

## Respective Role of the Vein Wall and Valves for the Development of the Varices.

### *Les rôles respectifs des parois et des valvules veineuses dans le développement des varices.*

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*Presented at the Annual Meeting of the French Society of Phlebology November 2010*

#### Summary

Varicose veins are superficial vessels that are abnormally twisted, lengthened, or dilated and are usually caused by inefficient or defective valves within the vein [1].

#### An intriguing question is how do varicose veins form?

The end result is reflux with incompetent valves leading to chronic venous disease (CVD).

Thinking about this process we must ask how the valves become incompetent.

1. Are there structural, biochemical, and physiological abnormalities in the venous wall that become dilated and valvular reflux is an epiphenomenon?
2. Are there changes in the structure of the valves making them “leaky” with progressive reflux that then induce changes in the venous wall and resultant CVD?

The evidence appears to favor the former. There are clinical and basic scientific studies that will help us understand the pathogenesis of varicose vein formation.

**Keywords:** varicose veins, reflux, chronic venous disease, smooth muscle, collagen, elastin, matrix metalloproteinase, hyperpolarization, potassium channels, integrin, receptor, angiotensin, phenylephrine, calcium.

#### Résumé

*Les varices sont des veines anormalement tortueuses, allongées ou dilatées, et secondaires le plus souvent à la présence de valvules incompetentes ou défectueuses à l'intérieur des parois veineuses [1].*

#### **Une question reste intrigante : c'est celle de savoir comment les varices se forment-elles ?**

*Le résultat est-il dû à un reflux dû aux valves incompetentes qui conduisent à l'insuffisance veineuse chronique (IVC) ?*

*Et, dans ce cas, on doit se demander comment les valves deviennent-elles incompetentes ?*

1. Existe-t-il des anomalies structurelles, biochimiques et physiologiques dans les parois des veines qui se dilatent et pour lesquelles le reflux valvulaire n'est qu'un épiphénomène ?
2. Existe-t-il des changements de structure des valves qui commencent à « fuir » avec un reflux progressif, qui à la suite provoque des changements des parois veineuses, ce qui conduit à un IVC ?

*Des preuves existent qui viennent en appui de la première hypothèse. Un nombre plus important d'études cliniques et scientifiques devrait nous aider à comprendre la pathogénèse des varices.*

**Mots-clés :** varices, reflux, insuffisance veineuse chronique, muscle lisse, collagène, élastine, matrice, métalloprotéinase, hyperpolarisation, canaux potassiques, intégrine, récepteur, angiotensine, phenylephrine, calcium.

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Accepté le 22 décembre 2010

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### Duplex ultrasound evidence of an ascending process: valvular dysfunction

*Primary venous reflux can occur in any superficial or deep vein of the lower limb.*

*Venous reflux can occur separate from a retrograde process and the reflux can be local and segmental* [2].

In an interesting study reflux in non-truncal superficial venous tributaries was evaluated in 62 patients (84 limbs) with CVD signs and symptoms (CEAP class 2, 71%; class 3, 14%; class 4, 6%).

None of the patients had evidence of reflux in the truncal saphenous vein, deep veins, perforators or muscular veins, or superficial or deep venous thrombosis.

All patients were evaluated with color flow Duplex US.

In this cohort, the prevalence of tributary reflux was 9.7% (84/860 limbs), and of all tributaries studied 19.9% had reflux.

The most common tributaries with reflux were in communication with the great saphenous vein (65%), small saphenous vein (19%), or with both (7%).

This study concluded that reflux confined to the superficial tributaries occurs throughout the limb, and, importantly, tributary reflux can develop in the absence of reflux in truncal superficial or deep veins or perforator veins [3].

*In support of the concept that venous insufficiency can occur in an ascending (antegrade) progression, a study evaluated the pattern of superficial venous reflux in two distinct groups:*

- 82 patients less than 30 years of age,
- and 183 patients aged greater than 60 years.

In the < 30 year-old group, limb reflux was present in tributaries (25%), non-saphenous superficial veins (36%), and in the saphenous vein (39%).

In the > 60 year-old group, reflux in the saphenous vein was more common (62%), and reflux in the tributaries was frequently associated with reflux in the saphenous vein.

**It was concluded that the presence of saphenous vein reflux is not required for primary varicose vein pathology to occur.**

Also, the different patterns observed in young vs. older patients raise the interesting hypothesis that CVD may progress in an antegrade fashion, starting in the tributaries and affecting the saphenous vein [4].

Thus CVD may begin in essentially any vein segment of the limb and can progress to affect other tributaries, superficial, and perforator veins, as well as have an ascending and segmental pattern that does not need a retrograde axial reflux to cause CVD.

***These concepts would favor that vein wall changes are a precursor to valvular insufficiency.***

### Basic scientific and translational evidence for primary vein wall changes: valve dysfunction as a secondary process

*The observations that venous reflux occurs both segmental as well as in an ascending fashion suggest that changes in the vein wall may take place before valve dysfunction.*

In fact early studies demonstrated that truncal varicosities were present below competent valve, and that normal valves were found between varicosities suggesting that dilation precedes valvular incompetence.

In a study evaluating the collagen and elastin content of non-thrombophlebitic greater saphenous varicose vein and normal appearing saphenous vein in proximity to varicose vein, were compared to normal saphenous veins. The study found an increase in collagen, a significant decrease in elastin, and an increased collagen: elastin ratio in both varicose veins and competent saphenous vein segments in proximal to varicosities compared to normal saphenous vein. This study demonstrated that in patients with varicose veins collagen and elastin changes were present, but importantly normal appearing saphenous vein in proximity to varicose veins also demonstrated connective tissue changes in the venous wall.

***These findings supported that vein wall changes precede valvular insufficiency*** [5].

An interesting study evaluated segments of varicose veins in patients using duplex ultrasound of the greater saphenous vein.

The saphenous vein segments had a dilated varicosity that was proximal to a competent venous valve and adjacent to a normal appearing distal vein segment.

The study evaluated the rigidity of the vein wall, matrix fibers, and elastin in the varicose vein and compared it to the continuous normal appearing vein. It was demonstrated that the rigidity was the same in both the varicose vein and normal vein, and both vein segments had increased matrix fibers and fragmented elastin. It was concluded that the role of venous valve pathology in varicose veins is secondary to the vein wall changes [6].

The same group evaluated the matrix proteins in the vein wall of varicose veins in 372 specimens, and compared them to normal control veins in 36 specimens.

The varicose veins demonstrated a significant increase in wall matrix proteins that included collagen, laminin, and tenascin, and nearly significant fibronectin increase. Importantly, in varicose vein patients normal appearing segments of vein just inferior to the varicose vein had the same biochemical profile as the adjacent varicose vein [7].

***This study provided further evidence that alterations of structural proteins in the vein wall occur in normal appearing veins taken from contiguous segments of a varicose vein, and precede the changes of venous valve reflux during varicose vein formation.***

## **Evidence for vein wall inflammation, Smooth muscle cell dysfunction, Matrix metalloproteinase expression and venous dilation**

***A significant finding in varicose veins is the role of inflammation as a consequence of inflammatory cell infiltrate.***

Several studies have evaluated inflammatory cell and activation in varicose veins and normal control veins. In human saphenous vein specimens from patients with CVD, have demonstrated an increased number of monocytes/macrophage infiltration in the venous wall and valves.

The inflammatory invasion is an important step in the events leading to inflammation, cytokine and proteinase production and structural changes in the vein wall architecture. Elevated intercellular adhesion molecule-1 (ICAM-1), a marker of activation of leukocytes to adhere to the endothelium, has also been detected in CVD vein specimens but not in normal veins [8].

This would suggest that certain predisposing factors and/or stimuli cause the vein endothelium to express ICAM-1 leading to leukocyte activation and infiltration. In fact, a study evaluating patients with venous hypertension, demonstrated that there was sequestration of activated neutrophils and monocytes in the microcirculation. ***This persisted even after elevating the limbs and decreasing the venous hypertension, which indicated that leukocytes were adhering to the endothelium*** [9].

In another interesting study, plasma collected from patients with CVD caused significant granulocyte activation, which was more prominent in advanced stages of CVD (skin changes and ulcer).

In addition, there was increased hydrogen peroxide production from activated granulocytes in the patient's plasma than the control patient plasma [10].

***These data suggested the presence of a circulating activating factor in the plasma of patients with CVD that may be important in the pathophysiology of CVD.***

Immunohistochemical studies of smooth muscle cells cultured from varicose veins were found to have decreased number of cells staining for collagen type III and fibronectin compared to control veins, although the transcriptional products of these two proteins were not dissimilar in varicose veins versus control vein.

In addition, the synthesis and deposition of collagen type III but not type I was significantly lower in varicose veins.

When matrix metalloproteinases (MMPs) -1, -2, and -9, and the natural tissue inhibitors of MMPs (TIMPs) -1 and -2 were analyzed from the supernatant of confluent smooth muscle cells, no differences were observed.

***These data suggested that the regulation for both collagen type III and fibronectin in smooth muscle cells was altered during post-transcriptional events*** [11].

Although there were no differences in MMP and TIMP in the supernatant tested, this did not exclude the possibility that altered expression, activity, and other types of MMPs exist in whole tissues including TIMP.

***Further work in this area demonstrated that varicose greater saphenous vein had a smaller spiraled collagen distribution specifically in the intima and media.***

To investigate the latter findings the same investigators demonstrated that inhibition of MMP with marimastat (BB-2516, non-selective MMP inhibitor) resulted in partial restoration in the production of collagen type III in smooth muscle cells from varicose veins.

In addition MMP-3, which degrades fibronectin, was elevated in both transcription product and protein expression.

It was concluded that the mechanism involved in collagen type III and fibronectin degradation in the smooth muscle cells cultured from varicose veins is likely linked to the expression of MMP-3 [12].

***Type III collagen is important for blood vessels elasticity and distensibility.***

The abnormal production of type III collagen in both smooth muscle cells cultured from varicose veins and fibroblasts cultured from dermal biopsies of patients with CVD raises the possibility that varicose veins pathology may arise from abnormal matrix collagen deposition and is likely a systemic disease [13].

Furthermore, to identify factors involved in the lack of distensibility in varicose veins, a study evaluated the content of hydroxyproline and quantified collagen types I, III, and V.

It was found that in both smooth muscle cells and fibroblast of patients with varicose veins as compared to control, there was an increase in hydroxyproline content, indicating increased collagen; however, the proportion of collagen type III was significantly reduced despite normal mRNA transcript.

***These data offered an explanation for the loss of distensibility in varicose veins, and suggested that the defect is generalized, supporting a genetic basis for the alterations in varicose vein patients*** [14].

Taken together these findings suggest that at least in cultured smooth muscle cells from varicose vein, there is an imbalance of collagen production with dysregulation and increased type I collagen but suppressed type III collagen production.

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Because of normal expression of mRNA for type III collagen the reduction in synthesis is related to post-transcriptional events.

The inhibition of type III collagen synthesis could be a result of degradation/inhibition by MMP-3 and may explain changes in the mechanical properties (elasticity and distensibility) of the vein wall leading to varicose vein formation.

### Matrix metalloproteinases and effects on endothelial and smooth muscle venous function

***A possible explanation for venous dilation and tortuosity may be from the influences of MMPs and TIMPs, which lead to venous wall remodeling and subsequent dilatation and valve incompetence.***

Several authors have found an increased expression, localization, and activity of MMPs in the venous segments of varicose veins and in veins with thrombophlebitis compared to control veins [15, 16, 17].

It is unclear if MMPs are present because of a secondary process due to cellular inflammatory infiltration, or directly involved in the formation of varicose veins. Other studies have investigated the ratio of TIMP-1/MMP-2 and found a three-fold ratio increase in varicose veins compared to normal veins.

***It was concluded that proteolytic inhibition and extracellular matrix accumulation may account for the pathogenesis of varicose veins*** [18].

Animal models of venous hypertension utilizing a femoral artery and vein fistula, have demonstrated an increased venous pressures above 90 mmHg and significant abnormal structural changes in the vein valve and wall.

In addition there was significant expression of MMP-2 and MMP-9 at 6 weeks [19].

In a similar model untreated veins developed venous hypertension with reflux and morphologic changes in the vein wall and valve, but in veins treated with flavonoid (Daflon, reduces inflammation by modulating inflammatory cells) there was reduced physiologic and anatomic changes in response to venous hypertension [20].

***It is possible that MMPs may have acute and chronic effects on the vein wall.***

Early changes may cause functional and metabolic changes to the vein wall, while the later stages may alter wall matrix composition to such an extent where dilation and tortuosity becomes the prominent morphological feature [21].

In recent studies evidence for how MMPs acutely influence venous wall dilation was determined. In a rat IVC model where exogenous MMP-2 was added changes in vein wall relaxation and contraction was recorded.

MMP-2 caused time-dependent venous relaxation.

However, MMP-2 induced venous relaxation was essentially abolished in 96 mmol/L KCl depolarizing solution, which prevents outward movement of K<sup>+</sup> from the cell through K<sup>+</sup> channels.

In order to define which K<sup>+</sup> channels were involved we tested the effects of K<sup>+</sup> channel agonists and antagonists on MMP-2 induced venous relaxation. MMP-2 caused further relaxation of vein segments in the presence of activators of ATP-sensitive potassium (K<sub>ATP</sub>) channel, indicating that MMP-2 was not working through the K<sub>ATP</sub> channel during cell hyperpolarization (a condition of negative membrane potential caused by outward potassium ion movement leading to smooth muscle cell inhibition resulting in venous relaxation).

In contrast, blockade of the large conductance Ca<sup>2+</sup> dependent K<sup>+</sup> channels (BK<sub>Ca</sub>) with iberiotoxin, significantly inhibited the MMP-2 effect on venous relaxation, suggesting that MMP-2 actions in part involve hyperpolarization and activation BK<sub>Ca</sub>.

MMP-2 induced activation of K<sup>+</sup> channels likely causes smooth muscle hyperpolarization, and leads to decreased Ca<sup>2+</sup> influx through voltage-gated channels. Taken together, these data demonstrate novel effects of MMPs on venous tissue function and suggest that protracted MMP-2 induced venous relaxation could lead to progressive venous dilatation possibly influencing the venous wall before changes in the valve occur, and leading to varicose vein formation and CVD [22].

In the same rat IVC and venous isometric contraction apparatus, it was determined that MMP-2 attenuates [Ca<sup>2+</sup>]<sub>e</sub>-dependent vascular smooth muscle (VSM) contraction (by inhibiting Ca<sup>2+</sup> entry into the smooth muscle), without affecting Ca<sup>2+</sup> release from intracellular Ca<sup>2+</sup> stores.

In addition, in an effort to determine the mechanism of MMP-2 induced vasorelaxation, it was found that MMP-2 induced VSM relaxation does not involve the generation of RGD or activation of αvβ3 integrin receptor (RGD contains the Arg-Gly-Asp tripeptide known to activate integrin receptors and lead to membrane hyperpolarization).

***From this study, it was concluded that MMP-2 induced inhibition of the Ca<sup>2+</sup> entry mechanism of VSM contraction may play a role in the venous dilation associated with varicose vein formation*** [23].

From previous data it is known that MMPs are found in the wall of varicose vein [15, 16, 17], and that MMP-2 can cause venous relaxation by hyperpolarization [21].

However, the relation between venous pressure, MMP expression and venous dysfunction is unclear. A study to test the hypothesis that prolonged increases in vein wall tension cause over expression of MMPs and decreased contractility, which in turn promote venous dilation was performed in rat IVC.

The results demonstrated that increases in magnitude and duration of wall tension, was associated with reduced contraction and over expression of MMP-2 and -9 (both are gelatinases). There was a direct correlation between the expression of MMP-2 and -9 with decrease vein contractile function.

Taken together, MMP-2 (as well as MMP-9) promotes IVC relaxation, indicating that protracted increases in venous pressure and wall tension increase MMPs expression, which in turn reduce venous contraction and lead to progressive venous dilation [24].

## Contractile abnormalities in human varicose veins

Although experiments with MMP-2 and human varicose veins and normal controls have not yet been performed, previous studies have evaluated the contractile response in isolated varicose vein segments with variable results [25, 26, 27, 28].

The variability in the results could be related to differences in the vasoconstrictor stimulus used and/or the vein segment examined. For instance, some studies evaluated the effects of norepinephrine which is known to stimulate  $\alpha$ -adrenergic receptor to cause vasoconstriction [26, 27] but could also stimulate  $\beta$ -adrenergic receptors to cause vasodilation, making it difficult to evaluate the specific changes in the  $\alpha$ -adrenergic contractile response in varicose vein smooth muscle.

**Recently the evaluation of the contractile properties of human varicose saphenous vein and varicosities, as well as control saphenous vein was conducted.**

The objective of the study was to test the hypothesis that the different degrees of venodilation in different regions of varicose veins (proximal, distal, varices) reflect segmental differences in the responsiveness to receptor-dependent vasoconstrictive stimuli and/or in the post-receptor signaling mechanisms of vasoconstriction.

The results demonstrated that compared with control veins, different regions of varicose veins display reduced angiotensin II (AngII)-mediated venoconstriction, which may play a role in the progressive dilation in varicose veins. Interestingly, the post-receptor  $Ca^{2+}$ -dependent contraction mechanisms remained functional in varicose veins, as tested by KCl contraction (a receptor independent  $Ca^{2+}$ -dependent response mechanism involving voltage gated channels).

In addition, AngII receptors ( $AT_1R$ ) were the same in varicose veins and control veins, indicating that the decreased AngII constriction in varicose veins is not due to decreased  $AT_1R$  expression, but appears to involve decreased sensitivity of  $AT_1R$ -mediated contractile signaling pathways.

Because the AngII contractile abnormalities were also seen in the distal part of the great saphenous vein, which on inspection, the veins were neither dilated nor tortuous and appear normal; **this finding would support the hypothesis that venous wall changes precede valvular insufficiency.**

In addition, the study found that there was a maintained  $\alpha$ -adrenergic response (using phenylephrine which is a purely  $\alpha$ -adrenergic agonist) in the distal and varix segments of the varicose vein, but a reduced constriction in the upstream proximal varicose segments, indicating further variations in venous response to contractile agonists.

The study concluded that AngII is an important contractile agonist in venous physiology which is disrupted in varicose vein, and that the maintained  $\alpha$ -adrenergic response may represent a compensatory adaptation of human venous smooth muscle to facilitate venous return from the dilated varix segments of varicose veins to the deep veins and saphenofemoral junction.

**Furthermore, the results highlight the importance to explore a potential relation between changes in the renin-angiotensin system and the incidence of varicose veins.**

**The results also suggest that genetic manipulations or pharmacological tools to enhance the activity of venous  $AT_1R$  may represent a potential approach in the management of varicose veins** [29].

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