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The Complex Structure of the Smooth Muscle Layer of Spermatic Veins and Its Potential Role in the Development of Varicocele Testis

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Abstract

Objectives: Varicocele, a dilatation of the pampiniform venous plexus, is considered to cause male infertility. The exact mechanism of varicocele development is not clarified yet. This study focused on the structure of varicocele veins, compared with normal spermatic veins, and its potential role in varicocele development.

Methods: Morphologic and immunohistochemical studies using antibodies against vWF and neurofilament-200 (NF-200) were performed on spermatic vein fragments of 20 varicocele patients and 40 normal spermatic cords. Casting preparation of veins was performed on five normal spermatic cords.

Results: Casting preparation frequently revealed circular constrictions of normal spermatic vein lumina. Histologic evaluation showed a strong longitudinal smooth muscle layer in the adventitia of large veins in addition to the circularly organised tunica media. Serial sections showed smooth muscle fibres branching from the outer longitudinal into the inner circular layer. Immunostaining for vWF revealed high vascularisation of this outer layer. Interestingly, the number of nerve fibres marked by NF-200 immunostaining was considerably higher in large veins compared to the testicular artery. The longitudinal smooth muscle layer was significantly degraded in the presence of varicocele grades I and II, and did not even exist in varicocele grade III. Correspondingly, the number of vasa vasorum and nerve fibres was reduced in varicocele veins.

Conclusions: Our data show a complex smooth muscle organisation of spermatic veins, which serves the basis for a contractile mechanism, providing an effective blood transport through pampiniform plexus. This mechanism is obviously damaged in the varicocele. Molecular processes behind this impairment remain to be clarified.

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1. **Introduction**

Varicocele is a pathologic dilatation of the pampiniform venous plexus of the spermatic cord and occurs in approximately 15–20% of males and in up to 40% of infertile males [1,2]. This condition occurs most frequently on the left side and can cause decreased testicular function [2,3]. There are several theories on the etiology of varicocele, ranging from anatomic reasons, including the angle at which the left testicular vein enters the left renal vein, to functional deficiencies regarding the lack of valves at the juncture of the testicular vein and renal vein [4]. Despite the existing studies and ongoing research, the mechanisms of varicocele development remain controversial and are not fully understood. Also, still controversial is the impact of surgical or radiologic intervention in varicocele treatment [5–7]. Several researchers have shown that only a few or even no valves exist in the spermatic veins [8–10], raising the question how the venous return can be ensured and if impairment of this mechanism can possibly lead to dilatation of the spermatic veins. The dilatation of spermatic veins can be assessed by the Valsalva maneuver, and ultrasound examination enables an exact determination of vein diameter [11]. To date there have been only a few studies on the microscopic structure and histology of normal spermatic veins and varicocele veins. In a former study we demonstrated that the pampiniform venous plexus consists of veins with different diameters and different vein wall anatomy [12]. However, there is no detailed analysis considering these differences in spermatic vein anatomy and comparing them with varicocele anatomy.

The aim of this study therefore was to evaluate whether there are anatomic and structural differences between normal spermatic veins and varicocele veins, and whether these differences might serve as a basis for an explanation of the development of varicocele.

2. **Methods**

2.1. **Patients and tissue samples**

Fragments of human spermatic veins were obtained from 20 patients (5 patients with varicocele grade I, 5 patients with grade II, and 10 patients with grade III) aged between 13 and 44 yr and undergoing antegrade sclerotherapy for left-sided varicoceles [13,14]. The grades were classified clinically according to Dubin and Amelar: grade I (palpable only during the Valsalva maneuver), grade II (palpable in standing position), grade III (visible without palpation). In addition, venous reflux was proven by CW-Doppler sonography in each patient to confirm the clinical diagnosis.

Normal spermatic veins of the left spermatic cord were obtained from 40 patients aged between 39 and 78 yr with prostate carcinoma undergoing orchietomy. Patients with varicocele or with hormonal pretreatment were excluded from this group. The material was obtained from temporal patients after informed consent. The study was approved by the ethics committee.

During the procedure of antegrade scrotal sclerotherapy, which has been described previously in detail [14], fragments of the dissected veins of the pampiniform plexus measuring 3–5 mm in length were preserved before injection of the sclerosing agent. These veins are embedded characteristically in a yellowish fat opposite the spermatic cord. A similar vein dissection was performed in prostate carcinoma patients before scrotal orchietomy. Tissue blocks of the whole normal spermatic cords were embedded in paraffin, and sectioned for histologic and immunohistochemical analyses. Tissue pieces were fixed in Bouin’s solution for paraffin embedding and in 5.5% phosphate-buffered glutaraldehyde for Epon 812 embedding.

2.2. **Casting preparation**

Casting preparation is a suitable method to gain an overview of the vascular organisation. Similar to our previous studies, in five cases, after rinsing with phosphate-buffered saline, the spermatic cord was cut close to the testicular hilus, and the distal end of the spermatic cord was clamped. The proximal end of a vein was then cannulated, and Mercox-B (blue) was injected through this cannula into the venous system [12]. The tissue was subsequently kept at room temperature to allow the polymerisation of the Mercox. The tissue was then immersed in a 35% KOH solution in which it remained for 48 h at a temperature of 80 °C, until maceration was complete and the vascular skeleton became visible. The tissue was rinsed with water several times to detach the tissue remnants. The venous net thus obtained was studied with a macroscope (Leica, Bensheim, Germany) and photographed.

2.3. **Light microscopic analyses**

To analyse the vascular structure by light microscope, in 40 cases of normal spermatic cord tissue and 20 cases of fragments of spermatic veins of varicocele patients, we stained paraffin sections with classic hematoxyline-eosine procedure. In 20 cases of varicocele vein fragments and in 12 cases of spermatic cord tissue, semi-thin sections from Epon 812-embedded tissue pieces were obtained and stained with toluidine blue-pyronine [15,16]. Light microscopic evaluation was performed with the use of a Leica microscope (Leica DM/LB) equipped with a digital camera (Leica DC 320). The first aimed comparison of diameter of the veins by light microscopic analyses was not usable for distinguishing large and small veins, since varicocele veins, in particular, exhibited big differences in diameter within the same vein. On the basis of the presence or absence of the outer smooth muscle layer, the spermatic cord veins were subclassified into two groups: large veins presenting an outer smooth muscle layer and small veins, since varicocele veins, in particular, exhibited big differences in diameter within the same vein.
veins without such a layer. We measured relative thickness of the outer smooth muscle layer of three veins on each section respectively.

2.4. Immunohistochemistry

To determine whether there are differences between normal and varicocele veins regarding vasa vasorum and nerve supply of the vascular wall, we performed immunohistochemical studies using antibodies against von-Willebrand-Factor (vWF) (DAKO, Glostrup, Denmark) and neurofilament-200 (NF-200) (Sigma, St Louis, MO, USA) on paraffin-embedded tissue sections of the normal spermatic veins and varicocele veins. Tissue specimens were fixed in Bouin’s solution for 24 h at 4°C and further processed for embedding in paraffin. Immunostaining using the two antibodies and controls were performed as described recently [15,16]. Particularly the vascularisation and the nerve supply of the large veins presenting an outer smooth muscle layer were compared.

3. Results

3.1. Spermatic veins of large diameter exhibit frequent constrictions of their lumina

The large spermatic veins visualised by means of cast preparations showed constrictions of their lumen frequently (Fig. 1A), although venous valves were either not present or spaced at longer intervals. Longitudinal histologic sections through the wall of large spermatic veins confirmed these constrictions (Fig. 1B). To analyse their morphologic basis, we performed detailed microscopic and immunohistochemical analyses of the venous wall.

3.2. Large spermatic veins of the pampiniform plexus consist of a special and complex venous wall structure

Sections of normal veins of the pampiniform plexus stained with hematoxyline-eosine revealed two main types of spermatic vein wall assembly. Spermatic veins with a large diameter showed the following anatomic structure: the intima of the vein wall consisted of an endothelial layer and subendothelial muscle pad. In addition to the circular smooth muscle layer of the tunica media, there were strong smooth muscle bundles arranged longitudinally in the adventitia (Fig. 2A). The wall of large spermatic veins appeared stronger than the vessel wall of the artery (data not shown). Tissue pieces embedded in Epon 812, cut into semi-thin sections (1 μm thick), and stained by toluidine blue-pyronine confirmed the observations on paraffin sections and allowed a detailed evaluation of the muscle layers of large spermatic veins. These sections revealed oblique running muscle fibres branching from the outer longitudinal smooth muscle layer into the inner circular smooth muscle layer of the venous wall (Fig. 2B). Longitudinal semi-thin sections through the venous wall clearly demonstrated outer smooth muscle fibres, which branch off into the inner smooth muscle layer interlacing the inner and
outer smooth muscle layers (Fig. 2C). The drawing (Fig. 2D) summarises our findings and shows the relation between both muscle layers of large spermatic veins. The constrictions were primarily seen at sites where the muscle fibres of both layers come together (Fig. 2D). Small spermatic veins were classically structured with no additional smooth muscle layer in the adventitia. Both venous groups only rarely exhibited venous valves.
3.3. Degradation or absence of the outer longitudinal smooth muscle layer in varicocele veins of different grade

Detailed morphologic studies on tissue sections from the fragments of veins obtained from varicocele patients (Fig. 3A) revealed degenerative structural changes within the wall of all studied veins in comparison with the structure of normal spermatic veins, which did not show these changes. These studies demonstrated that the changes, which preferentially concerned the outer smooth muscle layer, correlated with the degree of varicocele testis. In cases of varicocele grade I, the thickness of the smooth muscle layer was reduced by 30–40%, and in cases of varicocele grade II by 45–60% in comparison with normal veins. Large spaces between smooth muscle bundles (Fig. 3B and C) were found. In varicocele grade III, the outer smooth muscle layer was mostly reduced by 70–80%, but in some cases it was almost not detectable as a complete layer. Furthermore, the inner smooth muscle layer of the tunica media was affected by the degenerative process, particularly in varicocele grade III (Fig. 3D). The whole extent of degenerative vein

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Fig. 3 – Semiquantitative evaluation of the degenerative changes in varicocele veins demonstrates that all varicocele veins studied (n = 20) exhibit structural changes of their venous wall (A). The following two cross-sections of veins (B, C), which had been taken out during antegrade sclerosing of varicoceles grades I and II, reveal a degraded outer muscle layer compared (OM) with normal veins. Another venous cross-section demonstrates changes of varicocele grade III with only a few outer smooth muscle bundles (OM) and an additional degradation of the inner muscle layer (IM) of the tunica media (D). (Hematoxyline-eosine; ×250).

Two semi-thin sections from veins of varicocele grade I (E) and III (F) document the gradual differences in the degree of degenerative changes of the venous wall: the spectrum of changes ranges from low-grade changes of the outer smooth muscle layer (OM) (E) to an intense degeneration of the two smooth muscle layers of the venous wall (OM, IM) (F). (Toluidine blue-pyronine; ×250).
wall changes of large spermatic veins became even more evident in semi-thin sections (Fig. 3E and F). Whereas in light changes (varicocele grade I) the reduction of the spermatic venous wall thickness resulted primarily from a degradation of the outer smooth muscle layer (Fig. 3E), both smooth muscle layers were affected in high-grade changes (varicocele grade III) (Fig. 3F). In these cases the venous wall was reduced in such a way that the venous wall assembly could hardly be appreciated (Fig. 3F). The normally observed smooth muscle branches from the outer into the inner smooth muscle layer could barely be identified in veins of varicocele grade III (Fig. 3F).

3.4. **Vascularisation and innervation of the outer longitudinal muscle layer of spermatic veins and varicocele veins**

To analyse the vascularisation and innervation pattern of the spermatic vein wall, we visualised blood vessels by immunostaining for the endothelial marker vWF and marked nerve fibres by immunostaining for NF-200. Immunohistochemistry for vWF on paraffin sections of normal human spermatic cord demonstrated, in a semiquantitative evaluation, a higher number of vasa vasorum within the strong longitudinal smooth muscle layer in the adventitia of the wall of large veins (Fig. 4A) compared with those

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**Fig. 4 – Immunohistochemistry of human spermatic cord tissue for vWF (endothelial marker) shows the following.**

- The outer smooth muscle layer of the venous wall (V) (A) exhibits a much higher vascularisation (arrowhead) than the wall of the artery (Art) (B).
- Small veins (sv) of the spermatic cord near the artery do not show an outer longitudinal smooth muscle layer (B) (×250).
- Immunohistochemical staining for NF-200 reveals, in comparison with normal spermatic veins (C), a gradual reduction of nerve density (arrows) in the wall of varicocele veins (D–F) (×400).
of the wall of the testicular artery (Fig. 4B). Neighbouring small veins showed only a circular smooth muscle layer in the tunica media (Fig. 4B). Immunostaining for NF-200 revealed a higher number of nerve fibres within the wall of the large veins (Fig. 4C) compared with that of the testicular artery wall (data not shown). The number of both the vasa vasorum (data not shown) and nerve fibres (Fig. 4D–F) within the vein wall, particularly within the outer longitudinal smooth muscle layer, was significantly reduced in varicocele veins. Interestingly, the reduction of nerve fibre density seemed to correlate with the degree of degenerative changes (Fig. 4D–F).

4. Discussion

The present data show, to our knowledge for the first time, the exceptional anatomic structure of large spermatic veins of the pampiniform plexus composed of a strong longitudinal smooth muscle layer in the tunica adventitia with established oblique running muscle fibres reaching the inner circular smooth muscle layer of the tunica media. This assembly of muscle layers probably leads to a mechanism for venous blood transport similar to peristaltic waves. We postulate that the coordinated concurrent contraction of both muscle layers builds an important basis for the venous return, which may not be solely explained by the existence of spermatic venous valves that exist only in a low amount according to our studies or were sometimes not even found in previous studies [9,10]. The distinct amount of blood vessels and nerve fibres in the outer smooth muscle layer supports our hypothesis and probably serves to allow adequate contraction of the smooth muscles. Decrease of vasculature and nerves as it occurs in cases of varicocele might be associated with the degraded longitudinal smooth muscle layer in varicocele veins and loss of contractile function. Whether the degradation of the smooth muscle layer or the reduction in vasculature and nerve fibres comes first cannot be decided at this time. To determine whether the degradation of the outer smooth muscle layer, the reduction of vasa vasorum density, and the reduction of nerve density in varicocele veins correlate with each other and the grade of varicocele testis, it is necessary to perform additional studies on a higher number of normal and varicocele spermatic veins.

The existence of an additional muscle layer as we observed in all cases of normal large spermatic veins is known in veins of the lower leg or foot as it has been illustrated in several anatomic teaching books. The reason for such an additional supporting contractile structure may be the high hydrostatic pressure to which the veins of this part of the body are exposed. Analogous to this observation, it can be assumed that spermatic veins are also exposed to a high pressure caused by existing and haemodynamic active arteriovenous anastomoses [12]. In addition, the enormous increase of blood volume to be passed through the spermatic veins after adolescence may also contribute to venous failure leading to reflux and varicocele testis [17–19]. It would be of value to clear structural and molecular similarities and differences between the veins of varicocele testis and leg varicose veins by future studies.

Although there is an age difference between the studied normal population and varicocele patients, we assume that this probably has no influence on the observed structural particularities, since the vein structure of varicocele grade I is more similar to normal structure than varicocele II and III. Also we do not see any changes between the normal veins of the younger and the normal veins of persons with high age. Taken together these observations may suggest that the outer smooth muscle layer is an essential structural component of the spermatic cord vein wall and does not change substantially in higher age. Our data show clearly that mainly the longitudinal smooth muscle layer is degraded or completely missing in the presence of varicocele testis and that this degradation apparently correlates with the degree of varicocele testis. The impairment of the anatomic architecture and subsequently of oblique muscle fibers connecting the longitudinal layer with the tunica media, as we observed in all studied venous fragments from varicocele veins, might be the cause of venous insufficiency with venous reflux, an important sign in the clinical diagnosis of varicocele testis [6,11]. However, we are not able at this time to determine whether such a structural impairment reflects the consequence of varicocele or the cause of it.

In a few scientific publications a thickening of the smooth muscle layer of the internal spermatic vein or even an acquired development of a longitudinal smooth muscle layer found in patients with varicocele has been described [3,20]. This finding cannot be confirmed by our extensive analyses on varicocele vein tissue. We basically see the opposite. The existence of a longitudinal smooth muscle layer in normal large spermatic veins can be appreciated in basic anatomy textbooks and atlases. We can approve the existence of normal small spermatic veins with a missing longitudinal smooth muscle layer, but these veins are not the ones primarily involved in varicocele.
development. The large spermatic veins are the veins responsible for the main venous backflow into the testicular and renal vein. Our results clearly show a defective smooth muscle assembly in varicocele veins with a rarefied longitudinal smooth muscle layer and missing extensions into the circular muscle layer, probably leading to a reduced venous backflow. This mechanism may improve our understanding regarding the development of varicocele and provide new potential in this research area concerning the molecular basis for the venous defect. Whether a primary disturbance of the angiogenic vascularisation or innervation of the vein wall (vasa vasorum) is involved in these anatomic changes of the varicocele veins needs further analyses.

5. Conclusions

Our data show a complex structure of human spermatic vein organisation composed of an outer smooth muscle layer organised longitudinally to the vein wall in addition to the normally existing circularly organised smooth muscle layer of the tunica media. Both muscle layers are connected by oblique smooth muscle pads. We hypothesise that this complex vein wall structure contributes to the venous transport mechanism in the pampiniform plexus. This contractile function apparently is disturbed in varicoceles by morphologic changes of the venous wall that may lead to impairment of blood return of the veins, promoting the development of varicocele.

Conflicts of interest

There are no commercial relationships such as consultancies, stock ownership or other equity interests, patents received and/or pending, or any commercial relationship that might be in any way considered related to this article to disclose.

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Editorial Comment
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This paper demonstrates the anatomic structure of the veins of the pampiniform plexus and the changes that occur during varicocele formation.

The normal structure, as demonstrated by light microscopy and casting, is composed of intricate layers of smooth muscle with the outer longitudinal fibres interlacing with the inner circular layer to form "venous constrictions." With the rich vascular and neural network within the muscle layers it is likely that the muscular network plays an important peristaltic role to enable venous return.

In the varicocele veins there is a progressive degeneration of the outer and then inner smooth muscular layers with the loss of the intricate interlacing and a reduced vascular and neural network, with the end result being venous dilation and reflux.

As stated in the paper, it is difficult to know whether the pathologic findings are the cause of the venous reflux or are the end result of it. In varicocele veins venous hypertension and reflux are found, similar to those found in varicose veins of the leg [1]. The resultant structural degeneration that occurs in the wall of the saphenous is also likely to occur in the veins of the pampiniform plexus [2].

Many research reports suggest that there are fewer valves in varicocele veins and that this is the original cause of the reflux [3]. Are the venous constrictions caused by the muscular arrangement the "valves" that are depicted by radiologic studies? As the venous constrictions become less prominent with varicocele development, this then may explain why fewer valves are seen.

This paper demonstrates the normal and pathologic anatomic features beautifully, but we hope the follow-up study will determine whether the venous hypertension or the muscular degeneration is the primary pathology.

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