lifestyle advice, and compression therapy, respectively. Most patients prescribed compression therapy did not wear it daily; 24% wore hosiery most days, 27% intermittently, and 19% not at all. At the follow-up visit, 42% were wearing their hosiery incorrectly. The Vein Act Program detected several factors that influence patient compliance with CVD treatment, and this information could help educate physicians and patients about better ways to manage CVD.

Medicographia. 2016;38:175-180 (see French abstract on page 180)
Methods
The Romanian VEIN Act program is a prospective, multicenter, observational survey. Patients who consulted a doctor for any CVD-related clinical presentation and who complained of pain in the lower limbs were eligible for participation in the study. VEIN Act was conducted in accordance with the principles of the 7th revision of the Declaration of Helsinki (Seoul 2008). Patients were informed and verbal and written consents for participation in the study were obtained. Screening for inclusion in the program was carried out using the following set criteria: age over 18 years (male or female), not currently treated for CVD, not consulting for an emergency or for the acute episode of an ongoing condition, and free of any concomitant disease that might interfere with venous treatment. Patients who met these criteria were asked about venous signs and symptoms and then underwent a leg examination. If they presented with at least one venous symptom or venous sign, or both, the following information was collected: clinical presentation, clinical history, presence of CVD signs and/or symptoms, and nonoperative treatment prescribed (including all treatment characteristics). They were asked to come back for a follow-up visit during which compliance, treatment effects, and patient satisfaction were assessed. In those patients who were noncompliant the reasons for noncompliance were sought.

Characterization of CVD symptoms and signs
The Vein Consult Program identified four symptoms of CVD (“heavy” legs, leg pain, a swelling sensation, and cramps) and three main circumstances of onset (after prolonged standing, at the end of the day, during the night). At least two of these three aggravating factors had to be present to confirm that the symptoms reported by the patients were actually related to CVD. The presence of signs such as those described in the clinical section of the clinical, etiological, anatomical, pathophysiological (CEAP) classification were noted and used to assign patients to the correct CEAP class (C0: no visible signs; C1: telangiectasias, reticular veins; C2: varicose veins/C3: edema/C4a: Skin changes, angiodermatitis/C4b: Skin changes, atrophie blanche/C5: Healed ulcer/C6: Active ulcer).

Assessment of chronic venous disorder symptoms
Patients were asked to assess the intensity of their symptoms using a visual analogue scale (VAS) and to report their frequency with a 5-point verbal scale (0=never, 1=rarely; 2=occasionally; 3=regularly; 4=all day and night).

Results
Enrollment in the VEIN Act program
The Romanian arm of the VEIN Act program took place between December 2013 and September 2014 and involved 248 investigators from the Romanian health care system. These physicians came from a wide range of medical specialties and included general practitioners, dermatologists, internists, vascular surgeons, rheumatologists, rehabilitation physicians, and gastroenterologists. A total of 2496 patients were enrolled at V0, and 2444 patients returned for the follow-up visit (V1). The mean time between inclusion visits (V0) and follow-up visits (V1) was 88 days, ie, approximately 3 months. There was no statistical difference between men and women regarding the interval between visits (P=0.97).

Patient characteristics at V0
Participants were predominantly female (74.9%), had a mean age of 56.1±14.1 years, and were overweight (BMI 27.67±5.13 kg/m²). Most of them were in the 50-59 years age group (29% of women and 29% of men).

Symptoms of CVD
Symptoms were present in 96.1% of patients, and at least 91.2% of them reported having symptoms over the previous 4 weeks. In order of decreasing frequency the symptoms re-
ported were: heaviness (91.2%), sensation of swelling (81.6%), leg pain (75.2%), and cramps (52.7%). Patients complained of 3±1 symptoms on average. Average symptom intensity was 5.4 cm on the VAS. Women reported symptoms more frequently than men (97% in women, vs 95% in men, \( P=0.028 \)), in particular "heaviness," albeit at a lower intensity (5.3 cm in women vs 5.4 cm in men) (Figure 1). Symptoms were experienced "regularly" in 70% of patients and "occasionally, rarely or never" in 30% of cases, and were more intense at the end of the day (83%) and after prolonged standing (82%). The prevalence of local pain and cramps over the previous 4 weeks increased with age in females, but not in males. Symptom intensity increased with both increasing BMI and increasing CEAP class in both sexes. The frequency of symptoms also increased with age.

**Self-reported signs of CVD**
Telangiectasias were reported in 80% of cases, edema in 62%, and varicose veins in 61%. There was no difference according to sex for edema (\( P=0.0084 \)) and telangiectasias (\( P=0.0028 \)), but more men than women sought treatment for varicose veins (72% in men vs 57% in women; \( P<0.0001 \)); this was also the case for skin changes (35% in men vs 17% in women, \( P<0.0001 \)) and venous leg ulcers (12% in men vs 2% in women, \( P=0.0001 \)) (Figure 2).

**Physician-reported signs (CEAP classification)**
Most patients seeking treatment had chronic venous insufficiency and were assigned to high CEAP classes: C3, 38.6%; C4 (skin changes), 21%, and C5-C6 (venous leg ulcers, 4%), while there were fewer patients in mild stages: C0s, 3%; C1, 16.2%; and C2, 15.3% (Figure 3). In the C1 and C2 classes, most patients were under 34 years old, while patients in the C3 to C6 classes were older and had a higher BMI.

**Treatment for CVD**
A total of 551 patients (22.7%) reported that they had previously consulted a physician for venous leg problems (68.4% women and 31.6% men), but only 328 patients (13.5%) had received a treatment (69.5% women and 30.5% men). These figures significantly increased with older age, and increasing BMI, symptom intensity, and CEAP class, regardless of sex (\( P<0.0001 \)). Nearly all patients (99.6%) who consulted for leg problems at V0 were prescribed a treatment, irrespective of their CEAP clinical class, including those in C0s (\( P=NS \)). Approximately 40% of them (42.4%) were prescribed a treatment combining lifestyle advice, vеноactive drugs, and compression therapy, and another 41.9% were prescribed a combination of vеноactive drugs and advice. A few patients were prescribed a single treatment (<1%). The type and combination of treatments did not vary according to the patient profile.

Most of the 51.4% of patients who were prescribed compression therapy were prescribed “mild” compression (15-22 mm Hg; 34.0%) and “moderate” compression (23-32 mm Hg; 38.6%). Stockings (85.3%), particularly at thigh level (52%), were preferred to bandages. Most patients were prescribed compression therapy for more than 12 weeks.
Assessment of compliance to treatment at V1

Venoactive drugs and lifestyle advice
Analysis of treatment compliance revealed that 99% of patients followed their prescription for venoactive drugs in terms of trade name, posology, and treatment duration, and that 92% followed the lifestyle advice they were given. The reasons evoked by patients for not taking the recommended venoactive drugs were “I forgot” or “I took another drug.” In general, older patients were more likely to switch to another drug ($P<0.0001$). The reasons given by patients for not following lifestyle advice were “lack of time” (49%), “too difficult to follow” (47%), and “ineffective” (4%). There were no differences according to sex, age, BMI, CEAP class, or symptom intensity.

Compression therapy
Just over three-quarters of patients (77%) purchased the strength of compression hosiery they were prescribed. The remaining 23%, who did not buy the prescribed strength, gave the following reasons: “unavailability at the place of purchase” (14%), and “switched to a different strength by the pharmacist” (5%), but most patients gave no reason (83%).

Only 58% of the patients who had been prescribed compression therapy attended the follow-up appointment wearing the compression hosiery correctly, and 30% reported that they had worn the hosiery as prescribed. A majority of patients (70%) wore hosiery either most days (24%), or intermittently (27%), or did not wear it at all (19%). A significant percentage of patients (42%) did not use the recommended compression therapy. Their reasons for not wearing compression hosiery were that they found it “uncomfortable” (48%), “too difficult to put on” (35%), “too hot” (22%), “itchy” (21%), “unattractive” (13%), or “ineffective” (2%) (Figure 4).

Compliance with compression therapy differed according to sex, age, BMI, and symptom intensity. Most women preferred mild compression (37% of women vs 20% of men), while men preferred moderate and strong compression (80% of men vs 63% of women). Women felt that the stockings were unattractive, while men found them too hot. Young patients preferentially bought mild-strength stockings ($P<0.0001$). In young people noncompliance was related to the perceived unattractiveness or discomfort of wearing stockings, while older patients and those with advanced CVD (CEAP classes C3-C6) complained of having difficulty in putting the hosiery on. Patients with mild symptoms bought light-compression stockings, and those with advanced symptoms purchased moderate- and strong-compression stockings ($P<0.0001$), which they found difficult to put on ($P=0.03$).

Discussion

CVD is often underdiagnosed by doctors and neglected by patients, which is why the cost of care for patients with CVD is very high (particularly in severe stages), accounting for at least 2% to 3% of community health care budgets. CVD is a chronic, progressive, and debilitating disease; greater patient compliance with prescribed treatments and early diagnosis are key in controlling its progression and improving the quality of life for patients. Findings from the VEIN Act program have confirmed that although CVD affects millions of people worldwide, it is still underdiagnosed and dismissed by some patients despite its negative impact on the quality of life.

The Russian arm of the VEIN Act program found that a very small percentage of patients diagnosed with CVD prior to enrollment had been prescribed a treatment. Similarly, in our study, 551 patients (22.7%) reported having made at least
one visit to a doctor for CVD-related problems prior to entering the study, though only 328 patients (13.5%) had received a treatment. This was also the case in the RELIEF study (Reflux assessment and quality of life improvement with micronized Flavonoids), which found that a very low percentage (21.8%) of the intention-to-treat (ITT) population had previously been treated, despite showing obvious symptoms of chronic venous disorders. These findings confirm the fact that some patients underestimate CVD and that their compliance with treatment is poor. Therefore, more efforts are required to increase public awareness about the chronic, progressive, and potentially disabling nature of CVD, and patients should be reminded that a correct diagnosis and prompt treatment initiation can control the disease and prevent its progression to more severe stages.

Findings from the VEIN Act program show that very few CVD patients (3.3%) are diagnosed in CEAP class C0, and that most patients are diagnosed in stage C3 (38.6%). Similarly, the Vein Consult Program has showed that CVD is most often diagnosed when patients are in the advanced stages of the disease. These findings emphasize the need for a sustained effort to actively detect CVD and—why not—provide additional training for both GPs and specialists, so that therapeutic decisions can be made in the early stages of CVD. One of the risk factors for CVD is obesity. The Romanian arm of the VEIN Act Program has identified a significant positive correlation between BMI and incidence of CVD, especially for severe stages of the disease. Multiple studies have shown that patients with a high BMI tend to have a higher risk of CVD and leg ulcers. The VEIN Act program has also identified a strong correlation between BMI and the time needed to obtain symptom relief with vеноactive drugs. Little is known about how obesity affects the management of CVD. A recently published study carried out in obese patients concluded that the treatment of CVD is affected by BMI and class II obesity (ie, BMI between 35.0 and 39.9), and that morbidly obese CVD patients are less compliant with compression therapy but are willing to undergo surgical procedures and use topical agents. Another question is whether the usual doses of vеноactive drugs are sufficient for CVD patients with a very high BMI. It is important to emphasize that in obese CVD patients, lifestyle changes and weight loss are imperatively recommended in addition to long-term vеноactive treatment.

Short-term treatment with vеноactive drugs may be responsible for their low therapeutic efficacy and the occurrence of CVD complications. Thus, incomplete control of the signs and symptoms of CVD could be responsible for both the progression to severe stages and a decrease in quality of life and, consequently, poor patient compliance with the recommended treatments. Therefore, it may prove useful to promote awareness among physicians of the potentially detrimental effects of prescribing vеноactive drugs for too short a period of time.

References


Keywords: chronic venous disorders; compliance; compression therapy; vеноactive drugs
Évaluation de l’observance des traitements non chirurgicaux de la maladie veineuse chronique : le VEIN Act program en Roumanie

L’observance des traitements non chirurgicaux de la maladie veineuse chronique (MVC) a été peu étudiée. Le bras roumain du VEIN Act program, une étude internationale, observationnelle, prospective, multicentrique avait pour but d’évaluer l’observance et les effets des traitements non chirurgicaux de la MVC (conseils d’hygiène de vie, médicaments veinotoniques et traitement par compression) chez les patients symptomatiques. Des patients adultes souhaitant être traités pour une MVC et se plaignant de signes et/ou de douleurs veineuses des jambes ont été examinés lors de consultations ordinaires. Une fois le diagnostic de MVC confirmé et les patients inclus dans l’étude, les résultats de l’examen clinique, les antécédents des patients, ainsi que leurs symptômes et les traitements non chirurgicaux leur ayant été prescrits ont été notés. Une consultation de suivi a permis d’évaluer l’observance et les effets du traitement, ainsi que la satisfaction des patients. En Roumanie, environ deux-tiers (64%) des 2 424 patients ambulatoires atteints de MVC inclus dans l’étude (1 816 femmes, 608 hommes) présentaient des stades C3 à C6 de la CEAP et très peu d’entre eux (3%) un stade C0s (seulement des symptômes) ou un stade C1 (des signes légers ; 16%). Un traitement a été prescrit à presque tous les patients (99,6%); 42 % ont reçu des conseils d’hygiène de vie, des médicaments veinotoniques et un traitement par compression ; et 42 % ont reçu des médicaments veinotoniques et des conseils. À la visite de suivi, 99 % des patients ont dit suivre la prescription de médicaments veinotoniques, 92 % les conseils d’hygiène de vie et 30 % le traitement par compression. La plupart des patients auxquels un traitement par compression avait été prescrit ne le portaient pas tous les jours ; 24 % portaient leurs bas la plupart du temps, 27 % par intermittence et 19 % pas du tout. Lors de la visite de suivi, 42 % des patients portaient leurs bas de façon incorrecte. L’étude VEIN Act a permis d’identifier plusieurs facteurs influant sur l’observance des patients et cette information pourrait servir à éduquer médecins et patients afin d’améliorer la prise en charge de la MVC.
The optimal duration of VAD treatment in the initial stages of CVD is 8 weeks or more. In patients with advanced CVD—ie, with chronic venous edema or trophic skin lesions—VAD treatment can be prescribed for longer periods. However, in about one-third of patients (31.9%), VADs were administered for a period of 8 weeks or less, despite the fact that there is a direct correlation between the duration of VAD treatment and the duration of symptom remission.

The VEIN Act Program is an international study aimed at investigating the use of nonoperative treatments for chronic venous disease (CVD), assessing their efficacy and safety, and monitoring patient compliance. The Russian arm of the study revealed that vеноactive drugs (VADs) are highly popular, both among physicians and patients, and that this is a factor that explains the high adherence to VAD treatment by Russian patients. The advice on lifestyle modifications was followed by more than 80% of patients, but often incompletely. Compliance with compression therapy was quite low and characterized by a reduction in the class of compression stockings and their irregular use in more than 30% of patients. The benefits of nonoperative CVD treatment in Russia were proven by the high level of patient satisfaction with treatment and also by a statistically significant decrease in the severity of CVD symptoms in the vast majority of patients.

Nonoperative treatments have undeniable benefits in the treatment of chronic venous disease (CVD) of the lower extremities. On the one hand, this is due to the epidemiology of CVD and the predominance of symptomatic forms that do not require surgical treatment. On the other hand, contemporary phlebology has extended the indications for nonoperative therapy in patients at high risk for CVD (long periods of time spent in the standing or sitting position, use of contraceptive hormones, hot weather, etc…) or undergoing interventional procedures (thermal ablation, sclerotherapy, phlebectomy, etc.).

Nonoperative treatment requirements, and compliance with such treatments, are determined by factors that are difficult to modify. This is why many epidemiological and clinical studies are designed to assess the various techniques of nonsurgical treatment of CVD. The VEIN Act Program, which was initiated by the European Venous Forum, is such a study. Its aim was to study the use of nonoperative CVD treatments, assess their efficacy and safety, and monitor patient compliance. This article presents the preliminary results of the Russian arm of this large-scale international project.

Methods
A total of 82 physicians from various regions of the Russian Federation participated in the VEIN Act Program and enrolled 1607 patients (325 males, 1282 females) aged between 18 and 89 years (mean age: 45.7 ± 14.1 years). The distribution of patients according to age is presented in Table I (page 182).
Patients were screened using the following inclusion criteria: confirmed primary CVD, presence of CVD-specific symptoms, and absence of any condition that could affect the study results. At the first visit (V0)—which sometimes coincided with the screening visit—patients were prescribed nonoperative treatments for their CVD. At the follow-up visit (V1), the physicians assessed the effects of the prescribed treatment and compliance with the treatment regimen. A 10-point visual analog scale (VAS) was used to assess symptom intensity.

Treatment outcomes were assessed after 60 to 90 days. The mean follow-up period was 87.2 ± 22.1 days. A total of 18 patients (1.1%) did not show up for the final visit (V1), and were therefore excluded from the analysis. Statistical analyses were performed by an independent expert using parametric and nonparametric methods.

Results and discussion
Patient characteristics are known to play an important role in the development and progression of CVD. The mean body mass index (BMI) of the patients included in the study was 26.0 ± 5.0 kg/m², and 53.2% of them were either overweight or obese (BMI ≥25.0 kg/m²).

◆ CVD symptoms at inclusion
The study included 1607 patients with various CVD-specific symptoms or signs of CVD at V0 (Tables II and III). Among them, 626 (39%) patients had already consulted a doctor for their leg problems, and 494 (31%) patients had already been treated for venous leg problems prior to inclusion in the study.

At baseline (V0), the severity of CVD symptoms was assessed using a 10-point VAS scale. The most distressing symptoms for patients were “leg heaviness,” “leg pain,” and “sensation of swelling” (Table IV). Symptom intensity was maximal at the end of the day in 87.2% of patients, after prolonged standing in 58.3% of patients, and/or during the night in 18.2% of patients. CVD-specific symptoms occurred regularly in 55.9% of patients, occasionally or rarely in 39.5% of patients, or persisted all day and night long in 3.6% of patients.

◆ Nonoperative treatments prescribed
The study protocol specified that after assessing the phlebological status of their patients, doctors had to determine

### Table I. Patient distribution according to age.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Number of patients (N=1607)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20 years</td>
<td>9 (0.6%)</td>
</tr>
<tr>
<td>20 to 29 years</td>
<td>221 (13.8%)</td>
</tr>
<tr>
<td>30 to 39 years</td>
<td>352 (21.9%)</td>
</tr>
<tr>
<td>40 to 49 years</td>
<td>357 (22.2%)</td>
</tr>
<tr>
<td>50 to 59 years</td>
<td>362 (22.5%)</td>
</tr>
<tr>
<td>60 to 69 years</td>
<td>210 (13.1%)</td>
</tr>
<tr>
<td>70 to 79 years</td>
<td>58 (3.6%)</td>
</tr>
<tr>
<td>≥80 years</td>
<td>14 (0.9%)</td>
</tr>
<tr>
<td>Age not specified</td>
<td>24 (1.4%)</td>
</tr>
</tbody>
</table>

### Table II. Prevalence of chronic venous disease–related complaints at V0.

<table>
<thead>
<tr>
<th>Sign</th>
<th>Number of patients (N=1607)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No visible signs</td>
<td>70 (4.4%)</td>
</tr>
<tr>
<td>Telangiectases, reticular veins</td>
<td>1075 (66.9%)</td>
</tr>
<tr>
<td>Varicose veins</td>
<td>993 (61.8%)</td>
</tr>
<tr>
<td>Edema</td>
<td>714 (44.4%)</td>
</tr>
<tr>
<td>Skin changes, angiodermatitis</td>
<td>149 (9.3%)</td>
</tr>
<tr>
<td>Skin changes, atrophie blanche</td>
<td>48 (3.0%)</td>
</tr>
<tr>
<td>Healed ulcer</td>
<td>19 (1.2%)</td>
</tr>
<tr>
<td>Active ulcer</td>
<td>24 (1.5%)</td>
</tr>
</tbody>
</table>

### Table III. Signs of chronic venous disease at V0.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Number of patients (N=1607)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heaviness</td>
<td>4.56±2.59</td>
</tr>
<tr>
<td>Leg pain</td>
<td>3.23±2.90</td>
</tr>
<tr>
<td>Sensation of swelling</td>
<td>3.28±2.97</td>
</tr>
<tr>
<td>Cramps</td>
<td>1.73±2.59</td>
</tr>
</tbody>
</table>

### Table IV. Severity scores of the main symptoms of chronic venous disease assessed with a visual analogue scale (VAS) at baseline (V0).
whether any treatment was needed. In 74.2% of cases a treatment was deemed necessary. Nonoperative therapy was prescribed in 65.2% of patients, surgery in 0.4% of patients, and a combination of these approaches in 33% of patients. Table V shows a breakdown of the nonoperative treatments prescribed. The term “other” in the table corresponds to those isolated cases where the use of various topical agents (ointments, gels, wound dressings) and/or pneumatic compression was recommended. Although many studies have found high rates of topical agent use in CVD patients, this was not the case in this study.

**Lifestyle advice**

The recent updates of the international and Russian guidelines for the clinical treatment of CVD have put emphasis on lifestyle recommendations to eliminate the risk factors that are known to contribute to the development of CVD. Table VI lists the lifestyle recommendations given to patients in the VEIN Act program.

**Venoactive drugs**

In our study, venoactive drugs (VADs) were prescribed to 1554 patients, but they were actually used by 1590 patients. It is interesting to note that a number of VADs that are widely used in clinical practice outside of Russia (ie, oxerutin, troxerutin, escin, proanthocyanidins, calcium dobesilate, etc) were not prescribed in this study. Micronized purified flavonoid fraction (MPFF) (brand name Detralex in Russia) was the most commonly prescribed VAD; it was prescribed in 93.9% of cases (Table VII). The international nonproprietary name (INN) of Detralex (ie, MPFF) seems to have confused many physicians, who either checked simultaneously the items “MPFF” and “Diosmin” or did not give any answer. Therefore, it is likely that the physicians who checked the “Diosmin” box also meant “MPFF” (Detralex).

The efficacy and safety of VADs depend on the dosage and duration of treatment, which are themselves determined by the choice of drug and the CVD class. The vast majority of patients took the standard daily dose of Detralex (1000 mg twice a day) (Table VIII). However, it should be noted that all patients who took 3 or 4 tablets of Detralex a day, as well as 10 of the 18 patients who took it once a day, had also been taking Detralex without a prescription. The reason for this is unclear, as the doctors involved in the study are highly qualified phlebologists who know firsthand how to prescribe the different VADs. Perhaps this was due to negligence in completing the questionnaires or to the use of certain “proprietary” regimens of VAD treatment. In addition, the only patient who had an adverse event during the study was a patient who had been taking 4 tablets of Detralex per day.

The optimal duration of VAD treatment in the initial stages of CVD is 8 weeks or more. In patients with advanced CVD—ie, with chronic venous edema or trophic skin lesions—VAD

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Number of patients (N=1607)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lifestyle advice</td>
<td>1403 (87.3%)</td>
</tr>
<tr>
<td>Venoactive drugs</td>
<td>1554 (96.7%)</td>
</tr>
<tr>
<td>Compression therapy</td>
<td>1473 (91.7%)</td>
</tr>
<tr>
<td>Painkillers</td>
<td>30 (1.9%)</td>
</tr>
<tr>
<td>Other</td>
<td>13 (0.8%)</td>
</tr>
</tbody>
</table>

**Table V. Nonoperative treatment prescribed at V0.**

<table>
<thead>
<tr>
<th>Lifestyle advice</th>
<th>Number of patients (N=1607)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Move your legs as much as possible</td>
<td>1152 (71.7%)</td>
</tr>
<tr>
<td>Choose the right sport (walking, cycling, swimming…)</td>
<td>1214 (75.5%)</td>
</tr>
<tr>
<td>Avoid sources of heat</td>
<td>849 (52.8%)</td>
</tr>
<tr>
<td>Lose excess weight</td>
<td>746 (46.4%)</td>
</tr>
<tr>
<td>Wear shoes with suitable heels</td>
<td>1011 (62.9%)</td>
</tr>
<tr>
<td>Aid venous return by leg elevation</td>
<td>1169 (72.7%)</td>
</tr>
<tr>
<td>Massage your legs as often as possible</td>
<td>537 (33.4%)</td>
</tr>
</tbody>
</table>

**Table VI. Lifestyle advice given to patients at V0.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number of patients (N=1607)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micronized purified flavonoid fraction</td>
<td>1509 (93.9%)</td>
</tr>
<tr>
<td>Diosmin</td>
<td>51 (3.2%)</td>
</tr>
<tr>
<td>Calcium dobesilate</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Oxerutin/Troxerutin</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Escin (horse chestnut seed extract)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Ruscus extract</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Ginkgo biloba</td>
<td>1 (0.06%)</td>
</tr>
<tr>
<td>Proanthocyanidins</td>
<td>4 (0.25%)</td>
</tr>
<tr>
<td>Name of drug not specified</td>
<td>25 (1.6%)</td>
</tr>
<tr>
<td>Venoactive drugs not prescribed</td>
<td>17 (1.1%)</td>
</tr>
</tbody>
</table>

**Table VII. Venoactive drugs prescribed to the patients.**

<table>
<thead>
<tr>
<th>Number of tablets</th>
<th>Number of patients (N=1607)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 tablet</td>
<td>18 (1.1%)</td>
</tr>
<tr>
<td>2 tablets</td>
<td>1314 (81.8%)</td>
</tr>
<tr>
<td>3 tablets</td>
<td>1 (0.06%)</td>
</tr>
<tr>
<td>4 tablets</td>
<td>12 (0.7%)</td>
</tr>
<tr>
<td>Dosage not specified</td>
<td>245 (15.3%)</td>
</tr>
<tr>
<td>Venoactive drugs not prescribed</td>
<td>17 (1.04%)</td>
</tr>
</tbody>
</table>

**Table VIII. Prescribed dosage of venoactive drugs (number of tablets per day).**
treatment can be prescribed for longer periods. However, in about one-third of patients (31.9%), VADs were administered for a period of 8 weeks or less, despite the fact that there is a direct correlation between the duration of VAD treatment and the duration of symptom remission.

Compression therapy
Compression therapy is a key component of non-interventional treatment for CVD. It is categorized according to the level of pressure applied at the ankle and the method of compression used (compression bandages or elastic graduated compression hosiery). For patients with CVD, optimal compression pressure at the ankle should be within the range of 20-30 mm Hg. The grade of compression can be reduced in early disease or increased in advanced disease. In our study, the appropriate grade of compression therapy was prescribed in 90.5% of patients. In 92.4% of cases, preference was given to compression hosiery. According to our data, 35 (2.2%) patients were prescribed both compression bandages and hosiery, but the reason for this is unclear. Perhaps the physicians felt that it was preferable to use compression bandages in the first stages of treatment in case of severe CVD (ie, chronic edema, trophic skin lesions). Another likely explanation is that the choice of compression method was left to the patients’ discretion. Detailed analysis of the type of compression prescribed showed that the use of compression therapy in Russia differs from that of the rest of the world. None of the physicians prescribed rigid bandages—though they are more effective—and in 3.4% of cases bandages with moderate or high stretchability were chosen. The most frequently recommended compression garments were stockings and tights (prescribed to 74.9% of patients), which are expensive and difficult to put on. Below-knee compression socks/stockings, which are more user-friendly and cost-effective, were prescribed to only 14.5% of patients. It is interesting to note that in other countries, the ratio of prescription of compression socks to compression stockings/tights is reversed, and this appears to be for mostly pragmatic reasons, rather than financial ones. The main goal of compression therapy in CVD is to improve calf-muscle pump function, which can be achieved equally well using medical compression stockings/tights and below-knee stockings. Since the latter are easier to put on and more comfortable to wear at any time of the year for both males and females, compliance with compression therapy with knee socks is expected to be higher. In addition, the cost of compression hosiery is an important issue, and below-knee stockings/socks are definitely more cost effective.9

Treatment compliance
Lifestyle recommendations
One of the important goals of the VEIN Act Program was to evaluate treatment compliance. Life-style recommendations were followed by 1321 (82.2%) patients. Those who were not compliant gave the following reasons: lack of time, technical and organizational difficulties, and poor efficacy.

Venoactive drugs
In the study population, 93.7% of patients reported taking the VAD they were prescribed, and 87.4% of patients confirmed that they were compliant with the dosage regimen. However, more than one-third of patients followed their VAD treatment for less than 8 weeks, and 11.3% of patients reduced the duration of treatment of their own accord when the drug was prescribed for 9 weeks or more.
Compression therapy

The recommended compression hosiery was purchased by 78.4% of patients. There was also a trend—though it was not significant—for under- or overestimation of the recommended class of compression by some patients. To achieve a therapeutic effect, compression hosiery should be worn for the entire time spent in an upright position. This recommendation was followed by 67.6% of patients, albeit with small variations.

Treatment outcomes

Venoactive drugs

Following VAD treatment, 92.3% of patients experienced relief of their CVD-specific symptoms, and 67.2% of patients had a remission period of 3 weeks or more. In total, 8.8% of patients showed low compliance with VAD treatment due to various reasons.

Compression therapy

Relief from CVD symptoms was reported by 68.8% of patients who used compression therapy. Symptom relief lasting 3 weeks or more was reported by 62.4% of patients. Low compliance with compression therapy was recorded in 43.1% of patients.

Overall efficacy and patient satisfaction

Overall, the use of nonoperative treatment resulted in a statistically significant ($P<0.00001$) reduction in the severity of the main symptoms of CVD (Figure 1). There was also a statistically significant reduction in the number of patients experiencing symptoms (Figure 2). In addition, there was a statistically significant ($P<0.00001$) reduction in the frequency of CVD symptoms following completion of the treatment course (Figure 3).

The vast majority (95%) of patients were satisfied with the treatment outcomes. Only 4% of patients rated their treatment as having failed or as having low efficacy. In 1% of cases no data were available. Although the VEIN Act Program was not designed to assess tolerability, 1 patient (out of 1607 patients) reported an adverse reaction in the form of skin rashes and pigmentation. Data were available. Although the VEIN Act Program was not the main symptoms of CVD

Nevertheless, the VEIN Act program has objectively proven the usefulness of nonoperative therapy in reducing the severity of CVD symptoms in the vast majority of patients and this resulted in high rates of patient satisfaction.

Conclusion

In summary, the preliminary results of the Russian arm of the VEIN Act Program have highlighted a number of specificities regarding nonoperative treatment for CVD in Russia. First of all, VADs are extremely popular with both physicians and patients, and this explains the high adherence of Russian patients to VAD treatment. However, one in three patients does not follow the treatment regimen properly, and this is most often manifested by a reduction in treatment duration. Second, we were surprised to find that lifestyle recommendations were followed by more than 80% of patients, although often incompletely. Third, we found that compliance with compression therapy was low (ie, use of a lower class of compression and/or irregular use in more than 30% of patients), which is a shame given its effectiveness. Obviously, increasing the duration of treatment would lower the compliance even further. Nevertheless, the VEIN Act program has objectively proven the usefulness of nonoperative therapy in reducing the severity of CVD symptoms in the vast majority of patients and this resulted in high rates of patient satisfaction.

References

Le VEIN Act Program est une étude internationale dont le but est d’étudier l’utilisation des traitements non chirurgicaux dans la maladie veineuse chronique (MVC), d’évaluer leur efficacité et leur innocuité et d’apprécier l’observance des patients. Le bras russe de l’étude montre que les médicaments veinotoniques (MVT) sont très populaires, à la fois chez les médecins et les patients, ce qui explique en partie la forte adhésion des patients russes au traitement par les MVT. Quatre-vingt pour cent des patients suivent les conseils d’hygiène de vie, mais souvent partiellement. L’observance du traitement par compression est assez faible et se caractérise par une diminution de la classe de compression et une utilisation irrégulière chez plus de 30 % des patients. En Russie, les bénéfices du traitement non chirurgical de la MVC se traduisent par un taux élevé de satisfaction des patients ainsi que par une diminution statistiquement significative de la sévérité des symptômes de la MVC chez la majorité des patients.

**Keywords:** chronic venous disease; compression therapy; micronized purified flavonoid fraction; venoactive drug
THE QUESTION

Patients having undergone a superficial or deep venous procedure experience stable or improved hemodynamic and clinical status over time. However, after varicose vein surgery, up to 40% of patients may eventually develop neovascularization, and one-third, an extension of preexisting reflux or reflux in a new segment. Physicians across the globe share their own experience with the effect of varicose vein surgery in preventing the extension of venous reflux in their patients.

Can varicose vein surgery prevent extension of venous reflux with time?

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Reflux in the superficial veins, the most prevalent pathology in all chronic venous disease classes, is often collectively reported as great saphenous vein (GSV) or small saphenous vein incompetence. Labropoulos et al.\(^1\) show that primary venous reflux can occur in any superficial or deep vein of the lower limbs and in the absence of saphenofemoral junction (SFJ) or GSV incompetence. It is often found at different sites that may not communicate with or affect each other. This suggests that reflux is probably due to a local or multifocal process in addition to or separate from a retrograde process.

Patients with chronic venous disease often ask whether elective vein surgery could be delayed without consequences. Stripping or ablation of the GSV is a useful procedure in the management of varicose veins. In a study in which patients underwent either GSV stripping or SFJ ligation alone, stripping reduced clinical recurrence of varicose veins by two-thirds after 5 years.\(^2\) Perrin et al. in the REVAS study (REcurrent Varices After Surgery) identified several factors associated with recurrence and classified them as belonging to one of two groups: (i) those arising from inadequate or incomplete initial treatment, due to technical or diagnostic errors; and (ii) those arising from evolution or appearance of new sites of reflux secondary to neovascularization (defined as thin-walled, serpentine tributaries arising from a previously ligated SFJ), even after appropriate surgical intervention had been carried out.\(^3\)

According to Turton et al., venous flow must be rechanneled after venous surgery in an anterograde direction through the remaining superficial and deep veins of the leg.\(^4\) This sudden hemodynamic alteration to the return of blood in the superficial and deep veins causes redistribution of venous flow and could overload veins that had been competent. It is possible that the main source of reflux previously “masked” an inherent weakness in such veins or others that were more partially incompetent. This so-called neoreflux may appear in a substantial number of patients, despite complete abolition of the sites of reflux identified preoperatively.

van Neer et al.\(^6\) shows that 91% of patients who undergo a short stripping procedure (above the knee) will have persistent reflux in the remnant GSV branches below the knee after surgery. This incompetence of the distal GSV is independent from the proximal GSV part and from incompetent perforating veins (IPV). There seems to be a tendency toward worsening of clinical signs and symptoms between 6 months and 2 years after surgery, and this goes along with an increase in reflux and in diameters of the GSV remnants below the knee.

In patients with SFJ insufficiency, GSV varicosities, and slight reflux in the GSV below the knee, my preferred choice for surgical intervention is to carry out either complete stripping or ablation of the GSV above and below the knee, with mini-phlebectomy or foam sclerotherapy of remnants below the knee. All this significantly reduces development of the risk for postoperative reflux (duplex ultrasound should be performed 6 and 24 months after surgery).

Despite the new methods of treatment, the risk of development of recurrent varicose veins (based on 5-20 years of evidence) remains very high after surgical intervention. This finding indicates the necessity of further investigation and clinical trials.

References
The pathogeny of chronic venous disease (CVD) has more shades than previously believed. There are primary varices with retrograde development, but also with orthograde development, with a continent saphenous cross in an approximately similar percentage to suspended primary varices. The distribution of valves and their localization in the saphenopopliteal and saphenofemoral junction segments, in areas with highly mobile joints, and their ostial or subostial position are "discrete" elements (systemic "initial sensitivities"); the pathogenic involvement is difficult to assess at first glance. The muscle-pump activity, mainly in the calf and abdominal muscles, pushes venous flow distally. Thus, it influences active venous hypertension episodes, which have the biggest effect on the first upstream axial valve. When the common femoral vein has no valves, the pressure stress falls on the first (superficial) femoral valve, nearly always situated under the junction with the deep femoral vein. The ostial position of the great saphenous terminal valve directs the reflux preferentially toward the femoral valve and protects the crosse. The subostial valve position allows space for the pressure to be received within this largely distensible segment—the saphenous crosse. In the perforating vein, the first valve is extrafascial, sometimes in the ostial position, and the second valve is in a variable position, subfascial, providing conditions amenable to having pressure-reception "rooms."

In all these situations, conditions are right for a functional insufficiency, especially in the perforator veins, especially of the saphenous crosse. The better the terminal and subterminal valvular continence, the higher the stress on the (superficial) femoral valve. In a patient with an inveterate CVD (more than 15 years in duration), using ultrasound, I found a mixed longitudinal reflux in the proximal (superficial) femoral axis and in the saphenous vein as well (in 1 out of 6 cases); therefore, the patient had venous hypertension. Consequently, we are convinced that every patient should benefit from a multifaceted, personalized treatment. In these situations, any intervention that addresses only the saphenous system—identified by ultrasound—that will become more intense after crossectomy, saphenectomy, and varicose excisions, will lead to variceal relapse.

The pathophysiological argument encouraged us to propose a more complex surgical procedure, comprising a femoral tangent crossectomy, followed by venorrhaphy, with saphenectomy or intraoperative catheter-directed vein sclerotherapy, and varicose excision. The femorofemoral junction is wound with a synthetic rectangular patch about 5-6 cm high, so that the patch can slightly dip down. In the patch, we make a hole for the deep femoral vein passage. The patch descends about 2 cm onto the (superficial) femoral vein. We did not record any postoperative complications.

Patients were selected on the basis of ultrasound detection of valve and transvalvular reflux. Patients were excluded for having a post-thrombotic syndrome, valvular destruction, or parietal changes. The physiopathological objectives achieved include (i) restoration and maintenance of valvular continence, via a "fixed splint" (synthetic patch that is wound around the site); (ii) eradication of venous hypertension in the deep venous axis, and distal relapse prevention; (iii) cancellation of the saphenous "reflux gate"; and (iv) groin neovascularization prevention.

In up to 10 years of ultrasound monitoring of this group of 88 patients, no axial reflux greater than 0.3 seconds was found, and neither were any varicose relapses. Thus, we conclude that varicose vein surgery is effective in preventing the extension of venous reflux in rigorously selected cases, when the ultrasound diagnosis is complete and surgery occurs early and is adapted to the specific pathogenesis.
In my experience, the direct answer is yes. I do believe that varicose vein surgery has beneficial effects on the venous system in the leg and even on the segments that were not targeted by the surgery. Most of my patients have shown improvement in their venous competence, but in accordance with the pathophysiological concept that chronic venous disease is a progressive disease, some patients also may develop incompetence in other segments during their lifetime. I always tell my patients that varicose disease has an important characteristic: it is progressive and long lasting. I advise them to be evaluated periodically even after being treated. These patients must be followed-up with duplex scanning and evaluation for clinical criteria in order to assess the hemodynamics of the venous system after varicose vein surgery. Most studies have revealed that even with thermal ablation surgery (radiofrequency ablation [RFA] and endovenous laser therapy [EVLT]), the patients have showed improvement in venous hemodynamic abnormalities. We agree and believe that duplex ultrasonography is a fundamental component of the investigation of the lower limb venous system after treatment for varicose veins. The timing of such imaging depends on what we are trying to determine: immediate (1-4 weeks), either to know whether the intervention has achieved the goal or when intended to be a part of sequential treatments; short-term or mid-term (1-3 years), to detect newly developed incompetent veins or to reveal ultrasound features that predict longer-term outcome after thermal ablation treatment of great or small saphenous veins; and long-term (5 years or more), to assess the development of clinical recurrence, which may have arisen as a consequence of the incompetent veins detected by the short-term or mid-term scan. In addition to duplex ultrasound imaging, clinical findings should be evaluated according to a clinical score and quality of life assessment at the same time intervals. We also know that surgical treatment of calf perforator veins results in reduced deep vein reflux, and the improvement is most marked in cases of primary venous insufficiency. It is also important to emphasize—although we already know that selective stripping has the same rate of recurrence as standard stripping—that some studies have shown that complete stripping (long and short saphenous vein) abolishes deep vein reflux in a significant proportion of limbs, and that if failure of stripping occurs, new deep venous reflux may develop. These findings support routine stripping and suggest that the benefits of stripping may relate, at least in part, to a favorable impact on deep venous function.

References
Whereas compression therapy is the main treatment option for chronic venous disease (CVD), intervention therapy is considered a causal one.

Conventional surgery consists of crossectomy, stripping of the great or small saphenous vein (GSV or SSV), and phlebectomy. With crossectomy, a number of studies point out a risk of recurrence; however, study results differ significantly. In 2003, Van Rij reported that the recurrence rate 3 years after surgery reached 23%. On the other hand, in 2013, a large multicenter study called “LaVaCro-study” showed a recurrence risk of 2.57% with the same follow-up as in the previous study and a risk of 2.92% with a 4-year follow-up. In that study, the authors emphasized the great importance of the quality of crossectomy, during which the GSV is ligated directly to the femoral vein and so does not generate a stump in the junction. Furthermore, all branches that drain into the saphenofemoral junction (SFJ) and branches that also drain directly into the femoral vein are ligated and intersected. Subsequently, the hiatus saphenus is closed with nonresorbable material. De Maeseneer published an approach that creates a mechanical barrier of free endothelium at the site of crossectomy, using a special patch sutured onto the vein instead of a GSV ligation. The radical procedure is performed even for crossectomy of the saphenopopliteal junction. Despite the above-mentioned efforts, the recurrence of varicose veins still occurs. In the SFJ region, the cause seems to be neovascularization, in which vascular endothelial growth factor and its receptors play an important role. Our experience with conventional surgical procedures correspond to the above-described results. Therefore, to minimize the risk of recurrent varicose veins, the key is to perform a radical crossectomy.

Apart from conventional surgery, endovascular interventions have recently become popular, and most involve thermal ablation. Thermal ablation, such as endovenous laser therapy (EVLT) and radiofrequency ablation (RFA), aims to remove the saphenous veins, usually the GSV or the SSV. Based on the hemodynamic theory, this procedure eliminates the primary source of reflux from the saphenous vein, which leads to remodeling of the flow in epifascial varicosities and their subse-
quent regression. There are two approaches to the management of saphenous vein branches in endovascular surgery. In the first, epifascial varicose veins are treated simultaneously by ablation of the saphenous vein. In the second, varicose branches are treated in the second phase, assuming that the quantity and capacity will be reduced with time. However, according to the largest published meta-analysis, the results are not unambiguous, and for EVLT or RFA combined with mini-phlebectomy, there is a better outcome when the epifascial varicose branches are removed simultaneously with the saphenous vein. However, we have a different personal approach and perform surgery in two phases, with an endovascular ablation of the saphenous veins completed with a foam sclerotherapy of residual varicosities in the second phase. According to our results, on average, 11.11% of varicose branches completely disappear within half a year. These procedures are performed on an outpatient basis. They do not require hospitalization and are very comfortable for the patient. Similarly, we use this approach for perforator vein removal if the perforator vein is the primary source of reflux.

There are also saphenous vein–sparing procedures, such as CHIVA (Cure Hémodynamique de l’Insuffisance Veineuse en Ambulatoire) and ASVAL (Ablation Sélective des Varices sous Anesthésie Locale), which aim to remodel hemodynamics in the venous bloodstream and restore conditions for blood flow normalization. These procedures are generally considered to be very patient-friendly, with a significant benefit for the patient. However, we always expect a higher risk of residual varicosities with these procedures and a possible need for further treatment.

Generally, we consider intervention treatment for CVD, either conventional or endovascular, to be a causal treatment, despite the recurrence risk. This therapeutic approach has a long-lasting effect on the elimination of reflux.

References

Can varicose vein surgery prevent extension of venous reflux?
His is a difficult question to answer. On one hand, "yes," because when the vein is successfully ablated, reflux also disappears, and this can have a significant effect on reflux progression in other parts of the venous system. On the other hand, "no," because new reflux develops much more often than most of us would expect. However, this is just an opinion, an intentionally simplified view of such a challenging issue.

If we say "yes," that surgery prevents extension of reflux, then we have to believe that in the vast majority of patients, the reflux will never occur again. So, if we stop reflux extension with surgery, the patients should never have new varicose veins appear on their legs. This speculation seems logical, because reflux is considered to be the source of varicose veins. Indeed, there is a lot of evidence in the literature for such a view. Removing saphenous veins via any kind of invasive treatment is extremely effective. For thermal ablation techniques, the immediate consequence there will be no reflux, no varices. Indeed, there is a prospective study with a recurrence rate close to 100% if
there are no reflux, no varices. Indeed, there is a lot of evidence in the literature for such a view. Removing saphenous veins via any kind of invasive treatment is extremely effective. For thermal ablation techniques, the immediate consequence there will be no reflux, no varices. Indeed, there is a lot of evidence in the literature for such a view. Removing saphenous veins via any kind of invasive treatment is extremely effective. For thermal ablation techniques, the immediate consequence there will be no reflux, no varices. Indeed, there is a lot of evidence in the literature for such a view.

How should we treat other evidence from the literature? Rasmussen et al
points out recurrence rates of 47% at 5-year follow-up after laser ablation and 55% after stripping. What is even more impressive is that new reflux developed, respectively, in 18% and 10% of patients who underwent successful ablation of saphenous veins. This means that despite the majority of patients having no reflux after surgery, new varicose veins did develop in some cases. If such a high rate of technical or clinical failure (recurrence) was found in only one study, we could consider it to be an exception to the rule. However, we can easily find similar data elsewhere. For example, Proebstle et al report varicose vein recurrence after radiofrequency ablation in 41% of cases at 5-year follow-up. We can take into account the author’s remark that in the region of an ablated vein, the recurrence rate was only 6%, but this seems to be too simple an explanation for a highly complicated issue. So, one could say that surgery is not effective in preventing reflux extension.

Moreover, to answer this discussion question as thoroughly as possible, I looked into our postsurgery outcomes. As I was convinced that the recurrence rate in our institution would support the answer "yes," I was surprised to find records of residual veins, recurrent varicosities, or target vein recanalization in 36 of 97 (37%) cases at 1-year follow-up, and that we had performed an additional treatment in 29 (30%) cases.

In conclusion, I would prefer to avoid choosing between a clear yes or no. It may be that both answers are correct. First of all, I believe that we should include more than just reflux in the discussion. Results from many studies convince us that "no reflux" does not mean "no varices," and also that the presence of reflux does not necessarily mean the presence of varices. We must learn to determine the value of both new reflux and recurrent or residual veins, and, in particular, the associated risk of thrombosis or skin changes. The variations in assessment of such events lead to differences in the statistics and treatment approaches. Finally, despite all of the above, including the high recurrence rate, surgery provides a high level of patient satisfaction. In my opinion, though surgery may not be that effective in elimination of reflux, it does provide an adequate solution for clinical issues.

References
In the treatment of chronic venous insufficiency (CVI), high ligation (HL) and stripping of the saphenous varicose veins, and percutaneous phlebectomy have been the main options for many years. Modern management of CVI includes treatment of the cause (reflux) and result (varicose veins). Reflux should be treated before varicosities because if the cause is not eliminated, the varicose veins will recur. Generally, the elimination of reflux has been accomplished with surgery. The new thermal ablation techniques, such as endovenous laser ablation therapy (EVLT) and radiofrequency ablation (RFA), have the advantage of being performed with only local anesthesia, but they have the potential to leave residual saphenofemoral reflux due to incomplete ablation of all side branches of the saphenofemoral junction (SFJ). These techniques can obliterate only the main trunk of the saphenous vein, and complete disconnection of all of the side branches draining to the SFJ is never accomplished. Classical surgical HL has also been shown to lead to recurrent varicose vein development due to neovascularization, but this is not completely true; real neovascularization demonstrated by Doppler is rare and is mostly related to the SFJ and side branches not being divided properly. Thus, the primary cause of varicose vein recurrence after surgery is inadequate surgical technique, and neovascularization is never the only cause of recurrence. Recurrence also has an incidence of 7.1% after EVLT and 2.2% after RFA. The development of arteriovenous fistulas and the intensity of the inflammatory response might be responsible for recanalization of ablated venous segments. This effect is due entirely to inadequate SFJ or side-branch division in the initial procedure. In support of the importance of complete SFJ ligation, small vessel networks and GSV recanalization at the SFJ have more commonly been found in patients undergoing RFA without ligation (46%) than RFA with ligation (14%). In a systematic review comparing recurrence rates, overall complication rates, and symptom relief, it was found that surgery was not inferior to endovenous procedures. Our past experience and results of previous studies comparing the results of three different surgical techniques (complete stripping with HL, partial above-knee stripping with HL, and HL alone) have shown that the best recurrence rate, best improvement in CEAP class (Clinical-Etiology-Anatomy-Pathophysiology classification), and best event-free survival rates are achieved using complete stripping with HL. The rate of residual reflux and recurrence after partial stripping can reach up to 20%, and this complication is found more often with patent below-knee saphenous veins than with incompetent perforator veins (IPVs) undetected preoperatively. Given that these techniques are theoretically equivalent to stripping with low ligation of the proximal saphenous vein, it is not wise or completely true to claim that their recurrence rates and effectiveness are better than those of complete stripping with HL. Past experiments have shown that, for HL with complete division of the SFJ and all side branches, full-length obliteration of the saphenous vein for insufficiency was necessary to prevent recurrence.

An incompetent SFJ, an incompetent small saphenous vein, and incompetent superficial vessels in the thigh, if not treated by HL, female sex, and post-thrombotic deep vein incompetency were all associated with greater risk of recurrence after a technically correct surgery. Our study on 372 patients showed that preoperative CEAP class, bilateral limb disease, occupation, family history or genetic predisposition, prior deep vein thrombosis (post-thrombotic etiology of varices), older age, and preoperative IVs were predictors of early postoperative and later clinical status, outcome, and other events. The predictors of postoperative symptom recurrence and clinical and Doppler examination findings depend mostly on the preoperative characteristics of individual patients, and varicose vein surgery can prevent extension of venous reflux with a 5-year symptom-free survival rate of 51±0.8%.

References
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Management of varicose veins (VVs) depends upon its etiology, whether it’s primary, secondary, or part of a congenital vascular malformation. Treatment options for VV with superficial venous incompetence include high ligation with and without stripping of the refluxing venous segment, ambulatory phlebectomy, endovenous thermal ablation, and sclerotherapy. Venous duplex imaging to assess venous reflux in both superficial and deep vein systems has a Grade 1A recommendation in the Clinical Practice Guidelines. Reflux (diagnosed by duplex imaging) with a duration of >0.5 seconds in the superficial system and >1 second in the deep system occurs when the valves are absent or incompetent due to degenerative processes in primary venous disease or as a sequela subsequent to an episode of deep vein thrombosis. The correlation drawn from duplex imaging findings between the clinical picture and the severity of reflux is controversial. Whereas some studies deny such a correlation, others show one, suggesting that clinical signs of disease also correlate with great saphenous vein (GSV) diameter, with increasing diameter being associated with greater disease severity.

Venous reflux after VV surgery—manifesting as VV recurrence—may occur, eg, as in post-thrombotic syndrome causing secondary VV or progressive reflux. Recurrent VVs develop in up to 40% of patients on long-term follow-up, and a 65% recurrence rate after treatment of secondary VVs is observed, regardless of the modality of saphenous vein ablation. No effect on deep venous system reflux has been observed after VV surgery for secondary VVs caused by post-thrombotic syndrome. Laser thermal endovenous ablation procedures have lower saphenofemoral reflux rates than surgical ligation and stripping in short- and mid-term timeframes and have shown improvement in venous hemodynamics. One year after such ablation procedures, residual saphenofemoral junction (SFJ) tributaries have been reported to be non-refluxing and clinically insignificant. Radiofrequency ablation (RFA) treatment of VVs and duplex imaging estimation of the postoperative effect on reflux was studied, comparing the incidence of neo-vascularization at the SFJ after RFA and open high saphenous ligation and stripping of the GSV. In the open surgery group, limbs (11%) showed clear evidence of tortuous refluxing veins related to the SFJ; in the RFA group, no limbs showed neovascularization at the SFJ.

Another study evaluated phlebectomies alone as primary prevention in selected patients. This prospective, multicenter study, including 94 patients, aimed to analyze hemodynamic, clinical, and patient-reported outcomes after phlebectomies in order to determine predictors of treatment success, that is, restoration of GSV competence. One year after treatment, GSV reflux had disappeared in 50% of patients (P<0.01), and GSV diameter had decreased significantly (P<0.01).

Another study investigated the effects of either aggressive or less-aggressive treatment for superficial venous disease on deep venous insufficiency. Aggressive therapy included high ligation with partial selective perforation-invagination, axial stripping of the GSV, ambulatory stab phlebectomy of the VVs, and transdermal treatment of spider veins; less-aggressive treatment included all but the spider vein treatment. Results showed a marked decrease in the size of the deep veins in 80% of patients and a decrease in the reflux closure time of the deep vein valves in 83% of patients. Only 28% of patients receiving less-aggressive treatment without transdermal laser therapy of the spider veins showed improvement in reflux-valve closure time; the remaining 72% showed either no change or deterioration.

Surgical treatment of VVs and of calf perforator veins results in reduced deep vein reflux. The improvement is most marked in cases of primary venous insufficiency.

**References**

in patients having undergone a superficial or deep venous procedure, the hemodynamic and clinical status remains stable over time, as opposed to those having only been treated with elastic compression. However, studies have shown that after varicose vein surgery, up to 40% of patients eventually develop neovascularization, and one-third of patients develop an extension of preexisting reflux or reflux in a new segment. In a randomized controlled trial by Sell et al, the effectiveness of compression therapy and surgical treatment was evaluated for superficial venous reflux. A total of 153 patients were randomized to compression stocking therapy (77 patients) and surgery (76 patients). Surgical treatment included ligation of the great saphenous vein (GSV) and all side branches, with GSV stripping. Insufficient perforator veins were ligated and cut, and all patients underwent local phlebectomy. After 2 years of follow-up, the authors found that surgical treatment had significantly better results than compression therapy for elimination of superficial venous reflux.

Blomgren et al evaluated the effect of surgical treatment for primary varicose veins on the development of venous insufficiency 2 years after surgery. In 293 patients, surgical treatment consisted of GSV or small saphenous vein removal, extrafascial perforator vein ligation and local phlebectomy. After 2 years, new vessel formation was seen in 40% of the patients with surgically obliterated saphenopopliteal junction and 11% of patients with treated saphenofemoral junction. A substantial number of patients in this study developed new reflux during the follow-up, possibly as a result of progression of the venous disease. However, redirection of venous flow via a weakened venous segment becoming varicose after GSV removal cannot be ruled out. The authors of this study found that the reflux in GSV below the knee was abolished after the above-knee GSV stripping, suggesting that the practice of below-knee GSV stripping is unnecessary.

Endovenous laser treatment (EVLT) is described as a less-invasive treatment option that provides a better cosmetic outcome than the standard surgical procedure. Myers et al assessed the efficacy of EVLT for the treatment of saphenous reflux with varicose veins. Over a 5-year period, EVLT was performed in 361 patients. EVLT was used for the proximal part of the saphenous vein, and ultrasound-guided sclerotherapy was used for the distal saphenous vein and tributaries. Primary failure was defined as failure to occlude the venous lumen, with reflux in part or all of the treated saphenous veins, whereas secondary failure was defined as failure to occlude the lumen, and reflux after the primary failure. The primary success rate at 4 years was 76% and the secondary success rate was 97%.

As radiofrequency ablation (RFA) is one of the potential ways to treat varicose veins, Salles-Cunha et al evaluated 106 extremities treated with RFA and concomitant ligation and division of the SFJ tributaries, except in 13 patients that were treated with RFA alone. The authors found a significantly lower prevalence of small-vessel networks at the SFJ level and of GSV recanalization in patients with combined RFA and SFJ ligation than in patients with RFA alone.

To conclude, although a high reflux rate could be seen in some studies following surgery, the majority of papers published thus far favor surgical treatment (open surgery, endovenous laser ablation, RFA as an appropriate option for varicose vein treatment, with better results than that seen with compression therapy only. Stripping or endovenous obliteration of the above-knee GSV combined with high GSV ligation and local phlebectomy seems to be a better option than stocking therapy alone for preventing extension of venous reflux over time.

Acknowledgments. This case report was partly funded by the Serbian Ministry of Science and Technological Development – Project No. 41002.

References
Can varicose vein surgery prevent extension of venous reflux?

Varicose vein surgery, namely venous stripping (VS), has been a standard procedure in varicose vein treatment for 100 years, though today its efficacy is questioned by some. In one study, up to 40% of patients still had progression of venous incompetence afterwards. While true that after VS, many patients have progressive venous incompetence, the author of that study believes it is still an effective means to treat patients with symptomatic varicose veins. On the basis of our own postoperative interview, we found that patients who have this operation are happy with the procedure. There are several reasons for this.

Firstly, after the operation, patient clinical outcome improves markedly. In the landmark randomized controlled trial by Rasmussen and colleagues, two study arms designed to use VS and endovenous laser ablation (EVLA) were compared. Outcomes included open refluxing in the great saphenous vein (GSV), recurrent varicose veins, venous clinical severity score (VCSS), quality of life (QOL) scores (based on the Aberdeen Varicose Vein Symptom Severity Score [AVVSS] and Short Form-36 [SF-36]). After a 5-year follow-up, the incidence of open refluxing in the GSV in the VS group and the EVLA group was 10.1% and 17.9%, respectively (P=0.22). However, in the early postoperative period (1-3 months) and onwards, the VCSS and AVVSS improved significantly in both groups: the mean VCSS before the operation was roughly 2.4, and 1 month afterwards was 0.2; the mean AVVSS before the procedure was around 16, and 3 months after VS was reduced to 8. Also, the SF-36 significantly improved compared with the pre-operative data in several aspects—namely, bodily pain, mental health, social function, physical function, and vitality, as well as in the physical and mental component summaries. In short, the recanalization of the GSV appears to have no significant impact on the severity score or QOL. It is also important to note that, in all aspects, the results of VS and EVLA are not significantly different.

Secondly, although the recurrence rate in VS reaches as high as 54.6% in a 5-year period, based on the Rasmussen study, this can be treated easily by foam sclerotherapy or mini-phlebectomy.

Finally, nowadays, VS is used to strip the GSV from groin to knee, and quite often surgeons leave persistent incompetent GSV below the knee and residual perforator incompetence. In the study by Sugiyama and colleagues, the effect of VS on the venous filling index (VFI) in the calf vein segment below the knee was assessed both 1 month and 1 year after the procedure; the study found that 1 year after VS, VFI was a little higher in patients who had residual calf incompetent GSV. Patient symptoms were significantly improved after the operations. This study suggested that ablation in the GSV below the knee is not necessary during VS.

In summary, it does not matter whether VS can prevent extension of venous reflux with time. Rather, as VS can make remarkable clinical improvements in terms of severity or QOL, it is a worthwhile therapy for symptomatic varicose veins.

References
The treatment of venous disease dates back to the beginning of medicine. These early learnings are the tools that we use in the current treatment of superficial venous disease. Today, many surgical options are available, including great saphenous vein (GSV) stripping and ligation, varicose vein ligation, phlebectomy, endovenous vein obliteration, and perforator vein surgery. Nowadays, the techniques and technology have evolved to enable us to improve the short- and long-term outcomes of vein surgery, but the real question is whether varicose vein surgery in any of its modalities prevents extension of venous reflux with time.

First of all, although varicose vein surgery is very common, data on long-term relief of symptoms and the incidence of recurrence are meager. What we currently know is based on the little data retrieved from a few trials; for example, in one trial in the United Kingdom in 2003, a consecutive cohort of 100 patients was reviewed 10 years after varicose vein surgery in order to detect rates of recurrence, symptom relief, and patient satisfaction. This study showed long-term symptom relief in 77% of patients 10 years after varicose vein surgery; however, the recurrence rate was high, with most patients having a few varicosities. Some studies report a clinical recurrence in 50% of patients 3 to 5 years after surgery. Although inadequate surgery is a considerable cause of recurrence, there are many other possible explanations. For example, after surgery, the early physiologic improvement is satisfactory, but the normalization of venous function is difficult to achieve, especially in patients with neovascular reconnection and persistent abnormal venous function, such as residual deep reflux and persistent incompetent perforator vessels. After varicose vein surgery, the failure to normalize venous function is more likely to result in recurrence.

Can varicose vein surgery prevent extension of venous reflux? Currently, the treatment of choice for a venous reflux in the GSV is endovenous laser ablation (EVLA), but there are few data on the long-term outcomes of this intervention. Disselhoff et al and Rasmussen et al developed the only two randomized clinical trials comparing conventional surgery and EVLA with a follow-up of 5 years. They found similar recurrence rates (90%-100% vs 82%-93%). Recently, van der Velden and colleagues (1 July 2015) published a new randomized clinical trial with 5-year results that compared the long-term outcomes of conventional surgery, EVLA, and ultrasound-guided foam sclerotherapy (UGFS) in patients with GSV varices. They demonstrated that absence of reflux in the GSV after 5 years of follow-up was more frequent with conventional surgery or EVLA than with UGFS. These trials show that varicose vein surgery, which has satisfactory results for early outcomes, can also avoid venous reflux in the long term.

Finally, current data for foam sclerotherapy, EVLA, and open surgery involving high ligation and stripping suggest that they have similar overall early and late outcomes; however, these findings lack robustness due to incompatibility between studies, which impedes more accurate analysis to establish whether varicose vein surgery has long-term benefits.

**References**

Deve a recognized genetic basis, many environmental factors are involved in chronic venous disease (CVD) development and progression. The most significant risk factors have been identified as age, obesity, sedentary lifestyle, lack of elastic stocking use, multiparity, and prolonged occupational exposure to heat sources. Ambulatory venous hypertension underlies the pathophysiological and clinical CVD condition, thus all the available treatments must be focused on restoring this parameter.

Despite a small number of clinical trials on the topic, a recent Cochrane review has demonstrated that the saphenous-sparing approach significantly reduces the recurrence of varicose veins, compared with the ablative techniques.\(^1\) The satisfying recurrence rate associated with the saphenous-sparing strategies\(^1\) together with the mini-invasiveness of the modern endovenous techniques have stimulated pioneering investigations regarding GSV competence restoration by means of endovenous devices.\(^2,3\)

In a survey involving 1978 patients over an observation period of 6.6 years, the Bonn study demonstrated that class C2 disease evolved toward a more severe stage in 31.8% of saphenous refluxing cases and in 19.8% of nonsaphenous refluxing patterns.\(^4\) Indirectly, these data point out the possibility that varicose vein surgery can reduce the evolution of CVD by suppressing venous reflux. Of course, a distinction must be made between primary and secondary varicosity evolution, the latter being faster. Moreover, venous reflux has recently been weakly associated with clinical outcome impairment, highlighting the poorly understood relationship between these two parameters.\(^5\)

In order to obtain an effective resolution of venous reflux and potentially of disease evolution, the presence of a physiological draining network has been shown to be a good prognostic factor for long-term results.\(^6\) A major cause of failure in CVD evolution control is an incomplete/adequate treatment, associated with lack of patient compliance.

CVD is by definition a chronic pathology, with both genetic and environmental components that determine a hemodynamic and clinically extremely polymorphic condition. In order to significantly impact such a heterogeneous scenario, a single treatment option cannot be the panacea for all patients. On the contrary, a tailored diagnostic and therapeutic multimodal plan must be customized for every single case.

The combination of an accurate hemodynamic assessment, a proper therapeutic strategy and technique, and a lifestyle targeted on managing the specific risk factors are mandatory in order to restore healthy lower limb venous drainage, thus decreasing disease evolution over time. According to our personal experience, a mini-invasive saphenous-sparing surgery presents a therapeutic option that is not only esthetically satisfying, but also effective in the reduction of recurrence and in the prevention of venous reflux extension with time.

References
Varicose disease is a progressive condition; if left untreated, it will become more extensive, resulting in more symptoms and a higher C-classification. Progression includes anatomic extension in most cases and may also involve development of reflux in new segments or a combination of both. Both clinical progression and progression of superficial venous reflux correlate significantly with age. Surgical treatments, such as high ligation and stripping, phlebectomy, and ligation of incompetent perforating veins, will remove the incompetent veins. More recently, these surgical treatments have often been replaced by endovenous techniques that are equally efficient in the treatment of venous insufficiency, but cause fewer side effects. After treatment, ulcers heal, varicose veins disappear, and symptoms decrease. Treatment should result in a lower C-score and fewer symptoms. Even incompetent deep veins can become competent after superficial treatment.

Studies have shown, however, that the recurrence rate after varicose vein surgery is high and can reach 40% after 5 years and 70% after 10 years. About 20% of surgical procedures are carried out to treat recurrent disease. There are various causes of recurrence. Poor surgery was historically seen as a major cause. Other causes are a poor preoperative patient assessment, incorrect duplex mapping, and anatomical variability. After endovenous treatments, recanalization of the treated vein may occur. Changes in hemodynamics after treatment can cause new forms of incompetence, such as insufficiency of the anterior accessory vein after great saphenous vein ablation. Soon after surgery, there is often an increased number of incompetent perforator vessels.

Varicose veins—primary or recurrent—can, however, develop throughout the entire lower limb. Reflux can develop in any vein with or without an apparent feeding source, and this as a part of disease progression. More recently, neovascularization—especially at the saphenofemoral or saphenopopliteal junction—has been confirmed to be a major cause of recurrent reflux. Initially, it was thought that neovascularization is caused by surgery, as a part of the normal process of wound healing. Neovascularization can also occur after endovenous ablation. The patterns of recurrence following thermal ablation of saphenous veins are different to those seen after surgery. Specifically, new reflux in other saphenous veins is responsible for most recurrent varicose veins and neovascularity seems to be less common after endovenous laser ablation.

Still, there seems to be no overall difference in recurrence rates between surgical treatment and endovenous thermal ablation. Although recurrence of varicose veins is frequent 10 years after the operation, surgery provides long-term relief of symptoms in a majority of patients. The effect of superficial venous surgery is dramatic. Improvement in symptoms, early patient satisfaction, and conditions favoring ulcer healing are consistent results.

Despite a fair number of ultrasound-detected recurrences, the overall long-term result from the patients’ point of view remains surprisingly favorable. The correlation between ultrasound-detected recurrence and residual symptoms and cosmetic results remains low. To enhance the durability of treatment, prevention—especially paying more attention to risk factors—could influence long-term results. However, regular clinical and ultrasound checks are imperative as part of a surveillance protocol. Early recurrences can be treated in a minimally invasive way with sclerotherapy or ultrasound-guided foam sclerotherapy.

We conclude that varicose vein surgery can prevent extension of venous reflux over time if there is regular follow-up after an efficient treatment and additional procedures are carried out where necessary.

References

Can varicose vein surgery prevent extension of venous reflux?
this is a provocative question, with more than one component to consider. Rather than jump immediately to whether or not varicose vein surgery can prevent extension of venous reflux, I’d first like to address a somewhat related issue, looking at compression therapy as a conservative treatment versus surgical intervention, as some might ask whether surgery is even necessary. Clearly, compression therapy alone will never offer patients the same comfort and assurance that surgical intervention can provide. From the viewpoint of evidence-based medicine, although there is no evidence of faster healing of ulcers after surgery, there is a significantly lower re-ulceration rate. Moreover, there is abundant evidence that quality of life is better after surgery—endothelial, traditional, as well as sclerotherapy—than after conservative treatment alone. I am in agreement with the latest American Venous Forum (AVF) recommendation that when there is a surgically treatable source of reflux, the surgical treatment must be preferred over conservative treatment.

After that, pharmacological treatment is also necessary for successful management of chronic venous insufficiency. The reason for this is that there is no surgical intervention that can address the leukocyte-endothelial–interaction component of the disease. Even after the best surgery, adhesion molecules will still be present and at this point, nothing besides pharmacological treatment can decelerate the inflammatory reaction occurring at the vein wall.

Next, in a brief nod to the neovascularization component of the question—indeed, the discussion about neovascularization seems never-ending and is controversial itself—I personally accept its existence if there is ultrasound evidence for it; however, many so-called neovascularizations are actually due to inadequate surgery.

Now, let’s consider extension of reflux to new segments. In a subgroup of patients with isolated reflux in great or small saphenous veins and without reflux in the deep veins, there is a legitimate anticipation that after correct surgery on the reflux source, the risk of neovascularization or propagation of reflux to the intact part of the venous system will be lower. Of course, in everyday surgery, we have no significant information about the levels of adhesion molecules present (eg, intercellular adhesion molecule [ICAM], vascular cell adhesion molecule [VCAM]), as well as other influential factors, known and unknown.

In a subgroup of patients with simultaneous reflux to the superficial and deep veins, the conditions are more complicated; to understand the benefit of a procedure, it is necessary to understand the proximal point of insufficiency, the reflux path, and the distal point of insufficiency. These are the elementary questions that need to be answered to understand the prognosis of the disease. From my point of view, this is not something that will be settled within a short discussion!

Last, but not least, isolated reflux in the saphenofemoral junction is more complicated than it looks at first sight. In most cases, we think about the range of terminal or preterminal maximal ostial valve sufficiency, but what about suprasaphenous valve and infrasaphenous valve incompetence in the common femoral vein? These are the controlling ports for the reflux to the stump after surgery and also the crucial entries to the superficial and profundal femoral vein.

In conclusion, the answer is not as simple as it might seem at first glance! In my opinion, as long as we do not have satisfying answers for these basic questions, and as surgical intervention will not address detrimental leukocyte-endothelial interactions, there is a very strong incentive to do what we can to protect the venous system after operation, for example, with effective medications, such as micronised purified flavonoid fraction (MPFF).
Increased venous pressure underlies all the clinical manifestations of chronic venous disorders. Venous hypertension is the result of incompetent venous valves in the superficial veins for most patients. A strong link between venous hypertension, valve failure, and venous inflammation has been evoked in pharmacological studies and confirmed in a variety of animal models. A cascade of inflammatory reactions, resulting in adverse changes in venous valves and the venous wall, eventually produces venous hypertension. Symptoms, telangiectasias, varicose veins, and ultimately venous leg ulcers appear to be a consequence of the changes induced by venous hypertension. Treatment to inhibit inflammation and hamper the development of venous hypertension may offer the greatest opportunity to prevent progression of chronic venous disease and related complications. Inflammation-dependent valve failure is considered a target for drugs. D aflon, a venoactive drug containing purified micronized diosmin, hesperidin, linarin, isorhoifolin, and diosmetin at optimized dosages, is the only drug with evidence for the preservation of valve structures in animal models and for the suppression of commissural transitory reflux that occurs in symptomatic patients after prolonged standing. D aflon has also been shown to protect the microcirculation in animal models. This protection translates into clinical benefits, such as reduction in edema, hematoma resorption after surgery, acceleration of ulcer healing, and improvement in lymphatic drainage. Although the role of D aflon in the attenuation of the various elements of venous inflammation is now better known, it is still worthy of a more in-depth exploration in future.

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D aflon and the protection of venous valves

by L. Pascarella, USA

Most veins of the superficial and deep system in the lower extremities are equipped with a series of one-way bicuspid valves that open to allow flow toward the heart and close to prevent reverse blood flow toward the feet. Venous valves, which were first described by Dutch physician Jacques Dubois and whose true function was later described by William Harvey,1 ensure that blood flows in the correct direction, particularly when the body is upright, traveling against gravity and other pressures.2 Venous pathology for most patients develops when venous pressure increases and blood return is impaired by incompetent valves in the deep or superficial axial veins, perforator veins, or venous tributaries. Chronic venous disorders may also result from venous obstruction or a combination of both valve incompetence and obstruction. These mechanisms lead to global or regional venous hypertension, particularly with standing or walking.3 The subsequent macro-
circulatory hemodynamic disturbances contribute to the large variety of clinical presentations seen in chronic venous disorders. In turn, prolonged periods of venous hypertension in the legs alter the microcirculation, resulting in dermal changes with hyperpigmentation, lipodermatosclerosis, and eventual ulceration.

The presentation of chronic venous disorders includes symptoms and signs. A recent large-scale epidemiological study has shown that the most commonly expressed chronic venous disorder–related symptoms include (in order of frequency): heaviness; leg pain; swelling sensation; nighttime cramps; sensation of “pins and needles” in the legs; and sensation of burning and itching.4 The CEAP (Clinical-Etiological-Anatomical-Pathophysiological) classification system is widely used in venous medicine to describe the signs of chronic venous disorders, including telangiectasia, varicose veins, edema, skin changes, and healed or active venous leg ulcers.5

Venous hypertension and valve failure

Venous hypertension is, in most cases, caused by reflux through incompetent venous valves (Figure 1).3,6 Close inspection of surgical specimens removed from limbs with chronic venous insufficiency and, more recently, direct observation with angiography have revealed that lesions involve the venous wall, valvular annulus, and valve cusps.7,8 By maintaining venous hypertension or further increasing it, valve failure and failure of the valvular annulus are responsible for disease progression. Monocyte/macrophage infiltration into the valve leaflets and venous wall of C2 patients with varicose veins has been demonstrated in immunohistochemical studies using a monoclonal antibody specific for monocytes and macrophages.9 Leukocyte infiltration appears to be greater at both the base of the valve leaflets and in the proximal venous wall, according to monoclonal antibody studies.

Regions of low shear stress with venous eddies and recirculation contain substantial numbers of venous valves (Figure 2),10,11 and these phenomena may explain why leukocytes are preferentially deposited in these regions. In the long run, macrophage-induced tissue damage softens the venous wall rendering the valve liable to damage and/or destruction.12 Venous valve failure, and the subsequent reflux that results in distal venous hypertension, is likely to contribute to chronic hypertension, which activates leukocytes in the endothelium of veins and promotes leukocyte-mediated destruction of skin and subcutaneous tissues in the lower limb.

In addition to leukocyte activation, mast cells infiltrate into the venous wall and this infiltration may play a role in the development of varicosity. Increased expression of intercellular adhesion molecule 1 (ICAM-1) and CD68 on the endothelial surface of venous walls in patients with venous insufficiency has been demonstrated, and this increased expression may be related to the development of varicose veins.12

**Figure 1.** Visualization of competent and incompetent venous valves. Competent (panel A) and incompetent (panel B) venous valves as schematic (left) and B-flow ultrasound images (right). In panel B, the valve sinus is distorted. The cusp above the dilatation is frozen and the adjacent cusp is prolapsed. The high-velocity retrograde streaming deviates laterally above a prolapsing cusp. From reference 6: Lane et al. Phlebolymphology, 2007;14:105-115. Image courtesy of the author.

**Figure 2.** Selected mechanisms that may control inflammation of the vein wall and valve leaflet. A normal vein valve and wall are shown in panel A. Valve leaflets may be subject to inflammatory damage by alteration in the magnitude and direction of fluid shear stress on the endothelium (panel B). Venous valve leaflets may become unable to close due to vein wall distension caused by elevated venous pressure (panel C) or by weakening of the vein wall due to proteolytic degradation of its extracellular matrix (panel D). Abbreviation: MMP, matrix metalloproteinases. From reference 11: Schmidt-Schönbein. Medicographia. 2008;30:121-126. Image courtesy of the author.
This finding suggests a continuous inflammatory reaction related to venous wall remodeling.13,14

In order for leukocytes to migrate through the endothelial cell layer into tissue, endothelial cells must be activated.12 Endothelial stretching of the vein, due to changes in blood flow and fluid shear stress, may induce activation of the endothelium. Fluid shear stress is a key regulator for endothelial cells, and a decrease in shear stress makes the adhesion of leukocytes to the endothelium easier.9

Clinical observations confirmed in animal models
Since the mechanisms responsible for venous valve failure in primary chronic venous disorders cannot be investigated in vivo in human beings, animal models have been developed for experimental research. Lalka et al described a simple, reproducible model of hind-limb valve disruption in the greyhound.15 After acute valve degeneration, animals developed an immediate increase in poststimulation segmental venous pressure that lasted up to 14 weeks. Despite establishing that reflux occurred in segments with disrupted valves, reflux did not extend into the tributaries and no evidence of varicose vein development was found. It was hypothesized that in these quadrupeds the hydrostatic column of the hind limb was relatively short, which could explain these findings.16

To elucidate possible mechanisms for valve remodeling in chronic venous disorders, an arteriovenous fistula (AVF) model has also been tested. Unfortunately, an arterialized pressure profile was observed in the distal veins, making this model unsuitable for studying this chronic disease. The combination of outflow obstruction and AVF to produce a model of sustained venous hypertension was developed by van Bemmelen17 and applied to the study of reflux development by Bergan’s team.18 In a series by Takase et al, rat saphenous vein valves were examined after prolonged exposure to venous hypertension; femoral venous hypertension was elevated for a period of 3 weeks using the van Bemmelen model. In this model, venous reflux developed in response to venous hypertension around 100 mm Hg.

Examination of vein morphology revealed that valve failure occurred as a result of venous wall dilation and valve leaflet shortening. As the leaflets shortened, complete valve closure became more and more difficult until this was no longer possible and reflux subsequently ensued. Evaluation of the valves for molecular markers of inflammation revealed that leukocyte infiltration with granulocytes, monocytes, and T lymphocytes had been enhanced. In addition, endothelial cells of the saphenous vein wall expressed more P-selectin and ICAM-1, endothelial cell membrane adhesion molecules.19 The leaflets were still able to close properly in the early stages of this trial after AVF placement, which indicates that pressure per se may not be responsible for compromising the leaflets. It was later observed, however, that a reduction in leaflet dimensions had taken place by the time the leaflets failed and reflux occurred.

It has been suggested that with dilation of the venous wall, there comes a moment when reflux develops across the leaflets. Long-term exposure to irregular fluid shear stress at the leaflet surface during venous reflux would be inflammation-inducing for the endothelial cells of the valve leaflets. Eventually, leaflets would be irreparably harmed and then destroyed, leading to a deleterious spiral of venous hypertension and venous inflammation.

A new low-flow/high-pressure animal model in veins is being developed by Bouskela’s team to avoid the pitfalls of previous models. The objectives of such a model are to: achieve long periods of observation; study alterations in venous pressure over time; assess changes in microcirculatory parameters; and determine the inflammatory profile of the model. It will allow for the assessment of venous pressure and how it evolves with time and for the exploration of microcirculatory parameters, with a Cytoscan® device and intravital microscopy.

Inflammation-dependent valve failure as a new drug target: the example of Daflon
Intervention in the inflammatory reaction that occurs as part of the progress of chronic venous disorders may be a new pharmacological target and, for this reason, the models of Bergan and Bouskela have been used to assess the effect of Daflon,20 a vеноactive drug (VAD) consisting of micronized purified flavonoid fraction.

Chemical family of Daflon
Daflon is produced from a plant extract from the epicarp of Citrus aurantium var amara. It belongs to the chemical family of flavonoids that are included in the six main categories of vеноactive drugs (Table I, page 204). Daflon contains purified micronized diosmin, hesperidin, linarin, isorhoifolin, and diosmetin at optimized dosages.20 Each of the active ingredients in Daflon contributes to its action and explains its superior beneficial effect in reducing capillary permeability versus other VADs.20

Daflon’s mode of action
The pharmacodynamic effects of Daflon and their clinical consequences are summarized in Table II (page 205).21-24

SELECTED ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>AVF</td>
<td>arteriovenous fistula</td>
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<tr>
<td>CEAP</td>
<td>Clinical-Etiological-Anatomical-Pathophysiological</td>
</tr>
<tr>
<td>CIVIQ-20</td>
<td>Chronic Venous Insufficiency quality of life Questionnaire</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>intercellular adhesion molecule 1</td>
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<tr>
<td>VAD</td>
<td>vеноactive drug</td>
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Daflon is the only VAD that has evidence for preserving valve structure in animal models and for suppressing commissural transitory reflux that occurs in symptomatic patients after prolonged standing. In two trials of pharmacological post-operative recovery in patients with varicose veins who underwent phlebectomy, Daflon helped attenuate postoperative pain and improve quality of life.

Protection of the microcirculation
Experimental in vivo models have been used to study the effect of drugs on the microcirculation. Microcirculatory preparations include: hamster cheek pouch; hamster or mouse skinfold; rat or hamster mesentery; rat, hamster, or mouse cremaster; etc. VADs enhance capillary resistance and decrease capillary filtration, both of which help check capillary leakage. Daflon improves microvascular reactivity and increases functional capillary density after ischemia-reperfusion injury; following this type of injury, it also induces a significant dose-related reduction in macromolecular permeability. These protective microcirculatory properties of Daflon result in clinical benefits. In a recent meta-analysis, ten studies published between 1975 and 2009 including 1010 patients were analyzed to determine whether Daflon, hydroxyethylrutoside, *Ruscus* extracts, and diosmin reduced edema. Mean reduction in ankle circumference was $-0.80 \pm 0.53$ cm with Daflon, $-0.58 \pm 0.47$ cm with *Ruscus* extract, $-0.58 \pm 0.31$ cm with hydroxyethylrutoside, $-0.20 \pm 0.5$ cm with single diosmin, and $-0.11 \pm 0.42$ cm with placebo. Daflon reduced ankle edema more than other VADs ($P<0.0001$). When used after varicose vein stripping, Daflon helped decrease postoperative hematomas and accelerate their resorption.

Complications of chronic venous disorders are related to chronic venous hypertension and are visualized in the skin, the final target of chronic venous hypertension. This hypertension is a cause of chronic inflammation, which manifests as the result of persistent and sustained injury. Ultimately, in limbs with chronic venous insufficiency the most severely impaired circulation is the dermal capillary circulation. In a meta-analysis of 5 randomized controlled trials containing 723 C6 patients, micronized purified flavonoid fraction was efficacious for healing venous ulcers when used as an adjunct treatment to compression therapy and appropriate local therapy, particularly for large (>5 cm$^2$ in area) and/or persistent (>6-month duration) ulcers.

Improvement in lymphatic drainage
The drainage function of lymphatic vessels is very important; lymphatic vessels transport 4 liters of efferent lymph into the

### Table I. The principal categories of venoactive drugs.

<table>
<thead>
<tr>
<th>Chemical group</th>
<th>Plant of extraction</th>
<th>Latin name (common name)</th>
<th>Major active ingredient (part of plant)</th>
<th>Drug trade name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids (flavons and flavonols)</td>
<td>Citrus species <em>Citrus aurantium</em> L. ssp <em>amara</em> (bitter orange)</td>
<td>Diosmin, (pericarp)</td>
<td>Daflon</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Ginkgo biloba</em> L. (ginkgo)</td>
<td>Quercetol, (rutoside (leaf)</td>
<td>Ginkor Fort</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vitis vinifera</em> L. (common grape vine)</td>
<td>Quercetol, isorutinoside (leaf)</td>
<td>Venoruton</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Sophora japonica</em> L. (Japanese pagoda tree)</td>
<td>Rutoside, troxerutin (bud)</td>
<td>Ginkor Fort, Venoruton</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Viburnum prunifolium</em> L. (blackhaw)</td>
<td>Amentoflavon (stem bark)</td>
<td>Jouvence</td>
<td></td>
</tr>
<tr>
<td>Flavonoids (flavanons)</td>
<td><em>Vaccinium myrtillus</em> L. (blueberry)</td>
<td>Hesperidin</td>
<td>Daflon</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Ribes nigrum</em> L. (blackcurrent tree)</td>
<td>Methylchalcon</td>
<td>Cyclo-3; Bi-Cirkan</td>
<td></td>
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<tr>
<td>Anthocyanins</td>
<td><em>Hamamelis virginiana</em> L. (American witch-hazel)</td>
<td>Anthocyanins (leaf, fruit)</td>
<td>Pycnogenol</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Anthocyanins (leaf, fruit)</td>
<td></td>
<td></td>
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<tr>
<td>Tannins</td>
<td><em>Pinus maritimus</em> (maritime pine)</td>
<td>Gallic acid, ellagique</td>
<td>Jouvence</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vitis vinifera</em> L. (common grape vine)</td>
<td>(stem bark, leaf)</td>
<td>Hamamelis Boiron</td>
<td></td>
</tr>
<tr>
<td>Procyanidolic oligomers (PCO), precursors of tannins</td>
<td><em>Aesculus hippocastanum</em> L. (horse chestnut)</td>
<td>Escin (stem bark, seed)</td>
<td>Endotelon</td>
<td></td>
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<tr>
<td></td>
<td><em>Vitis vinifera</em> L. (common grape vine)</td>
<td>PCO (branch)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PCO (grape seed)</td>
<td></td>
<td></td>
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<tr>
<td>Saponosides</td>
<td><em>Centella asiatica</em> L. (hydrocotyle)</td>
<td>Asiaticoside, centelloside, madecassoside (bud)</td>
<td>Madecassol</td>
<td></td>
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<tr>
<td></td>
<td><em>Ruscus aculeatus</em> L. (holly)</td>
<td>Ruscin (roots)</td>
<td>Cyclo-3</td>
<td></td>
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<tr>
<td>Coumarins</td>
<td><em>Melilotus officinalis</em> L. (yellow sweet clover)</td>
<td>Melilotoside (bud)</td>
<td>SB-LOT</td>
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</table>
Reduces the number of activated leukocytes in venous valves in an arteriovenous fistula (AVF) animal model
Maintains the valve diameter in an AVF model
Reduces reflux rate in an AVF model
Prolongs the vasoconstrictor effect of noradrenaline (norepinephrine) on the vessel wall, reduces the gap between valve leaflets, and reduces blood venous stasis in vitro
Increases mechanical tension on bovine metacarpal vein rings in vitro

- Eliminates the evening commissural reflux in C0 patients, decreases the vein diameter, resulting in beneficial symptom relief and quality of life improvement
- Compared with control, improves postoperative pain and quality of life of C2 patients having undergone stripping surgery

Reduces hematomas by 30% compared with controls in C2 patients after stripping

Inhibits synthesis of prostaglandin E2 or F2

Reduces visual analog scale scores of pain, heaviness, sensation of swelling, cramps, and paresthesia in C2 to C6 patients

30% reduction in the pain associated with venous ulcers

Significantly decreases upper limb circumference by 32% and shortens time to healing by 5 weeks in C6 patients

Inhibits oxygenated free radical production in zymosan-stimulated human neutrophils or mouse macrophages in vitro

As adjunctive treatment to compression therapy, accelerates ulcer healing by 32% and shortens time to healing by 5 weeks in C6 patients

Significantly reduces upper limb circumference by 32% and shortens time to healing by 5 weeks in C6 patients

As adjunctive treatment to compression therapy, accelerates ulcer healing by 32% and shortens time to healing by 5 weeks in C6 patients

<table>
<thead>
<tr>
<th>Pharmacodynamic effects</th>
<th>Clinical consequences</th>
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<tr>
<td>Micronized purified flavonoid fraction (MPFF) suppresses damage to and preserves valve structures</td>
<td>Eliminates the evening commissural reflux in C0 patients, decreases the vein diameter, resulting in beneficial symptom relief and quality of life improvement</td>
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<tr>
<td>Shows a decrease in ankle edema that is at least 25% more than ruscus extract, diosmin, or hydroxyethylrutoside in C3 patients</td>
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<td>Reduces hematomas by 30% compared with controls in C2 patients after stripping</td>
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<tr>
<td>As adjunctive treatment to compression therapy, accelerates ulcer healing by 32% and shortens time to healing by 5 weeks in C6 patients</td>
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<tr>
<td>Reduces diameter of capillary bulk and diameter of dermal papilla in premenopausal women compared with placebo, indicating a protective effect of MPFF against the morphological changes that occur in the capillaries and an ability of MPFF to prevent capillary leaks and edema</td>
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<tr>
<td>Maintains the number of functional capillaries in premenopausal women</td>
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<tr>
<td>Improves microvascular reactivity and functional capillary density after ischemia-reperfusion injury in the hamster cheek pouch</td>
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<tr>
<td>Prevents capillary leakage in a significantly higher proportion of capillaries than a single diosmin in hamster cheek pouch</td>
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<tr>
<td>Decreases permeability more than any of its single constituents, showing that the flavonoids present in its formulation have a synergistic action in the hamster cheek pouch</td>
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<tr>
<td>Inhibits the increase in microvascular permeability that is induced by bradykinin or ischemia in rat cremaster muscle, and induced by histamine, bradykinin, leukotriene B4, ischemia-reperfusion injury, or oxidant challenge in the hamster cheek pouch</td>
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<td>Decreases expression of CD11B, a neutrophil receptor, and CD62L, a monocyte and neutrophil ligand, in C2 to C6 patients</td>
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<tr>
<td>Inhibits intercellular adhesion molecule 1 (ICAM-1) expression in skeletal muscle ischemia-reperfusion injury in rats</td>
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<td>Inhibits leukocyte adhesion and/or migration after ischemia-reperfusion injury in hamster skin fold or rat skeletal muscle, oxidant challenge in hamster cheek pouch, and venular mesenteric occlusion and reperfusion in rats</td>
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<td>Inhibits oxygenated free radical production in zymosan-stimulated human neutrophils or mouse macrophages in vitro</td>
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<td>Inhibits synthesis of prostaglandin E2 or F2α and thromboxane B2 in inflammatory granuloma in rats</td>
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<td>Reduces visual analog scale scores of pain, heaviness, sensation of swelling, cramps, and paresthesia in C2 to C6 patients</td>
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<td>Halves postoperative pain and significantly reduces analgesic consumption in C2 patients</td>
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Table II. Overview of the pharmacodynamic effects and clinical properties of Daflon. Based on references 21-24.

Bloodstream daily. Fluid turnover every 24 hours (including the volume of fluid reabsorbed in the lymph nodes) is up to two thirds of the total volume of interstitial fluid. The skin of the lower extremities contains a more dense and extensive network of lymphatic capillaries than the skin of the upper extremities. Natural human posture, which is upright, means that the lower extremities have higher filtration pressure and fluid influx. Greater lymph transport in the lower extremities counterbalances the higher volume of interstitial fluid produced by orthostatism and gravity.

Lymphatic vessels spontaneously contract twice to four times a minute to transport lymph. In human legs, the contraction of prenodal lymphatic vessels propels the flow of lymph.
ternal extensions of lymphatic endothelial cells act as valves to prevent the return of lymph. Varicose veins are associated with lymphatic dysfunction and structural damage to the lymphatic network. When the transportation of lymph is disrupted, the lymph stasis that follows can promote inflammation. Lipid accumulation in the media of diseased veins may further damage adventitial lymphatic vessels.

Protein and extracellular fluid accumulation in lymphedema may be reduced by treatment with Daflon, which can also stimulate lymph contractility and flow and reduce excess protein in tissues with high protein edema. In a study investigating Daflon (n=46) or placebo (n=48) in the treatment of lymphedema over 6 months, patients in the Daflon group experienced a 7% reduction in lymph volume versus a 10% increase for patients in the placebo group. Discomfort was reduced in both groups, but the Daflon group also reported a significant reduction in the sensation of heaviness. In addition, Daflon was found to be efficacious in reducing edema in Bancroftian filarial lymphedema.

**Potent anti-inflammatory effect**

Disturbed venous flow patterns and chronic venous inflammation are two interlinked phenomena. It is thought that mediators resulting from disturbed blood flow, and subsequent inflammation, have an important role in the occurrence of venous pain. Locally released proinflammatory mediators, resulting from hemodynamic changes and hypoxia, can activate nociceptors located in close proximity to the microcirculation, including those in the venous wall, space between endothelial and smooth muscle cells of the media, and perivenous space.

The primary site of activation of venous and/or perivenous nociceptors may not be in large venous vessels, which is suggested by the fact that the occurrence of pain does not correlate closely with objective parameters of varicose vein remodeling, incompetent venous valves, and inflammation. The efficacy of Daflon in the treatment of patients with symptoms of chronic venous disease has been widely evaluated in comparative and noncomparative clinical trials.

There is substantial evidence from meta-analyses and the RELIEF study (Reflux as sEssment and quaLity of life improvEment with micronized Flavonoids), a large observational study, that Daflon effectively relieves venous symptoms and lower limb edema. In the latest recommendations for the management of active venous ulcers, the evidence for using Daflon as adjuvant therapy was assigned the grade 1B. It should not be forgotten that Daflon is also capable of reducing associated pain.

**Protection against inflammation-related valve damage in chronic venous disease with Daflon**

Animal models have revealed Daflon’s ability to attenuate or block chronic inflammatory effects in the circulation, whether at the micro- or macrocirculatory level. In a venous occlusion/reperfusion model, in which increased venous blood pressure augmented the inflammatory cascade and tissue injury, markers of inflammation decreased in a dose-dependent manner in Daflon-treated animals. Parenchymal cell death and leukocyte rolling, adhesion to postcapillary venules, and migration were also significantly reduced by Daflon. Important data supporting the macrocirculatory protective effect of Daflon have been provided by Takase et al. Reflux rate was significantly reduced in a dose-dependent manner in an-
Clinical trials
Treatment with Daflon 1000 mg/day over 2 months resulted in the elimination of transitory commissural reflux (Figure 4) observed in patients presenting with subjective leg symptoms without visible signs of chronic venous disorders, so-called CO2 subjects.26 Transitory elimination of reflux occurred in parallel with pain relief and improvement in quality of life. In this trial, consecutive CO2 subjects were enrolled and assessed for the following: (i) symptom intensity using a visual analog scale; (ii) quality of life using the Chronic Venous Insufficiency quality of life Questionnaire (CIVIQ-20); and (iii) saphenous reflux duration and saphenous vein diameter, with a Duplex scan examination performed twice a day (morning and evening). A total of 41 CO2 subjects were enrolled in the study and, of these patients, 15 had no reflux in either the morning or evening and 26 had transitory evening reflux with 22 being commissural and 4 intervalvular. Saphenous vein diameter was greater in the subgroup of patients with transitory reflux compared with patients without reflux (P<0.05).

After Daflon treatment, there was a trend towards a reduction in intervalvular reflux length, while transitory commissural refluxes (n=22) no longer appeared. Additionally, vein diameters returned to normal values. These results mirror the protective effect of Daflon on venous valve structure.

Venous valve protection and targeted pharmacological intervention
A practical purpose of elucidating the molecular steps involved in the development of valve lesions is to identify ways of intervening with a targeted treatment. Studies have focused on available molecules known to modify the sequence of events involving leukocyte adhesion, interaction with endothelium, activation, and migration, and the subsequent valvular damage in large veins, mainly the great saphenous vein, with which these processes are associated. However, studies on the pathophysiology of chronic venous disorders have not yet shown that this sequence of events extends down to venules, where valves and microvalves play an important role in venous hemodynamics, and that this sequence is not just limited to large veins, including the saphenous veins.

We know from recent findings that the majority of microvalves in lower limbs are present in channels with a luminal diameter <100 µm.33 The role that microvalves play is still unclear, and their location and arrangement in normal lower limbs suggest that they prevent blood flow back into the capillary bed (Figure 5). Although there appears to be no difference between lower limbs with venous ulcers and normal limbs with respect to the number and density of microvalves,30 microvalves in diseased limbs are stretched and incompetent, allowing retrograde flow from large veins into the dermal capillary bed. Vincent and coworkers have proposed two hypotheses linking the failure of microvalves with skin changes in venous insufficiency: (i) degenerative changes in very small veins in leg skin may be related to the appearance of telangiectasias, reticular veins, and corona phlebectatica; and (ii) valve incompetence in both larger proximal vessels and small superficial veins, at the level of microvalves, would account for the appearance of severe skin changes in the event of venous insufficiency.21

This review shows that Daflon currently possesses the most appropriate profile to protect venous valves, and perhaps microvalves, even if its role in vivo still remains to be explored in more depth.

Daflon is also registered as Ardium®, Alvenor®, Arvenum® 500, Capiven®, Detralex®, Elatec®, Flebotropin®, Vantor® and Venitol®.

References
venous hypertension; inflammation; microcirculation; lymphatic system; valve; microvalve
DAFLON ET LA PROTECTION DES VALVULES VEINEUSES

L’augmentation de la pression veineuse est à l’origine de toutes les manifestations cliniques de la maladie veineuse chronique. Chez la plupart des patients, l’hypertension veineuse résulte de l’incontinence des valvules des veines superficielles. Des études pharmacologiques ont suggéré que l’hypertension veineuse, l’insuffisance valvulaire et l’inflammation veineuse sont étroitement liées, et cela a été confirmé dans de nombreux modèles animaux. L’hypertension veineuse est le résultat d’une cascade de réactions inflammatoires conduisant à des changements délétères dans les valvules et la paroi veineuses. Les symptômes, les télangiectasies, les varices et, par la suite, les ulcères veineux de la jambe semblent être la conséquence de changements induits par l’hypertension veineuse. Un traitement capable d’inhiber l’inflammation et d’entraver le développement de l’hypertension veineuse offrirait donc le maximum de chances de prévenir la progression de la maladie veineuse chronique et des complications qui y sont liées. L’insuffisance valvulaire liée à l’inflammation est une cible thérapeutique. Daflon, un médicament veinoactif contenant de la diosmine purifiée micronisée, de l’hespéridine, de la linarine, de l’isorhoifoline et de la diosmétilne à doses optimisées, est le seul produit ayant démontré sa capacité à préserver les structures valvulaires dans des modèles animaux, et à supprimer le reflux transitoire comissural observé chez les patients symptomatiques après une station debout prolongée. Dans certains modèles animaux, Daflon protège aussi la microcirculation. Cette protection se traduit par des bénéfices cliniques, comme la diminution de l’œdème, la résorption des hématomes après chirurgie, l’accélération de la cicatrisation des ulcères et un meilleur drainage lymphatique. Le rôle de Daflon dans l’atténuation de l’inflammation veineuse est désormais mieux connu, mais il mériterait d’être étudié plus en détail à l’avenir.
Bringing focus to the discussion about an association of venous reflux and chronic venous disease, Professor Dr med Eberhard Rabe, Emeritus President of the Union Internationale de Phlébologie, from the Department of Dermatology at the University of Bonn, Germany shares his comments on what three important studies in this area bring to the table. Looking particularly at the Bochum studies, the Bonn Vein study, and the Kostas trial, here he discusses the finding that in many cases reflux precedes the development of varicose veins (and may be an early stage of varicose-vein development) and touches briefly on risk factors for such development—such as age and genetics—but also more generally on risk factors for progression of chronic venous disease, factors possibly more amenable to intervention through lifestyle changes and adequate use of compression stockings. We ask Professor Rabe to weigh in on what can be done to prevent varicose veins and what measures may beneficially influence risk factors for progression to chronic venous insufficiency.

Could you summarize the main characteristics and findings of the Bochum study, the Bonn Vein study, and the Kostas trial?

In the initial Bochum study, 740 school children aged 10-12 years were screened for venous abnormalities. They underwent clinical and ultrasound follow-up at the ages of 14-16, 18-20, and 29-31 years. None of the participants showed varicose veins during Bochum study I. In the follow-up investigation, it was shown that the manifestation of truncal varicose veins is preceded by venous reflux in the same veins ($P=0.039$). Venous reflux increased with age (Bochum I: 2.5%, Bochum III: 18.5%, Bochum IV: 25%). The presence of preclinical venous reflux presented a 30% risk (95% confidence interval, 13%-53%) of developing truncal varicose veins within 4 years.

In Bonn Vein study I and II, reflux in the saphenous veins was defined as an insufficient vein in clinical stage C2. However, we demonstrated progression of the severity of venous disease from stage C2 with reflux in the saphenous veins to chronic venous insufficiency (stages C3 and C4a) in 31.8% of participants in the 6.6-year follow-up period. Kostas et al examined the contralateral limbs of 73 patients undergoing varicose vein surgery after 5 years of follow-up. In that study, 48 new sites of reflux were identified and reviewed in 38 patients (52%). During that same period, CEAP (Clinical-Etiology-Anatomy-Pathophysiology clinical class) scores deteriorat-
ed significantly. Independent risk factors for progression of chronic venous disease in a binary logistic regression analysis were orthostatism, obesity, and incompliance with compression treatment, whereas multiparity and estrogen therapy were not identified as risk factors.

**What did these studies have to say about the cause of varicose veins?**

In the Bochum studies, the development of varicose veins was preceded by the detection of reflux. However, venous reflux is caused by venous valve dysfunction, which itself is a part of the varicose vein disease. The detection of venous reflux in truncal veins may therefore be an early stage of the development of varicose veins. In addition, truncal veins, e.g., the great and small saphenous veins, are located in a duplication of the muscle fascia, and they are usually not clinically visible even if they have developed reflux and dilatation. This means that a dilated insufficient saphenous vein may not be clinically visible, whereas the more superficially located side-branch varicosities are clinically diagnosed as varicose veins.

In the Bonn Vein study, we investigated the incidence of varicose veins during a 6.6-year follow-up period. The overall incidence for new varicose veins, increasing with age, was 13.7%—so, roughly 2% per year—equally in the male and female population. The only significant risk factors for the incidence of varicose veins were age and family history of varicose veins. Pregnancies showed a trend for a higher incidence, but this did not reach a significant level. This may be due to the fact that the number of pregnancies in our population is low.

**Where varicose veins were preceded by reflux, what was the latency period?**

This question refers to the Bochum study; in that study, venous reflux in truncal veins preceded the clinical manifestation of varicose veins in most of the cases. In 30% of the participants, the progression of reflux toward visible varicose veins was fast, with the latency period between 0 and 4 years. In other cases, this period was longer. In two cases, truncal reflux detected in Bochum study I did not transform into varicose veins during the 19 years of Bochum study IV.

**Did these studies identify individuals at risk for developing varicose veins?**

In the Bochum study, venous reflux occurred earlier in individuals with a family predisposition for varicose veins and displayed a higher prevalence and longer duration of reflux than in the control group with a family history negative for venous disease. Concerning chronic venous disease progression, the Kostas study showed that prolonged orthostatism, obesity, and incompliance with compression-stocking use are risk factors. However, this is not specific for progression of varicose veins, but represents risk factors for the overall progression of signs and symptoms in chronic venous disease.

In Bonn Vein study II, higher age and family history for varicose veins were significant risk factors for the development of new varicose veins. With regard to progression of the C-stages, the risk factors for progression from C0 and C1 to higher C-stages were higher age, obesity, and superficial and deep venous reflux. Risk factors for progression from C2 to chronic venous insufficiency (C3-C6) were higher age, higher body mass index, and swelling sensation.

**On the basis of these studies, are there any preventive measures you would recommend?**

To answer this question, we have to differentiate between the development of varicose veins and the development of chronic venous insufficiency with edema, skin changes, or venous ulcers. With regard to the development of varicose veins, it seems that we have no instruments to prevent this, as we cannot change age and genetic risk. Early detection of venous reflux, however, may lead to a higher awareness of signs and symptoms of venous diseases in patients and may also encourage early treatment of developing varicose veins.

Age is also one of the main risk factors for the progression of the disease toward chronic venous insufficiency with edema and skin changes. Additional risk factors for the progression toward chronic venous insufficiency are obesity as well as orthostatism and compliance with treatment measures. These are risk factors that can be influenced by weight loss and a change in working habits, as well as engagement in more sports activities. The Kostas study also showed a protective effect of the use of compression stockings in the progression of chronic venous disease. The same may also be true for the treatment of varicose veins.

Today, we understand varicose veins and chronic venous insufficiency to be inflammatory diseases or diseases with inflammation in the venous wall. In this context, the use of vеноactive drugs that are able to reduce the inflammatory reaction of the venous wall may also be protective for the progression of venous diseases. However, long-term longitudinal studies showing this protective effect clinically are still missing.

**Keywords:** chronic venous disease; risk factor; varicose veins; venous reflux
Le Professeur E. Rabe, du Service de dermatologie de l’Université de Bonn, Allemagne, Président émérite de l’Union internationale de Philébologie, nous livre ses commentaires sur le débat concernant l’association entre reflux veineux et insuffisance veineuse chronique. S’intéressant particulièrement à l’apport de trois études importantes (les études de Bochum, la Bonn Vein Study et l’étude de Kostas et al) il examine ici les résultats qui montrent que, dans de nombreux cas, le reflux précède le développement de varices (et peut représenter un stade précoce des varices). Il aborde brièvement les facteurs de risque d’un tel développement (comme l’âge et la génétique), mais aussi, de façon plus générale, les facteurs de risque de progression de l’insuffisance veineuse chronique, plus susceptibles d’être modifiés par un changement de mode de vie et le port de bas de compression. Nous avons demandé au Professeur Rabe de se prononcer sur les mesures de prévention des varices et sur celles qui pourraient influer de façon bénéfique sur les facteurs de risque de progression vers l’insuffisance veineuse chronique.

References
The exact role of the microcirculation in the physiopathology of chronic venous disease (CVD) is still not completely defined…. It is still possible to encounter professionals that think that the physiopathology in patients with varicose veins in the lower limbs is due only to mechanical alterations caused by reflux and venous hypertension seen on vascular echography, even though CVD has long been considered an inflammatory pathology.”

Venous hypertension plays an important role in the development of microangiopathy

by M. das Graças Coelho de Souza, C. E. Virgini-Magalhães, B. Senra Barros, C. L. Lascasas Porto, E. Bouskela, Brazil

Venous hemodynamic changes leading to venous hypertension are important in the development of microangiopathy. To understand microangiopathy in chronic venous disease (CVD), one needs to know about microcirculation: how it functions and how it can be studied. Here we discuss the concept of the microcirculatory unit, which is composed of small arteries and arterioles, capillaries, venules, and lymphatics. Small arteries and arterioles are responsible for the control of blood flow to organs and tissues via the modulation of contraction or relaxation of the vascular wall. Capillaries, the smallest vessels of the cardiovascular system, vary in number according to the metabolic activity of the tissue. Venules are capacitance vessels with well-developed elastic walls. Lymphatics are histologically similar to veins and responsible for lymph transport. Analysis of microcirculatory changes in CVD is challenging because of the lack of practical tools. The orthogonal polarization spectral (OPS) imaging technique is fifteen years old and seems to be suitable to study these patients. The equipment (Cytoscan or similar) has a small handheld probe that can be noninvasively applied to the internal perimalleolar region. Using OPS imaging and comparing CVD patients to healthy controls, we found that capillary morphology (percentage of abnormal capillaries per field) and capillary diameter were significantly different from C2 onwards. The largest diameter of the capillary bulk and of the dermal papilla also increased with progression of CVD and was significantly different from C3 to C5. Functional capillary density (number of capillaries with flowing erythrocytes per unit of tissue area) decreased significantly from C4 to C5.

Medicographia. 2016;38:213-218 (see French abstract on page 218)

The main role of the microcirculation is to provide energy and nutrients to cells and to remove the waste products resulting from metabolism. The microcirculation represents the smallest functional unit of the cardiovascular system, where the interaction between blood and tissue creates the necessary environment for cellular function. The main components making up the microcirculatory unit (Figure 1, page 214) are arterioles, capillaries, venules, and lymphatic capillaries. Each component has a different and specialized function and behavior; however, in all of them, the endothelium has an important function, as under physiological conditions, it assures local tissue homeostasis. Capillaries are very thin structures with a wall consisting of one layer of endothelial cells. As a whole, the human body has approximately 10 billion capillaries, with a total estimated area of 500 to 700 m² (around 1/8 of a football field). In fact, it is rare that any functional cell of our body is...
more than 20 to 30 μm away from a capillary. Capillary diameter ranges from 4 to 9 μm, which is just sufficient to allow the passage of water, electrolytes, and circulating blood cells. Capillaries can be referred to as arteriolar, middle, or venular, according to their proximity to arterioles or to venules. 2

There is marked heterogeneity in microvascular endothelium, depending not only on the tissue but also on the organ and vascular segment, as well as vascular branching. Pronounced differences in the vascular wall structure may be observed. For example, in arterioles, the number of smooth muscle cells tends to decrease with the diminution of the arteriolar diameter. Capillary wall structure is different in that it consists of a single layer of endothelial cells that are superimposed on a basal membrane. Furthermore, several well-controlled studies of organ models have shown that venules can be several times more permeable to water than the arteriolar part of the capillary. Moreover, it is well-known that venules are more susceptible to inflammatory agonists, which can elicit a marked increase in water and macromolecular permeability.

Microcirculatory unit

- Arterioles
Small arteries and arterioles are mainly responsible for the control of blood flow to organs and tissues via variations in contraction and relaxation of the vascular wall. Changes in the degree of contraction of the circular smooth muscle layer of these small vessels allow the regulation of blood flow to tissues and control of mean arterial pressure. The diameter of resistance vessels (arterioles) is determined by intravascular pressure. In special situations, for example, when there is a generalized sympathetic discharge, the contractile activity of vascular smooth muscle cells in small arterioles could completely close the lumen. This phenomenon differs in magnitude in different regions, favoring the distribution of blood to organs such as the brain and heart. Arterioles are responsible for the biggest resistance to blood flow in the vascular system, and they play a fundamental role in the control of mean arterial pressure. Ten to one hundred capillaries originate from successive ramifications in each arteriole, and the mean arteriolar diameter ranges from 8 to 50 μm. In some tissues, it is possible to find metarterioles, intermediary between arterioles and venules, that could form a non-nutritional deviation of blood flow, from arterioles directly to venules. 3

- Precapillary sphincter
The precapillary sphincter is where the last smooth muscle cell is located before the capillary itself. When metabolism in the tissue increases, for example, during physical exercise, a larger number of capillaries need to be perfused. Thus, in this case, precapillary sphincters would be predominantly open to allow blood to enter the capillaries.

- Capillaries
Capillaries are the smallest vessels of the cardiovascular system, and their number varies according to the metabolic activity of the tissue: where there is higher metabolism, there are more capillaries, and vice versa. On the other hand, they occupy the highest cross-sectional area of the vascular system with only 5% of the circulating blood; inside the capillaries, blood flows with a velocity of approximately 0.3 to 1.2 mm/s under resting conditions, but this could increase several times during physical exercise.

The capillary wall is only one-endothelial-cell thick, the capillary is very thin and relatively short, and the blood flows with a low velocity; thus, it is an ideal structure for exchange between blood and tissue. However, there are different types of capillaries, depending on the organ or tissue, and these are classified as continuous (brain), fenestrated (kidney), or sinusoid (liver) capillaries: (i) Continuous. These are present in skeletal muscle, lung, adipose and conjunctive tissues, and in the nervous system. They are made up of one to three endothelial cells forming a circumference supported by the basal membrane. (ii) Fenestrated. These are present in tissues such as kidney tubules and glomerulus, exocrine glands, and intestinal mucosa. They have holes roughly 50-60 μm in diameter between endothelial cells and are more permeable to water and small hydrophilic solutes than continuous capillaries. (iii) Sinusoid. These are present in the bone marrow, liver, and spleen, and have intercellular gaps of approximately 100 nm, making these organs permeable to plasma proteins.

Capillaries do not actively control their diameter, as there are no smooth muscle cells in the capillary wall; passive changes in diameter occur by alterations of pre- or postcapillary resistance. The thin capillary wall resists high internal pressures without disruption.

Capillary blood flow is normally nutritional, but it could also be non-nutritional. Nutritional flow occurs when there is an exchange of gases and solutes. In certain tissues, such as the skin, one can observe an arteriovenous functional deviation.
that could be morphological or physiological. The morphological deviation occurs when there is a direct connection between arterioles and venules, i.e., there is no passage through capillaries. The physiological deviation is characterized by an increase in blood flow through open capillaries. In tissues that have metarterioles, the functional deviation occurs during low metabolic activity. When there is an increase in the metabolic activity, the precapillary vessels dilate and the blood passes through metarterioles and becomes available for capillary perfusion.

In spite of capillaries having been traditionally considered to be mainly responsible for tissue oxygenation, recent data suggest that their primary role is the extraction of catabolite products from the tissues. The functional capillary density (number of capillaries with flowing red blood cells per unit of tissue area) varies according to the metabolic needs of each tissue. In the brain and myocardium, we find higher functional capillary density than in the skeletal muscle. In these organs, oxygen consumption is high and constant.

**Venules**

Venules are capacitance vessels with well-developed elastic walls. Vein compliance is around 24 times higher and its diameter 3 times bigger than the corresponding artery. The role of venules is to collect blood from capillaries and bring it back to the heart. Returning to the heart, capillary blood passes through venules, then from venules to bigger veins that decrease in number and change in vascular wall composition. The cross-sectional area is reduced and the velocity of the blood passing through these bigger veins increases. Venules and veins are the reservoir of blood in the vascular system. Owing to their high compliance and low resistance, they can store 60% of the total blood volume. Between 15% and 30% of the circulating blood volume can be easily compensated by the adaptation capacity of these vascular components.

**Lymphatics**

The network of lymphatic capillaries converges to transition into lymphatic vessels and later on, lymphatic trunks. Lymphatic vessels are histologically similar to veins: the lumen is formed by a layer of endothelial cells, and the thinner vessels are covered by a discontinuous layer of smooth muscle; this layer of smooth muscle becomes continuous in vessels closer to lymphatic trunks.

In lymphatic vessels, the pressure oscillates between 1 and 2 mm Hg, similar to what is observed in the adjacent subcutaneous tissue. Lymphatic smooth muscle cells can elevate this pressure to 5-10 mm Hg during their rhythmic contraction. This contraction is synchronized in segments between valves and tends to push the lymph toward the thoracic duct. Lymphatic vessels have valves that restrict the movement of the lymph that proceeds toward the thoracic duct. Some tissues do not have the lymphatic system, such as bone marrow and cartilage. In other tissues, such as the dermis and the genitourinary, respiratory, and gastrointestinal tracts, lymphatic vessels are numerous.

Proteins that eventually exit the vascular system through the microcirculation are removed from the interstitial space by lymphatic capillaries through the lymph, formed by the difference between capillary filtration and reabsorption. As a whole, 2 to 4 L of lymph is formed every day. Lymph composition is similar to blood plasma, except in the quantity of proteins, which may be half that found in plasma.

**Endothelium-leukocyte interaction**

Interactions between the endothelium and leukocytes, as well as increases in fluid and protein filtration, are restricted almost exclusively to postcapillary venules (mean internal diameter is between 9 and 16 μm). The nature and magnitude of these adhesion interactions between leukocytes and endothelial cells are determined by a variety of factors, including the expression of adhesion molecules on leukocytes and/or endothelial cells, products of leukocyte (superoxide, among others) and endothelial cell (nitric oxide, among others) activation, and physical forces originating from the movement of blood close to the vessel wall (Figure 2). Evidence pointing to leukocytes as mediators of tissue injury in different diseases is accumulating rapidly.
The exact role of the microcirculation in the physiopathology of chronic venous disease (CVD) is still not completely defined, and it has only recently been subjected to systematic investigation. In spite of all the progress in genetics and molecular biology, the impact of these new tools is also small. It is still possible to encounter professionals that think that the physiopathology in patients with varicose veins in the lower limbs is due only to mechanical alterations caused by reflux and venous hypertension seen on vascular echography, even though CVD has long been considered an inflammatory pathology.

Although clinical and experimental studies have yet to completely elucidate the physiopathology of CVD, it is well-accepted that venous hemodynamic alterations leading to venous hypertension play an important role in the development of the observed microangiopathy. Elevated ambulatory pressure manifests not only in the macrocirculation with the development of varicose veins, but also in the capillaries, causing chronic damage and, finally, disruption of the microcirculation. Cutaneous capillaries become progressively enlarged and tortuous, forming “true” skeins described in the literature as glomerulus-like capillaries (Figure 3). The endothelial cells themselves become enlarged, with bigger interendothelial pores making the capillary lumen irregular. These alterations provoke an increase in microvascular permeability, with extravasation of plasma, blood cells, and macromolecules, such as fibrinogen. In the interstitium, fibrinogen is activated, forming a barrier involving the capillaries and limiting the exchange of nutrients, though there is no consensus about this point. The persistence of venous stasis and hypertension results in chronic inflammation of the capillary bed and surrounding tissues and in edema. The reduction in the number of capillaries leads to trophic disorders and leg ulceration.

Hemodynamic forces, such as venous hypertension, circulatory stasis, and alterations in shear stress (acting on the vascular wall as a result of the tangential force produced by blood flow), seem to have an important role in the activation of the inflammatory cascade, promoting adverse reactions in the vascular wall, venous valves, and skin. As a consequence of venous hypertension, there is extravasation of blood fluid from the vessels, mainly from postcapillary venules. Plasma extravasation is responsible for the increase in the lymphatic content and edema, as well as the increase in viscosity and in the amount of red-blood-cell aggregates, leading to a decrease in red blood cells in the microcirculation.

Alterations in shear stress as a result of abnormal blood flow lead to changes in morphology, function, and gene expression in endothelial cells. When blood flow is pulsatile or laminar, the shear stress is normal, and factors that reduce inflammation, thrombus formation, and free radicals—such as nitric oxide, tissue plasminogen activator (tPA), thrombomodulin, and prostacyclin (PGI2)—are actively liberated. On the other hand, if the shear stress is zero or very low—as a result of whirl or even reverse blood flow—free radicals and proinflammatory and prothrombotic (e.g., plasminogen activator inhibitor [PAI]-1, von Willebrand factor, monocyte chemotactic protein [MCP]-1, angiotensin II, and endothelin-1) mediators are liberated.

CVD is also accompanied by an increase in leukocyte infiltration in the affected leg. Leukocytes infiltrate the microcirculation by being trapped in the capillaries or by adhering to venular endothelium. Trapping of neutrophils in the microcirculation reduces capillary perfusion, increases free radical formation, and induces the liberation of proteolytic enzymes. Adhesion of leukocytes in postcapillary venules or in bigger veins could be facilitated by the expression of selectins (P- and L-selectin), integrins, and members of the immunoglobulin superfamily, such as intercellular adhesion molecule (ICAM)-1.

In addition to the acute inflammatory process, where granulocytes infiltrate the venular and venous walls, there is also an infiltration of T and B lymphocytes. Monocytes/macrophages also infiltrate the venous valves and could be involved in their destruction. There is evidence for the involvement of ICAM-1, vascular adhesion molecule (VCAM)-1, L-selectin, E-selectin, and integrins in this process.
Activation of leukocytes is characterized by synthesis and liberation of several inflammatory mediators; these include leukotrienes, prostaglandins, bradykinin, free radicals, and cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, which regulate and perpetuate the inflammatory reaction by autocrine and paracrine mechanisms.²³

CVD of the lower limbs is a common public health problem worldwide that has had a highly negative impact on quality of life due to leg ulceration, pain, and sick leave. CVD is a multifactorial disease with many clinical presentations and is increasing worldwide. Although the CEAP (C for clinical evaluation, E for etiology, A for anatomic findings, and P for pathophysiology) classification was revised in 2004,²⁴ with enhancement of the pathophysiologic analysis, we still lack a large microcirculatory study. Methods, such as laser Doppler flowmetry, videocapillaroscopy, plethysmography, and fluorescence videomicroscopy have been used to visualize the microcirculation (directly or indirectly); however, the latest technique is orthogonal polarization spectral (OPS) imaging. OPS is based on intravital microscopy with incident polarized light that produces reflected depolarized light from hemoglobin. Cytoscan implements the OPS technique and was described for the first time in 1999.²⁶ Cytoscan and its followers, Microscan and Cytocam, can be used for noninvasive studies of all tissue surfaces without the use of fluorescent dyes, and OPS imaging has been validated in comparison with conventional videocapillaroscopy and intravital microscopy.²⁶ Nowadays, it is possible to quantify microangiopathic changes related to CVD with this equipment. It is important to evaluate the perimalleolar area in these patients as it is a gaiter zone where stasis ulcers usually appear.³

Quantification of microangiopathy in CVD: our experience

According to the concept that venous microangiopathy resulting from venous hypertension is one of the first signals of CVD, quantification of microcirculatory parameters can be used to monitor disease severity.²⁷,²⁸ In our laboratory, we have investigated several microcirculatory parameters in patients with CVD. We found that morphological alterations of the microcirculation that are characteristic of this disease increase according to its evolution, but they are already present in the C2 class of disease. The method to observe the cutaneous microcirculation in patients with CVD through OPS imaging was also developed in our laboratory.²⁹ Reproducibility of studied parameters showed <20% variability, which is acceptable due to normal expected variation of the measurements.²⁹ Functional capillary density, the number of capillaries with flowing red blood cells per unit of tissue area (mm²), is similar for C1 and C2 patients, but starting with C3 there is a gradual reduction in the number of perfused cutaneous capillaries leading to capillary rarefaction in more advanced stages of the disease. On the other hand, capillary limb diameter (μm) and capillary morphology (the percentage of abnormal capillaries in the total number of capillaries observed in each field) followed the progression of the venous disease, being significantly different from healthy subjects already at C2. Diameter of the capillary bulk (μm)—measuring the size of the skein in which the hairpin (capillary found in healthy subjects) was transformed—and of the dermal papilla (μm, to quantify the onset of edema)—the smallest functional skin unit—also increased with the progression of the disease and were significantly different from healthy subjects from C3 to C5.²⁹,³⁰

Capillary morphology seems to be a good parameter for evaluation of CVD patients, because already at C2, it was significantly different from controls. In healthy subjects, only 3.6% of cutaneous capillaries in the lower limb showed morphologic alterations; in patients with CVD, one can observe a gradual substitution of hairpin capillaries with glomerulus-like ones (Figure 3).

The increased diameter of the capillary may represent a change in shear stress and consequently elicit endothelial cell activation. The diameter of the capillary bulk also increased gradually, according to the evolution of the disease. The dermal papilla tended to increase when the capillary inside it became tortuous. It is possible that the initial increase was due to changes in capillary-tissue exchange and the appearance of a preclinical edema.³⁰ The decrease in functional capillary density can lead to tissue ischemia and, subsequently, cutaneous ulceration.

In conclusion, we have found that capillary morphology and capillary diameter differs significantly from those in healthy subjects from C2 onwards. Diameter of the capillary bulk and diameter of the dermal papilla also increases with disease progression and is significantly different from those in healthy subjects from C3 to C5. Functional capillary density decreases significantly compared with healthy subjects from C4 to C5.³⁰ Thus, our experience supports the idea that quantification of microangiopathy in chronic venous disease could facilitate monitoring of disease severity.
Les modifications hémodynamiques responsables de l’hypertension veineuse ont un rôle important dans l’apparition d’une microangiopathie. Comprendre la microangiopathie liée à l’insuffisance veineuse chronique demande de bien connaître la microcirculation : la façon dont elle fonctionne et comment elle peut être étudiée. Nous analysons dans cet article le concept d’unité microcirculatoire composée de petites artères et artérioles, de capillaires, veines et vaisseaux lymphatiques. Les petites artères et les artérioles contrôlent le flux sanguin vers les organes et les tissus en modulant la contraction ou la relaxation de la paroi vasculaire. Le nombre des capillaires, les plus petits vaisseaux du système cardiovasculaire, varie selon l’activité métabolique du tissu. Les veines sont des vaisseaux de capacitance à la paroi élastique bien développée. Les vaisseaux lymphatiques ressemblent histologiquement aux veines et transportent la lymphe. Il est difficile d’analyser les variations microcirculatoires dans la MVC en raison du manque d’outils pratiques. L’imagerie spectrale polarisée orthogonale (OPS) date de 15 ans et semble convenir à l’examen de ces patients. L’appareil (Cytoscan ou autre) dispose d’une petite sonde portable qui s’applique de façon non invasive sur la région péri-malléolaire interne. En comparant, grâce à l’imagerie OPS, des patients atteints d’insuffisance veineuse chronique à des patients sains, nous avons trouvé que la morphologie capillaire (pourcentage de capillaires anormaux par champ) et le diamètre capillaire diffèrent significativement à partir de la classe C2. Le diamètre le plus large de la masse capillaire et de la papille demeure augmentée également avec la progression de la MVC et diffère significativement de la classe C3 à la classe C5. La densité capillaire fonctionnelle (nombre de capillaires avec érythrocytes circulants par unité de zone tissulaire) diminue significativement de la classe C4 à C5.

Keywords: capillary; chronic venous disease; microangiopathy; microcirculation; orthogonal polarization spectral imaging
We certainly feel ambivalent toward tiny scurrying or flying critters: some butterflies or beetles are as beautiful as jewels (and indeed are sometimes worn as such), others elicit anguish or disgust, bordering on “Fear Factor”–like hysteria, such as cockroaches or spiders. No-one more than Jean-Henri Fabre, an obscure 19th-century French schoolteacher turned world-famous entomologist and precursor of ethology, has written so comprehensively and compellingly about the lives of insects and arachnids. By restoring the Harmas, Fabre’s house and laboratory in Provence, as a museum devoted to his life and work, Anne-Marie Slézec, of the French National Museum of Natural History, has become the custodian of his memory. Forensic entomology has a long history, starting with Song Ci, a magistrate in 13th-century China. As a science it came of age in France with a veterinarian, Jean Pierre Mégnin (1828-1905), and a doctor, Marcel Leclercq (1924-2008). This tradition of excellence continues today with ForenSeek, a cutting-edge international web-based platform for forensic entomology sample analysis, created by Damien Charabidze, at the University of Lille.
If the plaudits of your peers are a yardstick of accomplishment, then Jean-Henri Fabre was a high achiever. Charles Darwin wrote of Fabre as “that inimitable observer”; the biologist and science writer Jean Rostand characterized him as a “great scientist who thinks as a philosopher, sees as an artist, and feels and expresses himself as a poet”; and Maurice Maeterlinck, Nobel Prize in Literature in 1911 and amateur entomologist, called him “the insect’s Homer.” And if that were not enough, Maeterlinck went on to say Fabre was “one of the most profound and inventive scholars and also one of the purest writers and, I was going to add, one of the finest poets of the century that is just past” (i.e., the 19th). Yet Fabre could have attained even greater heights had the wherewithal to fund university studies been available to him. As it was, his formal education ended after teacher training and he was thwarted in his ambition to take the exams to become a university lecturer. Born in 1823 in southern France, Fabre lived in Provence from 1840 until his death seventy-five years later. Fame as an entomologist has tended to overshadow Fabre’s other accomplishments, manifest though they are among the pages of his Souvenirs Entomologiques, the ten-volume scientific and biographical work which has cemented his reputation as an outstanding botanist and zoologist, keen observer of nature, pioneer in the science of ecology, and stylish writer. Souvenirs Entomologiques has been translated around the world and in Japan is held in particularly high esteem for the way Fabre harnesses literature and science to shed light on the marvels of nature.

Jean-Henri Fabre: unveiling the fascinating world of insects

by A.-M. Slézec, France

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Medicographia. 2016;38:220-232 (see French abstract on page 232)
“Life has unfathomable secrets. Human knowledge shall be erased from the archives of the world before we possess the last word that the gnat has to say to us”


From my earliest childhood,” wrote Jean-Henri Fabre in his Souvenirs Entomologiques, “I have felt drawn towards the things of Nature. It would be ridiculous to suppose that this gift, this love of observing plants and insects, was inherited from my ancestors, who were uneducated people of the soil and observed little but their own cows and sheep. Of my four grandparents only one ever opened a book, and even so he was very uncertain about his spelling. Nor do I owe anything to a scientific training. Without masters, without guides, often without books, I have gone forward with one aim always before me: to add a few pages to the history of insects.” As it turned out, Fabre added rather more than a few.

Born on December 22, 1823, in Saint-Léons in the Aveyron department of southern France, Jean-Henri Fabre entered primary school in Avignon on a scholarship. Alert, intelligent, a brilliant student, he completed his school studies early, enjoining his teachers, as he later recalled in Souvenirs Entomologiques: “Do what you please with my goodwill, make of me what you will; as long as I can study, the rest is unimportant.”

And study he did—botany, mycology, zoology, geology, archaeological science—passions that remained with him throughout his long life. An incomparable observer, Fabre in his fine prose called upon hard-won knowledge of Latin and of Greek, of chemistry, mathematics, and physics, the only subject matter deemed worthy of professional recognition at the time.

Childhood and adolescence: from Rodez to Avignon

Fabre was born into want. “I was five to six years old. To reduce the number of mouths to be fed in our poor household, I was entrusted to the care of grandmother.” There, in the harsh climate of the Aveyron (called Rouergue at the time), living conditions on the paternal grandparents’ farm were tough and no doubt forged the combative nature that Fabre showed throughout his life. “I owe you much, dear grandmother […] Perhaps you passed on to me something of your robustness, of your love of work.”

Later, back in the paternal home, when Fabre turned seven, “…the time was come to go to school. […] How shall I name the room where I was to learn the alphabet? At one and the same time it was a schoolroom, kitchen, bedroom, refectory, at times a henhouse, a pigpen. In those days we scarce dreamed of [the] palace-like schools [that came later].”

Fabre’s parents moved often in search of work and his was an itinerant childhood. From Rodez, where he was altar boy at the seminary, to Aurillac, to Toulouse, where he completed his school studies, to Montpellier and Pierrelatte.

“Then suddenly it was farewell to my studies. Relentless bad luck struck us. Bread in danger of short supply at home[…] Scratch around as best you can to earn ten cents’ worth of fried potatoes. Your life will become a loathsome Gehenna. Let’s not dwell upon it.”

Between seasonal jobs to make ends meet, Fabre persevered with his studies and in 1840 won a scholarship: “The good fortune that never abandons the brave led me to a primary school in the Vaucluse, where I found guaranteed fodder: dried chestnuts and chickpeas. […] I had a head start on my fellow students and used the time to organize my haphazard knowledge of plants and animals…”
In the same year Fabre published his first collection of poetry, took part in the Sainte Croix pilgrimage in September,8 and, following the example of the 14th-century Italian poet and scholar Petrarch, climbed Mount Ventoux, from which he returned with one of the first flowering plants for his herbarium, the poppy *Papaver aurantiacum* Loisel.

**Schoolteacher and father**

As a freshly qualified 19-year-old primary teacher in Carpentras (Vaucluse), Fabre soon incurred the ire of his superiors for his teaching practices, which included outdoor nature classes. Teaching in those days was hard: the clergy and the laity taught subjects that were deemed “noble”; the natural sciences were not among them. To what then could aspire a penniless, self-taught school teacher who had never set foot in a university and who, what is more, was sidetracked into observing nature?

In October 1844 Fabre married fellow teacher Marie Villard, who over the next two years gave birth to a son and daughter, both of whom died in infancy, followed in 1850 by a daughter and over the next thirteen years three more children.

Fabre took a bachelor’s degree in mathematical sciences in 1847 and another the following year in physical sciences: “In those long gone days it was customary to precede science by serious literary studies.”9 “Twelve months of lengthy meditation at my little desk finally were worth a degree in the mathematical sciences. And here I am, half a century later, able to perform eminently lucrative functions as a surveyor of spider’s webs.”10

Fabre’s reading of *Natural History of Articulated Animals, Annelids, Crustaceans, Arachnids, Myriapods, and Insects*, by Blanchard, Castelnau, and Lucas, spurred his studies and observations, restricted as they were by his teaching duties. “With this foretaste of the natural sciences,… I left school more passionate than ever about insects and flowers. Yet natural history had to be abandoned. It could lead me nowhere. Teaching in those days kept it at a distance, deeming it unworthy of association with Latin and Greek. This left me mathematics, the tools for which were most simple: a blackboard, a stick of chalk, some books….”11

**A Corsican interlude**

Armed with his diplomas, which procured a higher professional standing, in early 1849 Fabre was appointed head of physics at the Collège Fesch in Ajaccio on the French island of Corsica in the Mediterranean. “In short, I was sent to teach physics and chemistry…. The sea full of marvels…. …I succumbed. My free time was divided in two. The largest part I devoted to mathematics, the basis of my academic future … the other I spent tentatively collecting plants, studying things from the sea…..” Times were hard at the Collège Fesch: no student passed the high school diploma and the local authorities’ verdict was damning: “The college has fallen into ruinous disrepute because many pupils have deserted it to take instead tuition at the small seminary.”

During Fabre’s Corsican interlude, his encounter with Esprit Requien, the Director of the botanical gardens and founder of the Natural History Museum in Avignon, proved decisive in his development as a scientist. Requien introduced Fabre to Alfred Moquin-Tandon, professor of botany at the botanical gardens in Toulouse, zoologist, poet, philosopher, and physician. Moquin-Tandon accompanied Fabre on botanical excursions, discussed science with him, encouraged him to follow his penchant for natural history, and urged him to continue to use and preserve the Occitan language. Fabre and Requien planned to write a book on the flora of Corsica, but the plan came to naught following Requien’s sudden death in 1851.12

Upon his return to mainland France, Fabre took up a position in January 1853 as assistant teacher of physics at the Lycée Impérial in Avignon, where he and his family rented a house and garden at number 14, rue des Teinturiers. In Avignon, Fabre became firm friends with Théodore Delacour,
who worked as crop manager and associate at the seed merchant’s Vilmorin–Andrieux on the Quai de la Mégisserie in Paris. Delacour was Fabre’s Parisian contact and smoothed the way to the publication of his articles in journals of natural science or at the Academy of Sciences. Delacour pro-

cured for Fabre the latest scientific works and journals and even asked him to teach courses at the Vaucluse Chamber of Agriculture. Fabre’s garden in Avignon and after 1879 his house and garden in Sérignan-du-Comtat were the scene of their discussions, horticultural tests, and attempts to acclimate new species to be introduced onto the market.13

In addition to his passionate interest in botany, Fabre devoted much time to studying insects, for which he invented living spaces wherein to observe them. He kept abreast of publications by fellow entomologists, including Léon Dufour’s work on Cerceris, a genus of wasps, on which Fabre published an article in the Annales de Sciences Naturelles in 1855, for which the following year he was awarded the Montyon Prize for experimental physiology at the Institut de France. At this time Fabre completed his university studies with a Bachelor’s Degree in Natural Sciences, and two doctoral theses (zoology and botany) defended at the Faculty of Sciences in Paris.

Always keen to improve his family’s living conditions, Fabre gave his career a new twist by deploying his qualities as a chemist. Using the roots of madder, which was much grown in Provence, he extracted alizarin, a natural red colorant used to dye cloth for soldiers’ trousers. He registered several patents at the Vaucluse Agricultural Academy, but his hopes were dashed when two German chemists synthesized alizarin and the market for the natural dye collapsed.

Ever resourceful and hard-working and making use of his talents as a teacher and writer, Fabre published in 1862 Lessons in Agricultural Chemistry, followed by over one hundred schoolbooks, from which additional revenue in the form of royalties eased the family’s financial situation.

Under Napoleon III (French Emperor between 1852 and 1870), teaching methods evolved on the initiative of the Minister of Public Instruction Victor Duruy, who initiated evening classes for adults. As a teacher, Fabre was ahead of his time and highly successful in this role, collecting plants in the countryside, studying and speaking of natural phenomena and mother nature in all her guises, including sex in flowering plants.

“This was too much. See indeed how dark was my crime: I was teaching these young people what the air and water are, where thunderbolts, lightning, and thunder come from… how a seed germinates and how a flower blooms, things eminently abominable in the eyes of some, who half-close their flabby eyelids against the brightness of day. As a matter of urgency, the little source of enlightenment had to be snuffed out, the nuisance banished.” Much valued by his students, but disparaged by his superiors, Fabre was the victim of a conspiracy and the family lost their lodgings and had to leave Avignon.14

By 1868 Fabre was earning royalties from his schoolbooks equivalent to his teacher’s remuneration and so he left teaching forthwith. The city of Avignon provisionally appointed him to the position of curator at the Musée Requien (natural history), which is where he met John Stuart Mill. Economist, theoretician of liberalism, and former director of the British East India Company, Mill had come to Avignon to spend his retirement collecting plants, writing, and visiting the Musée Requien. Recently widowed after only seven years of marriage, Mill was inconsolable and found solace in his conversations with Fabre. They shared a taste for nature and upon Mill’s initiative planned to write a book on the flora of the Vaucluse. Fabre was to deal with Cryptogams (plants and fungi that produce spores, and not seeds or flowers), starting with a...
study of the Spheriaceae (minuscule fungi that cover dead wood). But Fabre’s precarious financial situation hampered his work on the project and with great tact Mill came to his assistance. Deeply moved, Fabre made it a point of honor to repay his friend. Sadly, the projected flora of the Vaucluse never materialized as Mill died in 1873, and in the same year the city of Avignon stripped Fabre of his position, thus separating him from the plant collections to which Mill had greatly contributed. Fabre’s essay on the Spheriaceae of the Vaucluse Department was finally published in 1878.

At the outbreak of the Franco-Prussian War in 1870 the Fabre family moved to Orange, where they rented accommodation in town and then on the outskirts, in La Vinarde. There, over the next nine years Fabre wrote educational books and part of what would later become the first volume of his Souvenirs Entomologiques. City Hall in Orange entrusted Fabre with expert appraisals pending receipt of his royalties, payment of which was blocked in Paris because of the war. The relative peace Fabre found in La Vinarde, far from the hubbub of the city, was nonetheless troubled by illness and above all by the death at just sixteen of his son Jules. “Dear child,” wrote Fabre, “my collaborator so passionate about insects… Ah! How death is unbearable when it cuts down a flower in the full bloom of youth!”

In late 1878, during his convalescence from a bout of pneumonia, Fabre discovered that the magnificent line of plane trees on the approach to La Vinarde had been cut down on the orders of the owner. It was time to move and in February of the following year Fabre bought a two-storied house eight kilometers west of Orange, in the village of Sérignan-du-Comtat. “Hoc erat in votis” (This was among my prayers) he wrote in his Souvenirs Entomologiques, quoting Horace, the 1st-century BCE Roman lyric poet. What’s more, the house came with an overgrown garden or “harmas,” which Fabre described as “an untilled, pebbly expanse where hardly any plant but thyme
can grow. It is too poor to be worth the trouble of plowing, but the sheep pass there in spring, when it has chanced to rain and a little grass grows up." This was to be his laboratory where never had he "seen so large a population of insects at a single spot."

Thus began thirty-six intellectually rich and fruitful years at Sérignan, during which Fabre went into the village no more than a score of times, but climbed the slopes of Mount Ventoux on close to fifty occasions and, in his harms, his "field laboratory," recorded his observations of insects in the next nine volumes of his *Souvenirs Entomologiques*. Until 1909 he continued to write school texts and science books for the general public, poetry in Provençal (a variety of Occitan, a Romance language closely related to Catalan), and also painted over six hundred watercolor drawings of fungi for educational purposes.

Fabre corresponded extensively with Darwin on matters of natural history and jointly they devised experiments, which Fabre carried out. Darwin wrote: "Allow me to make a suggestion in relation to your wonderful account of insects finding their way home. I formerly wished to try it with pigeons; namely, to carry insects in their paper cornets about hundred paces in the opposite direction to that which you intended ultimately to carry them, but before turning round to return, to put the insects in a circular box with an axle which could be made to revolve very rapidly first in one direction and then in another, so as to destroy for a time all sense of direction in the insects. I have sometimes imagined that animals may feel in which direction they were at the first star carried."

One experiment on Chalicodoma (bees) led Fabre to posit that the Earth’s magnetic field influenced the return of the bees to their nest, a hypothesis confirmed in the 1980s by the mathematician and physicist Yves Rocard. Fabre wrote: "The method of experimentation seemed ingeniously designed to me… This result seemed all the more likely to me as the country people around me repeated facts likely to confirm my expectations… I related to the philosopher of Down [Darwin] how the peasants had anticipated the investigations of science…."19

Fabre’s excursions on Mount Ventoux provided evidence for plant species’ composition at different altitudes. "My barometer indicated the altitude of the main botanical sites […] The temperature becoming too low, little by little the olive tree, the holm oak, vines and almond trees disappear; followed by blackberry bushes, the walnut, the white oak. Box becomes abundant. We enter a monotonous region that extends from...

**Fabre the scientist: an experimenter and observer of life**


Fabre found that insects were easy to rear and observe in his closed garden, a sort of ecological reserve, and in his lab, and to study in the surrounding countryside, blessed as it was with a wide range of species. The instincts of insects were much debated at the time and of great interest to Fabre.17

"The instinct aroused by a chance act that proves favorable for the animal is an acquired habit. And on that, arguments invoke natural selection, atavism, the struggle for life. I see a good many big words, but I prefer a few small facts. And for forty years before long I have been gathering these little facts, examining them. And they don’t tally exactly with current theories. […] In this world, apparently, the evolution of the cell is not everything. […] I dismiss the modern theory of instinct. I see nothing there but a presumption in which revels a naturalist who ventures not into the field but rather fashions the world according to his imagination; in which, however, the observer, grappling with the reality of things, finds no serious explanation of what he sees."18
Insect collections in Jean-Henri Fabre’s study at the Harmas. © MNHN/Philippe Abel.
the end of the arable land to the lower half of beeches and where the dominant vegetation is mountain savory." These observations were later confirmed by Charles Henri Marie Flahault, the pioneer of phytogeography who drew the first vegetation map in 1894.

"On this memorable day, therefore, the 13th of December, 1895, I instituted the caterpillars' meteorological observatory." Thus did Fabre begin his studies of the pine processionary caterpillar and its sensorial perception. He observed that the caterpillar dreads inclement weather: "A drop of rain sets him in a flutter; a snowflake exasperates him. To start for the grazing-grounds at dark of night, in uncertain weather, would be dangerous, for the procession goes some distance and travels slowly. The flock would fare ill before regaining shelter did any sudden atmospheric trouble supervene, an event of some frequency in the bad season of the year. So that he may be informed in this particular during his nocturnal winter rambles, can the Pine Caterpillar be endowed with some sort of meteorological ap-

Town in Roussillon with Mont Ventoux in the distance. © Christophe Boisvieux/Corbis.
From the sum of my observations it appears that the Pine Processionary is eminently sensitive to atmospheric vicissitudes, an excellent quality, having regard to his way of life in the sharp winter nights. He foresees the storm, which would imperil his excursions.”21

The great peacock evening

Fabre bred the giant peacock moth (Saturnia pyri) and observed its behavior and whether sight, hearing, or sense of smell guides the males to a female sequestered in a wire-gauze bell-jar in his laboratory. “It was a memorable evening. I shall call it the Great Peacock evening. […] What are the organs of information that direct the rutting Moth on its nightly pilgrimage? […] One suspects the antennae, which, in the males, do in fact seem to be questioning space with their spreading tufts of feathers. […] Are there, in point of fact, effluvia similar to what we call odor, effluvia of extreme subtlety, absolutely imperceptible to ourselves and yet capable of impressing a sense of smell better endowed than ours?”22 Fabre needed nearly six seasons to affirm the probable existence of odor emitted by the female moth that our olfactory organs are unable to detect, but which is indispensable for the survival of the giant peacock moth. It was not until 1959 that the mystery was solved by the German biochemist Peter Karlson and the Swiss entomologist Martin Lüscher who coined the term pheromones for “substances which are secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction, for example, a definite behavior or a developmental process.”

Death comes to all living things, but what happens to their bodies abandoned in the woods or fields? In his chapter on burying beetles, Fabre carefully describes his observations “…the ant hastens there first and starts the dissection into pieces. Soon the odor attracts Diptera, which generate the odious maggot […] …in waves scuttling with mincing steps comes the shiny carrion beetle.” The cadaver soon teems with insects, each wave exploiting another niche, another part of the nutrient-rich food source. The burying beetle does what its name says and uses the cadaver as food for its offspring. It is “the first of the little ‘sanitizers,’ ‘cleansers’ of the fields.”23 “…to rid the soil of the stains of death and to put into the treasures of life the deceased animal matter, there are legions of enterprising butchers, including in this part of the world the bluebottle fly (Calliphora vomitoria) and the common flesh fly (Sarcophaga carnaria). […] To lay its eggs the bluebottle seeks open wounds or the mucous membranes of the mouth or eyes unprotected by an epidermis offering any resistance.”24 “He who says dung-beetle says a fervent friend of dung, in which the insect protects the provender of its offspring. But this is not the case of the Coprophanaeus mini, the scavenging dung-beetle of the pampa. […] It needs the pus of corpses. In Provence just one scavenging dung-beetle, Onthophagus ovatus, is found frequently on dead moles and lifeless rabbits.”25 Did Fabre perhaps imagine insects in the service of forensic science?

George Legros and the legacy of Jean-Henri Fabre

Fabre’s intuition, experiments, and patience underpin his meticulous and all-embracing observations, which often are found in scientific work that came after him. We are indebted to biographer Georges Legros for many pages on Fabre and through these for the entomologist’s enduring renown.26 A man of means, country doctor, surgeon during the Great War, Legros never forgot “his master” and as a long-serving parliamentary deputy (congressman) oversaw laws that saved national heritage sites like Fabre’s home and garden, which in 1922 were acquired by the National Museum of Natural History. A duty to remember.
Jean-Henri Fabre: le naturaliste qui révèle le monde fascinant des insectes

Forensic entomology: how insects solve whodunits

by D. Charabidze, France

Some fifty common necrophagous insect species hasten decomposition of the cadaver, leaving just skin and bone and bits of dried muscle. Certain species, such as dermestid beetles, specialize in the late phases of decomposition and complete the decay and recycling process of the body. During her lifetime each female dermestid may lay up to 800 eggs, which hatch after a time dependent on temperature and moisture level, and in favorable conditions the body is soon host to thousands of beetles.

Can three flies devour a dead horse as fast as a lion? Carl Linnaeus, the father of taxonomy, thought so. What nonsense I hear you say. But is it? Hyperbole it may be, but imagine myriad flies swarming around carrion and do the math: each fly lays 200 eggs, whence numberless maggots and short shrift. Which is why you’re unlikely to happen across a deer carcass in the forest or the stiffened corpse of a crow in your backyard. Because winging in after death come necrophagous insects. Which brings us to the centuries-old, but now high-tech science of forensic entomology. Fans of the CSI franchise and other TV crime series may pay them scant attention, but the fact is that insects can provide vital clues after murder most foul. Notably the time of death. As these insects only develop on cadavers, they lay eggs when the hapless victim has already shuffled off this mortal coil. So the entomologist collects larvae from the body, determines their age and hence when the eggs were laid, and estimates how soon after death the insect colonization began. In criminal investigations, such entomological evidence may prove crucial in convicting the guilty and exonerating the innocent.

What happens to our body after death? Sooner or later this unsettling question will cross everyone’s mind, inspire unease, turn the stomach, and prompt cries of: Spare us the details! Yet a corpse is a fascinating ecosystem teeming with predators, opportunists, and parasites. An ecosystem that with breathtaking efficiency recycles organic matter and in so doing nurtures larvae, flies, and beetles, which within days reduce a body to a heap of bones. An ecosystem which, when analyzed by an expert, speaks volumes and notably can be used to pinpoint the time of death in a murder investigation.

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Medicographia. 2016;38:233-241 (see French abstract on page 241)
“Boîte à escouades” ("wave box") created by Jean-Pierre Mégnin, and preserved at the Entomology Department of the National Museum of Natural History in Paris, showing the 10 successive waves of necrophagous insects that feed and lay eggs on a decomposing body.

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Insects and their role in the decomposition of bodies

Colonization of meat by flies and their larvae was first studied in Europe in the 17th century, against a backdrop of the scientific controversy surrounding spontaneous generation. In 1671, Francesco Redi showed that maggots on meat came from eggs laid by flies, and that the site of laying conditioned larval development. This refuted the idea, held since the time of Aristotle (4th century BCE), that life could arise spontaneously from nonliving matter. Yet nigh on 350 years later, many people still believe that “worms” emerge from a body after death.

When an animal dies, its body becomes a valuable source of protein- and nutrient-rich flesh and offal: first come first served. Flies of the Calliphoridae family generally lead the way. These common large metallic blue and green insects, known as blowflies, have a highly developed sense of smell: they can detect a dead body kilometers away. Fast-flying, they locate and colonize a corpse within hours after death. They are seeking food, but above all somewhere to lay their eggs, because while the adults are opportunistic feeders on many sources of organic matter, their larvae are solely necrophagous: they need flesh to grow. So gravid females are constantly on the lookout for dead animals on which to lay their eggs: rodents, larger mammals, or human cadaver.

Once they locate carrion, flies find a sheltered spot to lay a clutch of 200 or so eggs. The choice of egg laying (oviposition) site is crucial, as young larvae are fragile and soon desiccate in the air. They need immediate access to abundant and easily assimilated nourishment. Which is why flies tend to lay their eggs in natural orifices, notably the eyes and nostrils. Once hatched, the larvae feed and grow quickly, as do all necrophagous species. Since a cadaver is an ephemeral and ever-changing food source, insects must exploit it before changes occur or competing species arrive. Blowflies may be the first to colonize a dead body, but are soon followed by other species. Competition is relatively limited on large mammals, but on small carrion larvae may at any time be deprived of sustenance, and to limit this competition some species exploit particular ecological niches.

The premises of forensic entomology

The waves of species of necrophagous insects that arrive on a decomposing body are the nitty-gritty of forensic entomology. Far from being a recent discovery, the link between these insects and the chronology of death has been known since the 13th century, when it was established in China by the founding father of forensic science, Song Ci (宋慈), who has since inspired characters in books, comic strips, and films.1,2 The story goes that one harvest time a peasant was found murdered in a field, cut down with a sickle. Magistrate Song Ci mustered the villagers and ordered them to drop their tools at their feet. Attracted by unseen traces of blood, flies...
clustered on one particular sickle, whose owner confessed to the crime. Its historical interest apart, this case neatly illustrates how the observation of insects and their behavior has a role to play in forensic investigations.

In Europe it was not until the 19th century that forensic entomology was applied and developed. Anecdotal accounts report that in 1850 one Dr Bergeret in France used necrophagous insects to establish the time of death of a nursling, although his dating was wrong because of limited knowledge of corpse fauna at the time. It later fell to Jean Pierre Mégnin, an army veterinarian and amateur entomologist, to lay the foundations of present-day forensic entomology. Mégnin found only pioneer species on cadavers discovered soon after death, whereas a longer postmortem interval allowed for a greater variety of species. Mégnin painstakingly described this sequence of species and theorized the original concept of successions or waves of insects (see illustration of Mégnin’s “boîte à escouades” (display box of necrophagous insect waves) at the beginning of this article.

Noting that there is a succession of more or less specialized species on a cadaver, Mégnin described 8 “open-air” (and 2 “underground”) waves of colonization by necrophagous insects, each wave comprising a set of species colonizing the body at a given time during its decomposition. Mégnin’s overall timeline reflected a certain reality, but in a legal context it tended to be generalized and misused. Numerous experimental studies have since shown that insect succession is strongly affected by climatic conditions, time of year, and the body’s characteristics and geographical location. So there is no standard, predictable, and constant succession of insects on a cadaver. Nonetheless, the dating of waves of insects was used up until the end of the 20th century and the emergence of contemporary forensic science.

**Waves and dating of death**

As Mégnin observed, a body decomposes according to a chronology in which insects play a major part. When pioneer species have colonized the body, symbiotic bacteria in the
gastrointestinal tract alter the cadaver from within, releasing a strong stench of putrefaction. Colonized wounds and ori-fices teem with maggots, which attack deeper tissues and secrete digestive enzymes, which dissolve muscle and other soft tissues, releasing rich organic liquids and opening the carcass, leaving behind strands of ligamentous tissue, fat, and strips of flesh. Other species join the feast later, notably small Diptera of the genus Muscidae, as well as necrophagous or predatory Coleoptera, which prey on Diptera larvae when food is scarce or competition keen.

By consuming flesh and promoting the flow of fluids, this varied entomofauna (there are some fifty common necrophagous species in France) hastens decomposition of the cadaver, which gradually dehydrates, leaving just skin and bone and bits of dried muscle. Exploitation of these water- and energy-poor remains is difficult and requires special physiological capacities. Certain species specialize in these late phases of decomposition and complete the decay and recycling of the body. Notable examples are the dermestid beetles, such as Dermestes maculatus, which strip flesh from bones, a capacity exploited by forensic anthropologists to clean skulls before examination. During her lifetime each female dermestid may lay up to 800 eggs, which hatch after a time dependent on temperature and moisture level, and in favorable conditions the body is soon host to thousands of beetles.

Contemporary forensic entomology

A complete list of the species and stages associated with body decomposition can be used to estimate the postmortem interval. When this is short (body discovered soon after death), only pioneer species have been able to colonize the cadaver: their larvae are developing. In this case, the principle is to calculate the age of larvae so as to date egg laying. Larval growth rate is mainly a function of ambient temperature: when low, physiological processes stop and development is virtually nil; when high, development speeds up. This temperature–growth rate relation can be used to calculate the age of larvae, and hence the time of egg laying, ie, the minimum postmortem interval (PMImin). This PMImin indicates when the victim was already dead, but not necessarily the time of death. When climatic conditions are unfavorable or the body is inaccessible (in a closed apartment, for instance), it can take several days for the first insects to arrive.

In Europe, the postmortem interval is generally estimated using accumulated degree days (ADDs), a measurement of the thermal energy required for growth and development of an insect, based on 24-hour periods. Forensic entomologists use ADDs to estimate when insects first colonized a corpse and to calculate the minimum postmortem interval. As insects are cold-blooded, their development is affected by ambient temperature and each species has a threshold temperature above which development occurs and below which it stops. In the simplest method of calculating ADDs, the minimum and maximum temperatures for the day are averaged. If this average is above the threshold temperature, the latter is subtracted from the former to give the ADDs for that 24-hour period. For the blowfly Calliphora vicina, for instance, the threshold temperature is 2°C and a day spent at 20°C therefore corresponds to 20–2=18 ADDs. As it has been calculated that 388 ADDs are required for the development from oviposition...
to emergence of this species, *Calliphora vicina* will develop fully in $388/18=21.5$ days at $20^\circ$C. The same reasoning can be applied when the temperature varies. The age of larvae can also be estimated from their length. This method works well and gives a continuous reading of the age of individual larvae, unlike methods based on stages of development. However, larvae tend to contract on sampling, which can falsify the estimation of length, even after death. So they are scalded, which induces them to stretch and they can therefore be measured in a standardized manner.

Dr Marcel Leclercq, a Belgian medical examiner and entomologist, provided numerous examples of dating the time of death using insect colonization. Media-friendly, enthusiastic, an excellent communicator, Dr Leclercq worked for Belgian and French courts until 2005, served as an expert witness in 132 cases, and pioneered the development of forensic entomology in Europe. Kenneth G. V. Smith dedicated his book *A Manual of Forensic Entomology* (1986), which is generally considered as the reference work in the field, to Marcel Leclercq, along with Jean Pierre Mégnin and Pekkta Nuorteva, as “pioneers in the application of entomology to forensic science.”

An exemplar dealt with by Dr Leclercq concerned the case of a dead infant discovered in a house in the Belgian Ardennes on 21 May 1947. Wrapped in a linen cloth, the body was much decomposed and larvae had partially skeletonized the face. Dr Leclercq found *Calliphora vicina* pupae, a dead female, and numerous larvae in the final stages of growth, which he reared before noting the emergence of adult flies on 2 June. On the basis of previous work and the temperature of the house, Dr Leclercq inferred that it had taken 20 days for the formation of the pupae. This meant that the first egg laying had occurred on 1 May 1947 and that the child was already dead at this time. In view of the insects’ restricted access to the body (indoors, swaddled) and the prevailing spring temperatures, Dr Leclercq concluded that the corpse had been placed where it was found in the last week of April, soon after the child’s murder. The suspect’s subsequent confession fully corroborated Dr Leclercq’s conclusions. (The reader will find many more anecdotes and case notes in the fascinating memoirs of Dr Erzinçlioglu).

**Entomological evidence: its limitations**

A complex blend of specialist knowledge and technical expertise is needed to estimate time of death using necrophagous entomofauna. The conclusions of an expert witness can have serious implications in a criminal investigation and should therefore be both reliable and scientifically unambiguous. This is why sometimes it is impossible to estimate the time of death, notably when it occurred several months before and various generations of insects have since colonized the body.
It is therefore hard to find examples of such cases in the literature, as authors usually prefer to report simple cases in which their expert evidence led to accurate and reliable dating. In their *Traité d’Entomologie Forensique*, Wyss and Cherix illustrate this with problematic cases. The first case, which was typical of those involving long postmortem periods (old corpses), started with the discovery in mid-March of the skeletonized body of someone missing since the previous October. Developing larvae were present on what flesh remained. The egg laying was dated to 20 days before the discovery of the corpse, in other words late February. As noted by Wyss and Cherix, this minimum postmortem interval (different from the time of death), although accurate, added little to the investigation.

Wyss and Cherix describe a second case in which the partially skeletonized body of an unknown man was discovered in April 1996 in a low-altitude forest. They identified an empty pupa of *Chrysomya albiceps*, *Piophila foveolata* larvae, and various beetles (Nitidulidae, Histeridae, Dermestidae, and Staphyliniidae). Using Méggnin’s work, which was still frequently used at this time, Wyss and Cherix classified these insects in different waves. In the first (pioneer) wave was *Chrysomya albiceps*, which was especially interesting as this migratory species is only seen in Switzerland in August. Wyss and Cherix concluded that death had occurred in early August. Ten years later, writing in their *Traité d’Entomologie Forensique*, they admitted that they would no longer dare make such affirmations, adding that only Diptera of the Calliphoridae and Sarcophagidae (flesh flies) can help estimate a postmortem interval. The aim of current research in forensic entomology is, therefore, to bolster existing knowledge, but also to discover new methods of expertise.

Forensic entomology: a decision-making aid for experts
A French team has developed the first forensic entomology software, called ForenSeek, using a combination of entomology research and artificial intelligence (www.forenseek.org). ForenSeek constitutes a significant step forward in the analysis of larvae and establishing the time of death. A major difficulty in calculating the age of larvae stems from the use of experimental data. For each species, the relation between temperature and duration of development (ADDs, for example) is determined experimentally and is therefore subject to intrinsic interindividual variability. What is more, in comparisons for a given species, data from different sources vary between experiments. In other words, the data recorded by a researcher in one laboratory are never identical to those recorded by another scientist elsewhere. These variations can be ascribed to the strains used (genetic diversity), the rearing conditions, the reliability of the measurements, and so on. Be that as it may, the expert must come to terms with this variability and is not always able rationally to choose one data source rather than another. In addition, experimentally recorded data on development can never be used directly, but must be modeled (ADDs, for instance).
The choice of data and models can have a more or less marked effect on the estimated timing of egg laying, and so on the expert’s conclusions. It is therefore useful to know how these choices influence the final dating, i.e., to be able to compare estimates made using different data/model combinations so as to assess the resulting differences. Unfortunately, in practice this solution is extremely labor-intensive. ForenSeek was designed to overcome this problem. Developed by researchers as a platform for the sharing of knowledge and expertise, ForenSeek facilitates determination of the age of insects and comparison of calculation.

ForenSeek uses a simple step-by-step process. First, the thermal history is entered, since to calculate the duration of development we need to know the temperatures experienced by the larvae. This leads to the screen for the input of data, such as a stage 3 larva of the species *Lucilia sericata* collected on 2 April 2015. The software then shows for each sample the data available in the database and the user selects the desired development data and model. Once all the sample data have been entered, the calculations begin. For each insect, ForenSeek determines the compatible egg-laying times and provides the results in graphical form. A timeline indicates the date of the first egg laying and compares data differences for a given sample.

Although the ForenSeek software greatly facilitates dating, the results obtained must be interpreted by an expert. Note that the comparison of multiple data sets and modeling methods increases reliability, but lowers precision. So, timing egg laying to within a minute would be very precise, but clearly unreliable. Conversely, egg laying pinpointed to a one-week period would doubtless be reliable, but imprecise.

The challenge then is to be both reliable and precise. The expert must therefore base his or her conclusions not on all data, but only the most relevant. Lastly, the software estimates the time of egg laying, not the time of death, so the expert must reckon the precolonization period, i.e., the time between death and the laying of the first eggs.
Conclusion
Forensic entomology indicates the time of death, but may also reveal events that occurred before or after, such as poisoning or intoxication (entomotoxicology) or the transfer of a body. In addition to criminal investigations, in an archaeological setting, the study of necrophagous insects sheds light on the funerary practices and rites of ancient populations. Necrophagous larvae are also used to clean wounds in maggot (debridement) therapy: live disinfected larvae placed on a lesion eat the necrotic tissue and debride the wound. The larvae also excrete antimicrobial compounds, thus preventing infection, so this is a highly effective, albeit little used therapy. Lastly, myiasis, the presence of maggots on a living person—in a wound or a dirty diaper, for example—indicates poor hygiene and may be suggestive of neglect of a dependent adult or infant.

Necrophagous larvae still have much to teach us. Their primitive social organization (gregarious or aggregation behavior) seems to be a direct response to environmental constraints, and this poorly understood adaptive strategy is a promising and exciting field of research.

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Zoom in on paragraphs for more detailed information if needed.
"Chance favors only the prepared mind."

Pasteur

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Vol 38, No. 2
Pages 133-242
2016