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Histological analysis of forearm superficial veins structure

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Background: *The connection between the basilic and cephalic veins of the forearm shows considerable interindividual variation. Depending on its form, the most common types of venous connections are M-, N- or Y-shaped. This study aims to compare the metric traits of the basilic and cephalic veins and the relative content of smooth muscle/collagen fibres/elastic fibres in their walls and to determine the differences between the forearm venous systems.*
Materials and methods: *The study was performed on 42 veins collected from 26 deceased individuals between the ages of 19 and 50 years. Vein sections were fixed, embedded in paraffin blocks and used to prepare histological slides, stained according to pentachrome Movat's method. Venous metrics were assessed and the percentage of muscle, elastic and collagen fibres was determined using the Trainable Weka segmentation. Statistical analysis compared the M-type vein with the Y- and N-types, which were combined into one category.*
Results and Conclusions: *Analysis showed a greater tunica media thickness in the M-type vein, with a greater lumen circumference in the Y/N types. Correlation analysis showed a correlation of vein metrics with elastic fibre content and a weak inverse correlation with the tunica media thickness. It can be hypothesized that the increased performance of N- and Y-types may be related to elastic fibre content.* (Folia Morphol 2024; 83, 2: 374–381)

Keywords: superficial veins, histology, segmentation, microanatomy

INTRODUCTION

Large venous vessels, especially superficial ones, show considerable morphological variation in calibre, shape, the number of tributaries and architecture. In particular, the superficial veins of the upper limb show several morphological and individual differences in this respect [14, 15]. These veins two systems, superficial and deep, connected by numerous vessels penetrating the fascia. Blood can therefore use both systems equally or with a predominance of one of them. For example, during hard work, thickened muscles compress the deep veins and obstruct blood flow, in which case more blood flows through the cutaneous veins [6].

Venous system between the *basilic* and *cephalic* veins of the forearm shows considerable interindividual variation. Depending on the shape, the most common venous connection types are M-, N- or

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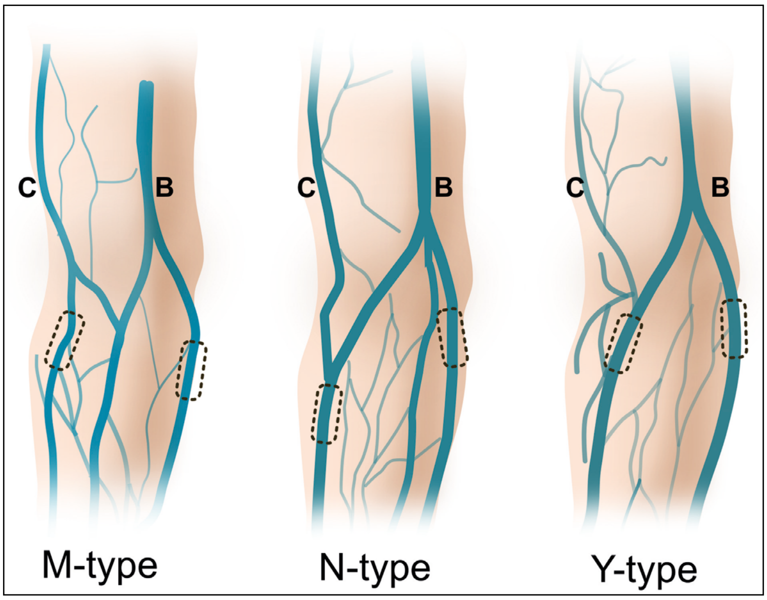

Figure 1. Superficial veins types divided into three categories: Y, N and M. The most prominent veins resemble letters, which are assigned to particular types. Cephalic vein is marked with letter C and basilica — with letter B. Dotted lines shows approximate material sampling location (from the upper part of the cubital fossa, above the junction).

Y-shaped [12, 19], as shown in Fig. 1. In addition to this classification, other more detailed classification systems (dividing the connection systems into eight types) are discussed in more detail elsewhere [29].

The forearm veins system is influenced by genetic and environmental factors or physical activity [1, 3]. One of the main factors influencing the variability of venous architecture seems to be the type of the work performed. Physical effort, in particular one of its forms — athletic training — is one of the crucial factors shaping the morphological structure of the body [5, 7]. The relationship between the efficiency of venous outflow from the upper extremity in relation to the architecture of the venous system and the velocity and force parameters of the extensor and flexor muscles is also important and not fully understood. According to one theory, type Y is more likely to form in people who work physically, as it is described by the lowest resistance to blood flow through the vessels forming the Y-type anastomosis. This is an example of the morphological and functional adaptation of the circulatory system. Hard-working muscles, richly vascularized by the arterial network, require a very efficient outflow system [11].

Histologically, veins are composed of three layers: the inner, i.e. *tunica intima* (composed of epithelium and basement membrane), the middle, i.e. *tunica media* (composed of smooth muscle cells, elastic and collagen fibres) and the outer, i.e. *tunica adventitia/ /externa* (containing collagen and circular elastic fibres, residual smooth muscle cells and vasculature) [16]. Of these, the middle layer is the most diverse [26].

Understanding the course of forearm venous connections is of clinical importance; historically, interindividual variability in this system has influenced the efficacy and complications of phlebotomy (bloodletting). They are currently used as common venous access in manufacturing dialysis ports [23]. Superficial forearm veins can also serve as material for autologous grafts [8], especially when the veins of the lower limbs show pathological alterations: varicose veins, thrombosis or venous insufficiency of the lower limbs [13, 18]. In this context, a detailed analysis of veins typologies can help to make specific clinical decisions [25].

There have been studies on the course and macroscopic characteristics of forearm superficial vein systems [2, 23]. However, to our knowledge, no studies have been published on histological differentiation of the forearm superficial veins.

This study aims to compare the metric traits of the basilic and cephalic veins and their relative smooth muscle/collagen fibre/elastic fibre content and to determine the differences between the forearm venous system types.

MATERIALS AND METHODS

Material acquisition

The study material consisted of 42 basilic and cephalic veins collected from 26 deceased individuals at the Department of Forensic Medicine, Wrocław Medical University. Only male cadavers aged between 19 and 50 were eligible for the study. Individuals with late post-mortem lesions, extensive injuries to the upper limbs or in the cubital fossae, including traces of intravascular insertions, were excluded. Due to the need to comply with formal requirements, it was also necessary to establish the identity of the deceased. In accordance with the Polish law, prior to each procedure, the position of the court or prosecutor supervising the autopsy regarding the acceptance of the procedure was obtained. It was established that the person from whom the material is to be taken had no objection during his lifetime. During the autopsy, the height and weight of the deceased were measured, and the type of anatomical structure was determined, dividing the subjects into pyknic, athletic and asthenic groups.

In addition to baseline parameters (age, height, weight), arm and forearm circumferences, subcutaneous fatty tissue thickness at the maximum circumference of the arm and venous type (divided into Y/N/M//unspecified [11]) were determined for each decedent. After identification of the basilic and cephalic veins, approximately 2 cm sections of these vessels were taken from the upper part of the cubital fossa (above the junction). Along with the vein, a small layer of surrounding soft tissue was also extracted, ensuring that the vein was not damaged during extraction.

Venous system type was determined after a thorough assessment of the anatomy of the region by dissection of the cubital fossa. Limb selection (right or left) was based on arm and forearm circumferences, with the assumption that the dominant limb should have larger circumferences than the non-dominant limb. In the case of identical arm and forearm circumferences, the right-handedness of the deceased was considered statistically more likely.

The dissection involved a vertical incision from the proximal third of the upper arm to the distal third of the forearm on their anterior surfaces, passing through the middle of the cubital fossa to the depth of the dermis only. Subcutaneous tissue was then peeled away laterally at the edge of the dermis to expose the superficial venous system. Immediately after collection, the sections were placed in sealed and pre-labelled containers filled with a 4% formalin solution. The slides were then transferred to the Department of Histology and Embryology, Wrocław Medical University, together with the information obtained. The above procedure was reviewed favourably by Wrocław Medical University's Ethics Committee, decision No. KB-258/2013, extended by decision No. KB-15/2014 (extension of the age range of the test subjects).

Histological preparation and examination

After dissection and collection, veins were fixed using a standard protocol [10]. Briefly, veins were fixed for at least 24 hours in 4% formaldehyde, dehydrated through graded alcohols and embedded in paraffin blocks.

Paraffin-embedded veins were cut into 4 μm histological slides and then stained using the pentachrome staining method developed by Movat [17, 22], which allows the differentiation of collagen, muscle and elastic fibres, stained with yellow, red and black colours, respectively.

Stained slides were scanned using a Pannoramic MIDI II (3DHistech, Budapest, Hungary) histological scanner and then the scans were then examined using Pannoramic Viewer 1.15.4 software (3DHistech). The following parameters were measured and/or estimated: lumen area, circumference, area/circumference ratio and maximum width; minimum, maximum and median thickness of the venous middle layer (tunica media).

Vein component segmentation

The Trainable Weka Segmentation plug-in for ImageJ was used to estimate the relative proportions of smooth muscle, collagen and elastic fibres [4]. Weka is a collection of machine learning algorithms designed for image analysis, including automatic image segmentation and data mining, The image segmentation was preceded by algorithm training — different parts of veins (muscle cells, collagen//elastic fibres) were manually marked by a human, and based on these guidelines, Weka created an algorithm designed to automatically distinguish vein components. The efficiency of each algorithm was evaluated by a human and additional guidelines were added, until the algorithm successfully marked all vein components.

After training segmentation schemes, a segmentation algorithm was applied to images of vein histological slides, converting them into four-colour images representing four classes: smooth muscle, collagen, elastic fibres and background (red, purple, black and yellow, respectively).

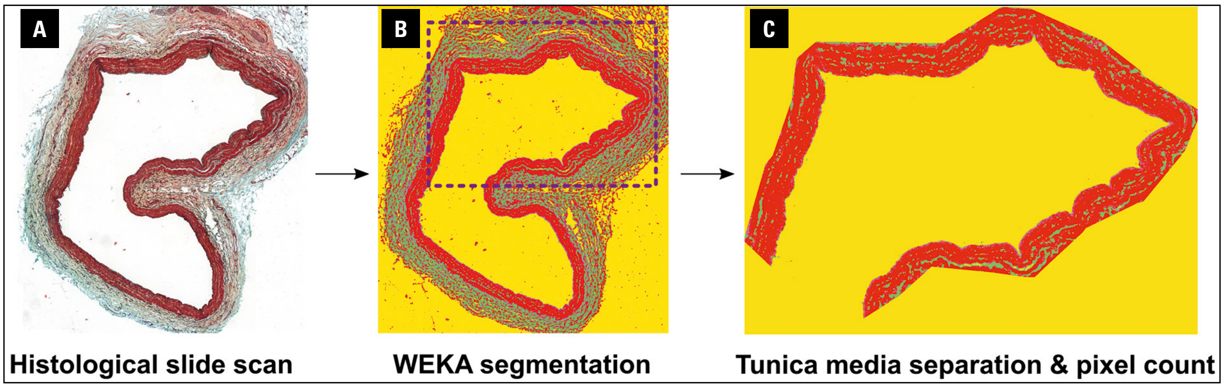

Figure 2. Vein segmentation workflow based on Movat's stain slides. The Trainable Weka Segmentation was used to separate muscles (red), elastic fibres (purple), collagen (green) and background (yellow). **A.** Scanned vein stained using Movat's method; **B.** Same vein after applying the Trainable Weka Segmentation; **C.** Separated tunica media used for pixel count.

Pixels of each colour were counted, and then the relative proportions of each class were determined.

The workflow for this procedure is shown on Figure 2.

Statistical analysis

Due to the lack of normal distribution of many parameters, non-parametric tests were used. The Mann-Whitney U test was used to compare the differences between two groups; for three or more groups — the Kruskal-Wallis ANOVA with an appropriate post-hoc test was applied.

Spearman's correlation test was used to analyse correlations [30]. Statistical analysis was supported by Statistica 13.1 software (TIBCO Software Inc., Palo Alto, CA, USA) and R statistical environment [20] with the ggplot2 package [28].

RESULTS

Histological examination and segmented vein analysis

Of the 26 individuals, eight were found to have M vein type, seven had N vein type and eight had Y vein type. Unfortunately, the venous type of the remaining two individuals was inconclusive and they were excluded from further study.

The remaining veins were segmented (as shown in Fig. 2), and the percentage composition of the elements building the middle layer of the vein was compared. However, no significant differences were found between the relative amounts of different fibres in the different vein types. The percentage of elastic fibres gave the p-value closest to the significance threshold, namely $p = 0.084$. The results for the other components are shown in Tables 1 and 2.

Venous type dependent variability

The Kruskal-Wallis ANOVA revealed statistically significant variability in the following traits: lumen circumference and minimal or median width of the middle venous layer. The *post-hoc* test revealed significant or almost significant differences between vein types M and Y or N and no differences between types Y and N (Table 1). Therefore, the further analysis included two groups: M-type and a merged group of Y/N-type veins.

The Mann-Whitney comparison of all basilic and cephalic veins (regardless of vein type) showed no statistically significant differences (data not shown). Therefore, this division was not included in further analyses.

After merging the groups, more statistically significant differences were found (Table 2), covering most of the metric traits. Figure 3 shows bar graphs representing significantly different traits. Y/N veins tend to have larger lumen circumferences, although their tunica media is thinner than in type M.

Correlation matrix

Table 3 shows that significant correlations between the relative fibre amounts and venous metric traits were only found for elastic fibres. Roughly, bigger and more prominent veins (with greater circumference, area, circumference/area ratio and maximum width) tend to have relatively more elastic fibres than the small veins (with R ranging from 0.321 to 0.399 and p-value ranging from 0.004 to 0.025). A weak but significant negative correlation ($R = -0.299$, p-value = = 0.038) was found between the minimum width of the *tunica media* and the relative amount of elastic fibre.

Table 1. Results of Kruskal-Wallis ANOVA test for comparison of 3 venous types (Y, N, M)

Variable	p-value Kruskal-Wallis ANOVA	p-value for post-hoc comparisons		
		M vs N	M vs Y	N vs Y
Lumens circumference [μm]	0.345	**0.068**	**0.084**	1.000
Lumens area [μm^2]	0.340	1.000	0.429	1.000
Area/circumference ratio	0.542	1.000	1.000	0.886
Maximum width [μm]	0.106	0.210	0.196	1.000
T. media minimum width [μm]	0.026	0.211	0.025	1.000
T. media maximum width [μm]	**0.071**	**0.075**	0.375	1.000
T. media median width [μm]	0.050	**0.095**	0.114	1.000
% of muscle	0.229	1.000	0.258	1.000
% of elastic fibres	0.211	0.590	0.275	1.000
% of collagen fibres	0.333	1.000	0.550	0.700
Collagen/muscle ratio	0.351	1.000	0.548	0.800
Elastic/collagen ratio	0.539	0.978	1.000	0.989

P-values 0.05 are marked red, below 0.1 — bolded

Table 2. Comparison of M and Y/N type veins

Variable	Rank Sum for M type	Rank Sum for Y/N type	p-value
% of muscle	460.5000	715.5000	0.136
% of elastic fibres	312.5000	863.5000	0.084
% of collagen fibres	351.5000	824.5000	0.381
Collagen/muscle ratio	349.5000	826.5000	0.358
Lumens circumference	233.0000	757.0000	0.010
Lumens area	283.0000	707.0000	0.181
Area/circumference ratio	323.0000	667.0000	0.728
Maximum width	252.0000	738.0000	0.035
T. media minimum width	443.0000	547.0000	0.009
T. media maximum width	424.5000	565.5000	0.032
T. media median width	436.0000	554.0000	0.015

Mann-Whitney test, highlighted p-value below 0.05

DISCUSSION

This study attempted to determine the microanatomical differences between the metric and histological traits of the veins of the superficial venous systems divided into types Y, N and M. Previous studies have shown significant differences in the architecture of the superficial veins of the upper limb depending on the type of the physical work performed [14].

The Kruskal-Wallis test suggests the most significant variability in the thickness of the middle layer of the vein; *post-hoc* tests revealed one statistically significant difference — between the tunica media thickness at its thinnest point in the M and Y systems (Table 1). This is consistent with previous studies and preliminary hypotheses that the M- and Y-types are the most differentiated, with the N-type being intermediate. Type Y is thought to have the highest power and M — the lowest [14, 31].

For this reason, the Y and N categories were combined into a single category, allowing the diversity in the venous structure of the different types to become more apparent (Table 2). The differences are in the circumference and width of the vein and the thickness of the tunica media. Surprisingly, no statistically significant differences were found in the percentages of the individual components of the middle layer of the venous wall — the closest to the confidence threshold was the percentage of elastic fibres. The differences in the content of these fibres were expected, as a large

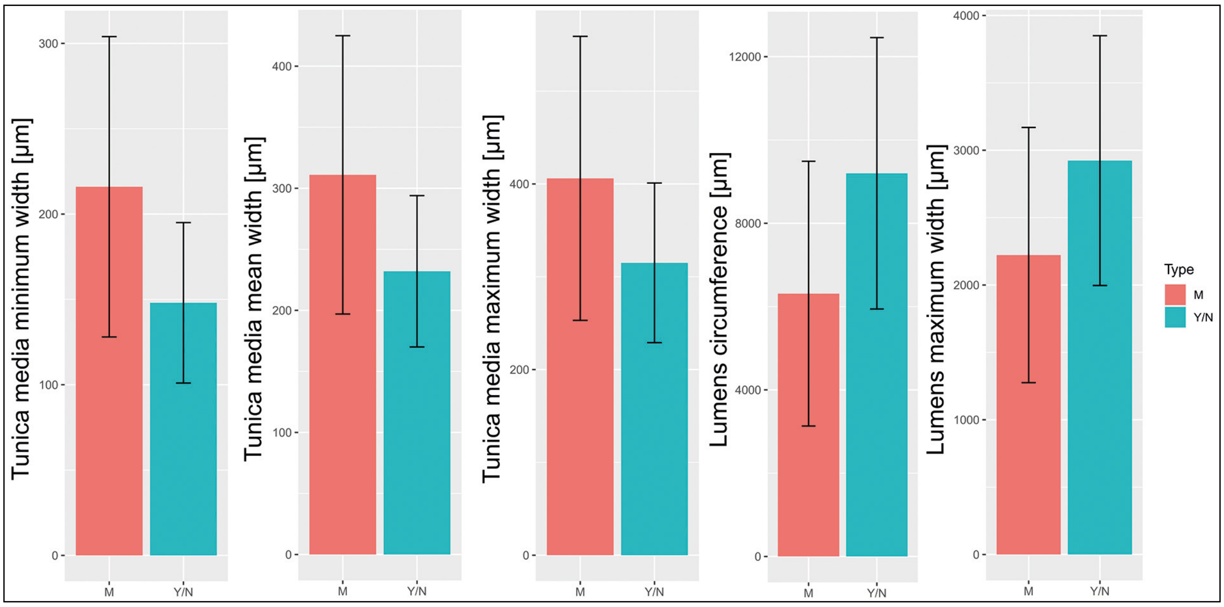

Figure 3. Differences between venous types Y/N and M (p-values and non-significant differences are given in Table 2).

Table 3. Correlation matrix for metric parameters and veins fibrous composition

Variable	% of muscles	% of elastic fibres	% of collagen	Collagen/muscle ratio	Elastic/collagen fibres ratio
Lumens Circumference	–0.053	0.362	–0.094	–0.074	0.258
Lumens area	–0.045	0.399	–0.157	–0.140	0.327
Area/Circumference ratio	–0.052	0.321	–0.142	–0.133	0.271
Maximum width	–0.075	0.367	–0.080	–0.058	0.243
T. media minimum width	–0.068	–0.299	0.188	0.182	–0.277
T. media maximum width	–0.161	–0.080	0.195	0.191	–0.191
T. media median width	–0.129	–0.195	0.211	0.204	–0.256

Highlighted results with p-value below 0.05

number of elastic fibres is characteristic of particularly strong blood vessels. However, this has little effect on the tensile strength of the wall, but does increase its elasticity [27].

Overall, the statistical analysis suggests that the differences between the different vein types are quite subtle in each of the respects studied, with many results hovering on the edge of statistical significance. This does not discredit their reliability; similar studies on larger numbers of individuals could demonstrate the significance of these differences, but the material limitations of the present study do not provide sufficient statistical power.

Counter-intuitively, type M veins have a thicker tunica media than the more efficient Y and N veins, although they also have a smaller circumference. It is suggested that the increased venous circumference and (presumably) greater tensile strength are not due to the thickness of the tunica media, but to the relative content of the individual fibres. This is supported by correlation analysis, which shows significant correlations between the venous metrics and the elastic fibre content. A higher content of elastic fibres correlates with a larger circumference, surface area and width of the vein and thus indirectly with the shape of the vein, which is inversely correlated with its thickness at the narrowest available point. The suggest that these fibres play an important roles in maintaining a large vessel lumen.

The increased thickness of the tunica adventitia in Y/N veins cannot be ruled out either. However, due to technical limitations, the tunica adventitia was subject to significant layer separation and destruction during preparation, and this issue remains unresolved.

The above results appear to be consistent with findings from previous studies. For example, Rowley et al. [21] showed that professional squash players generally have greater blood vessel thickness and more efficient blood flow. Similar findings have also been reported by Simmons et al. [24] and de Brito et al. [7].

It is difficult to relate our findings to similar studies of histological comparison of these or other veins due to the rather unique methodology and subject matter; to our knowledge, there are no publications to which we can refer. Nevertheless, although the results should be treated with some caution due to the small sample size of the study, they do provide a better understanding of the microscopic variability of superficial venous anastomosis types in the forearm.

ARTICLE INFORMATION AND DECLARATIONS

Acknowledgments

The authors of current study declare no conflict of interests. Data used in the current study may be shared by the corresponding author upon reasonable request. The authors would like to thank prof. Marzenna Podhorska-Okołów for valuable consultation. Authors would like to acknowledge the donors of the material for current study.

Conflict of interest: None declared.

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