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ANATOMIC STUDY OF SEPTOCUTANEOUS SYSTEM OF THE HUMAN FETUSES’ LOWER LEG: POSTERIOR TIBIAL ARTERY

The paper has never been presented before.

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Abstract

**Background:** Lower-leg septocutaneous system of perforating blood vessels represents the vascular basis of fasciocutaneous flaps. Additionally, it is of particular importance when designing distally based fasciocutaneous flaps which represent the "workhorse" in the reconstruction of the distal third of the lower leg and foot.

**Methods:** The dissection was conducted on 20 fetuses of both sexes and of gestational age from 20 to 28 weeks. The focus was on the vascular anatomy of posterior tibial artery and its septocutaneous (fasciocutaneous) perforating arterial vessels. Cluster analysis was applied to the data obtained.

**Results:** A total of 212 perforating arterial vessels was identified. The average number of perforating arterial vessels was 5.32 (ranging from 4 to 7). It was identified that septocutaneous perforating blood vessels are more likely to be found at certain levels ("Safe levels of finding perforators"). These are: second, third, fifth and sixth tenth (measured as a distance from intermalleolar line to popliteal crease).

**Conclusions:** The presence of septocutaneous system of perforating blood vessels and reliability of their localization even in the fetal period allows application of these findings in the lower leg reconstructions in children of early age. It also contributes to the greater level of understanding of anatomy of the lower-leg vascular system. Finally, it provides a basis for understanding the development of this system as it is now possible to compare results obtained on fetuses with those obtained on adults. Reduce the length of the conclusions. Organize Introduction and Conclusions in a different manner. Split out the text of Introduction and Conclusions. Provide more specific results.

**Key words:** septocutaneous system, perforating blood vessels, posterior tibial artery, fetuses, dissection
Introduction

Discovery of fasciocutaneous blood vessels system of the lower leg by Ponten in the eighties of the last century resulted in more profound understanding of skin vascularization. The main arteries of the body were re-examined with an emphasis on the perforating branches which provide direct skin vascularization. Flaps, that are based on the blood vessels passing through the septum duplication in the lower leg, consist of skin, subcutaneous adipose tissue, and deep fascia and are named fasciocutaneous flaps. They can be either direct or indirect flaps. The initial advancements in this area was followed by a period when numerous studies on this blood vessels system were conducted. This period was marked by two distinct pathways: solving disagreements and confusion in the nomenclature of perforator flaps and further exploration and understanding of new concepts of perforator flaps.

From the practitioners’ point of view, discovery of this type of flaps was especially important because of its structural characteristics and lack of reliable flap locations for lower leg area. Patient studies showed that flaps are the best choice when it comes to lower leg reconstruction. The greatest advantages are the simplicity of the procedure, very high success rate, and relatively small number of minor disadvantages. Furthermore, the procedure can be applied to wounds with different origins (e.g. fourth-degree burn injuries, blast and high-velocity projectile wounds, distal tibial fracture) and injuries of various size, location and depth.

Numerous anatomical studies on cadavers have been carried out in order to determine the localization of perforating blood vessels of the main lower-leg arterial trunk. The results, although often significantly different, were of great help in planning and designing the distally based flaps of the lower leg ("separated asseptocutaneous perforators
of the lower leg”). However, studies on fetuses that would shed some light on the vascular anatomy of this system of blood vessels are very rare and often inconclusive, despite the fact that pediatric cases are more complicated and require further theoretical knowledge. This research is, therefore, an attempt to provide a comprehensive, clear and conclusive overview of the lower-leg septocutaneous system of skin blood supply in fetal age. The results were statistically analyzed in order to enable comparison with other studies of this kind. Implications of this article go beyond contribution to the theoretical knowledge, as the information provided can be applied to cases of lower leg reconstruction in children.

**Methods**

The study was conducted on 40 lower extremities from 20 human fetuses. The fetuses were prepared by fixation in 10% formalin and blood vessels of 10 fetuses were injected with Micropaque solution (barium sulfate) (Merck, Darmstadt, Germany) for better visualization. Fetuses were collected in the Department of Anatomy between 1962 and 1985. All fetuses were medico-legally obtained from the Clinic of Gynecology and Obstetrics of the Faculty of Medicine in Nis, Serbia (mišljenje Etičkog komiteta Medicinskog fakulteta Univerziteta u Nišu od 22.09.2016. godine). No established anatomical deformities or systematic diseases were recorded.

Fetal age ranged from the third to the ninth lunar month and was established by measuring crown-rump length. Microdissection of the fetal lower extremities was performed under 5x magnifying lenses. Two horizontal cuts and one vertical cut of the skin were made (Figure 1 and 2). The first horizontal cut was made at the level of the popliteal fold and the second was made at the level of the medial and lateral malleolus (Figures and
2). The vertical cut extended from the middle of the upper horizontal cut at the level of the popliteal fold to the middle of the lower horizontal cut. After that, the dissection was going through the skin, subcutaneous tissue, and fascia, and then continued carefully medially and laterally until reaching medial and lateral septum of the lower leg. There, we noted the origin of arterial septocutaneous vessels from posterior tibial artery. Also, we can clearly measure the distance from lower horizontal cut to the origin of perforating vessels. Characteristic cases were photographed (Figure 1 and 2).

**Data analysis**

Cluster analysis was conducted on the raw data obtained from the primary research. Portable IBM SPSS Statistics v19 was used. K-means cluster analysis was conducted. Due to the small data set, the number of iterations was set at 10 in order to determine whether it is possible to run this type of analysis. SPSS conducted the command without any further notifications, meaning that the data set is large enough. The number of clusters was set at three and clusters were divided based on the number of perforators found in each area of fetuses’ lower leg. Clusters were divided into low, medium and high-density area. Additionally, cluster membership information was saved.

**Results**

Anatomical microdissection of fetal lower legs with (figures 1 and 2) or without contrast injected (figures 3) was used in order to define the number of septocutaneous perforator vessels and their localization.
Figure 1 and figure 2. Posterior tibial artery perforators (fetal dissections with injected contrast)
The examination of septocutaneous perforating vessels of posterior tibial artery confirmed
the existence of 4 such vessels in 6 dissections and 5 in 20 dissections. Furthermore, in 9
dissections there were 6 perforating vessels and in 5 dissections their number was 7 with an
average value of 5, 32 and with the standard deviation of 0, 88 (total of 212 perforating
vessels). Septocutaneous perforating vessels of posterior tibial artery originated between
flexordigitorumlongus muscle and soleus muscle.

Figure 3. Posterior tibial artery perforators (without contrast) from their origin to the deep fascia.
In order to statistically process the data, conduct the subsequent analysis and enable comparability of the results obtained by our research and the results of other similar studies, fetal lower legs were "divided" in 10 equal parts.

![Bar chart showing localization of septocutaneous perforating vessels of fetal posterior tibial artery]

**Figure 4.** Localization outbreak of septocutaneous perforating vessels of fetal posterior tibial artery

Levels at which perforating blood vessels are encountered as well as outbreak schedule of perforating vessels of posterior tibial artery and peroneal artery, obtained by dissection of the fetuses, are counted and shown in Table 1 and Figure 4.
Table 1. Number (and percentage) of fetuses with septocutaneous perforating vessels of posterior tibial artery and their percentage per each group

<table>
<thead>
<tr>
<th>1/10 length of the lower leg (from intermalleolar line – popliteal crease)</th>
<th>Number of dissections where perforators were found</th>
<th>Percentage of the total number of dissections (%)</th>
<th>Percentage of the total number of perforators (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>5</td>
<td>12.25</td>
<td>2.36</td>
</tr>
<tr>
<td>Second</td>
<td>36</td>
<td>90</td>
<td>16.98</td>
</tr>
<tr>
<td>Third</td>
<td>32</td>
<td>80</td>
<td>15.09</td>
</tr>
<tr>
<td>Fourth</td>
<td>19</td>
<td>47.5</td>
<td>8.96</td>
</tr>
<tr>
<td>Fifth</td>
<td>35</td>
<td>87.25</td>
<td>16.51</td>
</tr>
<tr>
<td>Sixth</td>
<td>30</td>
<td>75</td>
<td>14.15</td>
</tr>
<tr>
<td>Seventh</td>
<td>19</td>
<td>47.5</td>
<td>8.96</td>
</tr>
<tr>
<td>Eighth</td>
<td>27</td>
<td>67.5</td>
<td>12.73</td>
</tr>
<tr>
<td>Ninth</td>
<td>6</td>
<td>15</td>
<td>2.83</td>
</tr>
<tr>
<td>Tenth</td>
<td>2</td>
<td>5</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Descriptive statistics for the posterior tibial artery is presented in Table 1. In order to visualize the previously outlined results, a bar chart was constructed and it is presented in Figure 4.
Finally, K-Means cluster analysis was performed on the data set and the variable of interest was number of dissections where perforating vessels of septocutaneous perforating vessels of posterior tibial artery were found (labeled Variable 1). Analysis conducted on Variable 1 showed that, on average, 4 septocutaneous perforating vessels were found in cluster 1 (M = 4), 33 were found in cluster 2 (M = 33) and 22 were found in cluster 3 (M = 22). Final cluster centers are summarized in the Table 2. Analysis also showed that cluster 2 is represented with 4 cases while cluster 1 and 3 consist of 3 cases each. Cluster membership showed that the high density cluster is the Cluster 2. Low-density areas (Cluster 1) are the first, ninth and tenth 1/10 of the lower leg. Medium-density areas (Cluster 3) are fourth, seventh and eighth 1/10 of the lower leg, while high-density areas are the second, third, fifth and sixth 1/10.

Additionally, independent samples T-test was used in order to determine whether there is a statistically significant difference between distributions of dissections in areas where perforators were found as observed in adults (in our previous study\(^{10}\)) and fetuses. Age was used as a grouping variable with value 1 assigned to adults and value 2 assigned to fetuses. As the number of adult cadavers is higher than the number of fetal cadavers, instead of using absolute values, the test was run on percentages of the total number of dissections for each of the ten areas.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spv_posterior_tibial_artery</td>
<td>4</td>
<td>33</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 2. Final Cluster Centers: Posterior Tibial Artery
The results of the T-test showed that there is no statistically significant difference between adults and fetuses (t = .146, p = .866), which means that the distribution of dissections in ten areas where perforators were found is highly similar for fetal and adult cadavers.

**Discussion**

Lower leg area has been focus of interest of many different research papers and extensive studies. However, knowledge about this body area is still far from complete. While the importance of the topic has been recognized, it is still fairly insufficiently researched (for example, posterior lower leg skin and its vascular anatomy\textsuperscript{11,12,13} illustrate this statement). Implications of such theoretical gaps extend into the practice. Consequentially, numerous challenges arise, such as often quoted poor success rates of lower leg soft tissue reconstruction\textsuperscript{14}. A step forward in this area was made with the recognition of importance of faciocutaneous perforators and flaps. Technology development was one of the biggest obstacles and once when color duplex imaging was introduced, it became feasible to access deep fascia\textsuperscript{15}. Ponten was the first to recognize potential of faciocutaneous flap when it comes to surgical solutions for soft tissue defects. After that, a long stream of researches followed. Hupkens and colleagues conducted an anatomical study to localize and classify lateral lower leg perforators\textsuperscript{13}. Same author gave his contribution by researching medial lower leg perforators, their distribution and characteristics. Apart from anatomical studies focused on general characteristics, a number of published papers revolves around soft tissue reconstruction and the role of fasciocutaneous flaps in the reconstruction process\textsuperscript{14,15,16,17}. While it is evident that this area of research is becoming more complex and compatible with practical needs, vast
majority of studies has been conducted on adults. Studies conducted on children are quite rare (the study by Wahib is one of the very few ones exploring reconstruction of full thickness of soft tissue defect of lower extremities in children\textsuperscript{9}) but still more present than studies on fetuses. Lack of studies conducted on fetuses we identified as a serious research gap. Study undertaken by Ugrenovic and colleagues on neurovascular stalk of the superficial sural flap is one of the few ones conducted on fetuses\textsuperscript{18}.

We identified several reasons why this research gap should be bridged. Septocutaneous system of lower leg perforators is already very well developed in the fetal period. There is a rather clear clustering pattern of perforators at different levels which reflects the pattern we find in adults. Having this in mind, as well as the fact that this system represents the vascular basis of fasciocutaneous flaps, we can conclude that the skin and soft tissue defects of distal lower leg area can be taken care of during early childhood by implementing this reconstructive method\textsuperscript{19,20,21}. The significance of this research lies in the fact that now pre-operative mapping and finding perforators using ultrasound is significantly easier (and it’s almost not necessary at all) because the operative method requires presence of at least one reliable perforator and in this study we showed exact levels of emergence of the most distal perforators. Furthermore, this paper also contributes to increasing the level of understanding of anatomy of the lower-leg vascular system.

Independent samples T-test, applied to results of our research obtained in fetuses and adults, confirmed that there is no statistically significant difference between adults and fetuses, which means that the distribution of dissections in ten areas where perforators were found is highly similar for fetal and adult cadavers\textsuperscript{22}.

Finally, our research provides a basis for understanding the development of this system as it is now possible to compare results obtained on fetuses with those obtained on adults.
Despite the well-known fact that during embryogenesis and fetal development significant changes occur in the number and caliber of the lower leg vascular vessels as well as obliteration of some blood vessels and creation of new ones, the basic model of blood vessels pattern of this system stays rather stable until its final stage of development.

**Conclusion**

Results of this study is not of academic importance only but of practical one. Lower leg defects in children can be posttraumatic (motor vehicle accidents, burns, war injuries) or postoperative (after tumor removal). Many factors should be considered in reconstruction of this kind of defects in children. In addition to lack of children cooperation postoperatively and functional requirements, surgeon must be aware of anatomical limitations, such as small structures and state of growth and developments of vascular system of the lower leg.

Results obtained in this study clearly show that there is exactly defined schedule outbreak of septocutaneous perforators of posterior tibial artery.

**Limitations of the study**

Despite the fact that sample size for this kind of analysis is not as huge as it could be, it is sufficient in a way that we can draw some important conclusions:

1. Septocutaneous system of posterior tibial artery is well established in late fetal age already,

2. There is precisely defined schedule of septocutaneous perforator’s outbreak which is very similar to the pattern we found in adults (there is no statistical difference between these two study groups).
All this means that reconstructive surgeon can safely use this data to plan reconstruction of almost any kind of the lower leg and foot defects in children, using fasciocutaneous flaps (perforator flaps, flaps with reverse flow etc.) whose vascularisation relies on septocutaneous system of vessels.

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