Annals of Anatomy xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.de/aanat

Invited Review

# Distribution of orientation of smooth muscle bundles does not change along human great and small varicose veins

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ARTICLE INFO

*Article history:*
Received 25 June 2013
Received in revised form 22 October 2013
Accepted 22 October 2013
Available online xxx

*Keywords:*
Blood vessel
Histology
Great and small saphenous veins
Stereology
Tunica media
Volume fraction

SUMMARY

Wall remodeling in varicose veins is associated with hypertrophy of subendothelial tissue, increase in inner diameter, wrinkling and invagination of the endothelial layer. Due to structural alterations of the wall, the smooth muscle cells (SMCs) change their original circular and longitudinal orientations. Our aim was to quantify the volume fraction of circularly, longitudinally and obliquely oriented SMCs within both the inner and outer half of the wall of 11 great saphenous varicose veins and five small saphenous varicose veins. Using stereological methods applied on cross-sections of the vessels regularly gained each 5 cm along the vessel we determined the wall thickness ($846 \pm 319$ μm, mean ± standard deviation), the volume fraction of circular SMCs in the inner ($0.19 \pm 0.13$) and outer ($0.06 \pm 0.06$) layers, the volume fraction of longitudinal SMCs in the inner ($0.06 \pm 0.05$) and outer ($0.05 \pm 0.04$) layers, the volume fraction of oblique SMCs in the inner ($0.15 \pm 0.08$) and outer ($0.09 \pm 0.08$) layers, and the total volume fraction of SMCs in the inner ($0.4 \pm 0.1$) and outer ($0.21 \pm 0.09$) layers. The volume fraction of SMCs with circular and oblique but not with longitudinal orientation was greater in the inner layer compared to the outer layer. The SMC orientation distribution was uniform along the varicose saphenous veins. With increasing wall thickness, the volume fraction of longitudinal and oblique SMC bundles increased in both layers at the expansion of circular SMC bundles. The main differences in the orientation of the SMCs in the inner and outer wall layers should be taken into account when computational modeling of varicose saphenous veins is attempted.



**Contents**



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http://dx.doi.org/10.1016/j.aanat.2013.10.005

## 1. Introduction

Varicose veins are pathologically elongated, over-distended, tortuous veins with insufficient valves (Standring, 2004; Lim and Davies, 2009). In humans, varicosities mostly affect the great saphenous vein (GSV), while only 8–15% of the cases affect the small saphenous vein (SSV) (Almgren and Eriksson, 1990; Ravi et al., 2006, 2009; see also Beebe-Dimmer et al., 2005).

The pathogenesis of this vascular disease involves changes in extracellular matrix metabolism (Woodside et al., 2003; Lim and Davies, 2009; Oklu et al., 2012), in the endothelium (Lim and Davies, 2009), in smooth muscle cell (SMC) activity (Lim and Davies, 2009), and in adventitial vasa vasorum (Kachlík et al., 2003, 2007, 2008). Structural changes within the vessel wall developing due to these processes seem to be the primary cause of over-distension of the veins and the typical clinical picture of varicosities (Elsharawy et al., 2007; Lim and Davies, 2009; Oklu et al., 2012).

The wall of healthy blood vessels consists of three concentric layers: tunica intima as the innermost layer, tunica media, and tunica adventitia (Standring, 2004). In veins, these layers are less well defined than in arteries. The intima of larger veins consists of endothelium and a thin subendothelial connective tissue layer with one or more incomplete elastic lamellae. In the media, a prominent outer circular (Liu and Fung, 1998; Liu, 1998) and a thinner inner longitudinal SMC layer can be found. Oblique SMC bundles are scarce (Woodside et al., 2003). The connective tissue of the adventitia is well developed (Gallagher, 1992; Elsharawy et al., 2007). Saphenous veins of adult persons often show further histological changes without any clinical manifestations. These changes include a thickened intima with increased collagen content, elastic fibers and enmeshed SMCs, hypertrophy of longitudinal and circular muscle layers, increased connective tissue content in the tunica media, and, sometimes, formation of a third longitudinal muscle layer between the circular muscle layer and adventitia (Milroy et al., 1989; Gallagher, 1992; Elsharawy et al., 2007).

When compared to the normal vein, varicose veins show an increased diameter of the lumen and hypertrophy of the wall (Wali and Eid, 2002b; Elsharawy et al., 2007). The intima is considerably thickened, and its surface can be enlarged and invaginated with a sometimes discontinuous endothelium (Wali and Eid, 2002b; Elsharawy et al., 2007). SMCs of varicose veins may lose their fusiform shape and are often separated from each other by a marked increase in connective tissue in which many of the collagen and elastin fibers lose their normal structural arrangement (Travers et al., 1996; Renno et al., 2006). SMC density as well as elastin density decreases in comparison to that of healthy veins (Naim and Elsharawy, 2005; Wali et al., 2003; Wali and Eid, 2001). However, the absolute amount of SMCs can be higher in the walls of varicose veins compared to those of healthy veins.

SMCs can change their orientation in response to changes in the direction and intensity of loading or to the damage followed by regeneration of the tissue (Liu and Fung, 1998; Liu, 1998). Because the tonus of SMCs is responsible for the active response of the vein to distension due to loading (active tonus; Travers et al., 1996; Wali et al., 2003) and because the generated force is influenced by the amount and orientation of SMCs in the vessel wall (Holzapfel and Gasser, 2001), it would be very helpful to know the actual orientation of SMCs in varicose veins. Sophisticated biomechanical models of blood vessels (Holzapfel and Gasser, 2000) should be based on the morphometry of real tissue samples.

To our knowledge, there are no studies describing the distribution of SMC orientations in varicose veins. Therefore, our aims were (i) to determine the volume fraction of circularly, longitudinally, and obliquely oriented SMCs within the inner and outer layers of human varicose saphenous veins and (ii) to assess the relations between populations of SMCs with different orientations and wall thicknesses.

## 2. Materials and methods

### 2.1. Specimen preparation

The present analysis is based on the samples used in our previous study on vasa vasorum in human varicose saphenous veins (Tonar et al., 2012). In total, 11 great saphenous veins (GSVs) and 5 small saphenous veins (SSVs) were collected from 12 patients (six male and six female patients) who were undergoing surgery due to chronic venous insufficiency of their saphenous veins and fixed with buffered formalin immediately after their removal. Informed consent was obtained from all patients prior to surgery. The collection of saphenous vein samples for this study was approved by the Ethics Board of the University Hospital in Pilsen and the Faculty of Medicine in Pilsen, Charles University. The patients were 39–67 years of age ($50.2 \pm 10.6$, mean $\pm$ standard deviation). All patients except one were non-diabetics; the diabetic patient had well-compensated diabetes mellitus. Four patients were being treated with antihypertensive drugs. According to the CEAP classification based upon clinical class (C), etiology (E), anatomical distribution of reflux and obstruction (A), and the underlying pathophysiology (P) (Meissner et al., 2007; Partsch, 2009), 6 patients were classified as CEAP clinical class 2 (simple varicose veins only), and six patients were classified as CEAP clinical class 3 (ankle edema of venous origin).

Only veins without apparent ruptures caused by the stripping technique were analyzed. All of the varicose veins were divided into 5-cm-long segments in the proximo-distal direction. The length of the stripped veins ranged from 15 to 40 cm. Each vein was thus represented by 3–8 segments. A 1-cm-long tissue sample used for histological processing was taken from each segment. All tissue samples (92 in total) were dehydrated in graded ethanol solutions and embedded separately into 92 paraffin blocks. All tissue blocks were cut transversally; i.e., perpendicular to the axis of the vessel, into 5-μm-thick sections, mounted onto glass slides and stained with hematoxylin–eosin. Due to the irregular shape of the whole veins, the perpendicular cutting direction was assessed separately in each of the 5-cm-long segments. We did not use any special devices for embedding and the local orientation of the perpendicular cutting plane was done subjectively during the histological embedding. One section per segment was analyzed.

### 2.2. Microscopic quantification

Four micrographs ($2288 \times 1712$ pixels) per histological section were taken using a $10\times$ objective to include the whole vessel wall into quantification. The magnification was sufficient to clearly reveal the orientation of the SMCs.

The inner and the outer boundary of the vessel wall were marked manually on each micrograph with a Polygon tool plugin of the Ellipse software (ViDiTo, Košice, Slovakia). The cross-sectional area was then automatically divided by the software into two virtual

Please cite this article in press as: Kochová, P., et al., Distribution of orientation of smooth muscle bundles does not change along human great and small varicose veins. Ann. Anatomy (2013), http://dx.doi.org/10.1016/j.aanat.2013.10.005