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Histochemical, immunohistochemical and ultrastructural analysis of aortic wall in neonatal coarctation

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Abstract

The neonatal type of coarctation is characterized by the presence of the ductal sling and coarctational shelf placed proximally in relation to the ductal orifice. Those morphological features are not described in detail yet from immunohistochemical and transmission electron microscopy (TEM) aspects, so the aim of this study was to investigate the smooth muscle cells (SMCs) phenotype in aortic intimal thickening, presence of inflammatory cells and contents of intimal and medial, and adventitial connective tissue. We examined samples of coarctation segments excised at surgery after end-to-end anastomosis from 30 patients, ages from 14 days to three months, histochemicaly, immunocytochemically and by TEM. In all samples, it is noticed focal intimal thickening on the posterior aortic wall, with accumulation of SMCs, which show immunoreactivity on alpha-smooth muscle actin (α-SMA) and vimentin (but not on desmin) and also expressed proliferating cell nuclear antigen (PCNA) and S-100 protein. At TEM analysis, those SMCs show a fibroblast-like morphology, so their functions could be to proliferate and secrete extracellular matrix (ECM) components (a synthetic phenotype). In all studied samples of the coarctation, on the posterior wall, the immunocytochemical and TEM examination revealed the presence of SMCs of the synthetic phenotype. Results also showed an increase of the cell number in intima of this part of aortic wall, followed by proliferated SMCs in inner media and absence of inflammatory cells. This finding suggests that proliferation of the SMCs, their synthetic activity and increase of the cell number could lead to formation of the intimal thickening on the posterior wall.

Keywords: neonatal aortic coarctation, aortic intimal thickening, smooth muscle, cells phenotype.

Introduction

Coarctation of the thoracic aorta is defined as a congenital narrowing of the upper part of the descending aorta, next to the place of joining of *ductus* or *arteriosum* ligament, which produces a difference in the pressure above the narrowing and below it [1]. The aortic coarctation, seemingly morphologically and physiologically a simple entity, is not easy to understand. One of the most widely accepted classifications of the coarctation is its division based on the age of patients. According to this classification, coarctation is divided into the neonatal and adult type [2]. The neonatal type of coarctation is characterized by the presence of "the ductal sling" and "coarctational shelf" placed proximally in relation to the ductal orifice [3]. In newborn babies and younger nurslings, the shelf and neighboring parts of coarctational segment contain ductal tissue. The ductal tissue forms a "sling", which completely surrounds the juxtaductal aorta [4].

Aim

Those morphological features, the ductal sling and coarctation shelf, are not described in detail yet from transmission electron microscopy (TEM) and immunohistochemical (IHC) aspects, so the aim of this study was to investigate the smooth muscle cells (SMCs) phenotype in aortic intimal thickening, presence of inflammatory cells and contents of intimal and medial and adventitial connective tissue.

Materials and Methods

We examined samples of coarctation segments excised at surgery after end-to-end anastomosis, during 2007, from "Tirsova" University Children's Hospital in Belgrade, Serbia. These aortic segments were excised from 30 patients (18 boys and 12 girls), aged from 14 days to three months. Twenty samples were cases of isolated coarctation and 10 samples were cases of coarctation joint with the obstruction of left ventricular outflow tract. Coarctation segments were obtained according to the guidelines of the local Medical Ethical Board. Material was collected prospectively in accordance with the principles outlined in the *Declaration of Helsinki* and the present study was approved by the Institutional Review Board of "Tirsova" University Children's Hospital, Belgrade.

For light microscopy, the specimens were dehydrated in graded ethanol (70–100%), cleared in xylol, embedded

in paraffin and histochemically stained [with Masson's trichrome, Reticulin, Weigert–van Gieson, Orcein, Periodic Acid–Schiff (PAS) and Alcian Blue–PAS technique, at pH 2.5 and pH 1] by standard procedures [5]. Immunocytochemical staining was performed on 5 μm sections from formalin-fixed paraffin-embedded blocks, using a labeled Streptavidin–Biotin method with an LSAB kit (Dako). Sections were deparafinized and rehydrated. After microwave treatment of 21 minutes in citrate buffer, pH 6, endogenous peroxidase activity was blocked with 3% H_2O_2 for 15 minutes. The sections were first incubated with the primary antibody for 60 minutes [von Willebrand's factor (vWF), alpha-smooth muscle actin (α-SMA), vimentin, desmin, myosin heavy chain (MHC), cluster of differentiation (CD) 3, CD45, CD34, CD68, S-100 protein, laminin, fibronectin, collagen IV and proliferating cell nuclear antigen (PCNA)], then with biotinylated link antibody and finally with Peroxidase-labeled Streptavidin. Slides were counterstained with Hematoxylin, washed in water and mounted. For TEM, the primary fixative consisted of 2.5% glutaraldehyde in 0.1 M sodium cacodylate–HCl buffer (pH 7.4) for 24 hours, at 4ºC. The specimens were post-fixed for one hour, at 4ºC, in 1% osmium tetroxide in 0.1 M cacodylate buffer and 4.8% uranyl acetate for 24 hours, at 4ºC. The samples were dehydrated in graded ethanol (70–100%) and embedded in Epon 812. Ultra-thin sections were stained with 2% uranyl acetate and alkaline lead citrate.

Results

The biggest prominence of the intimal thickening and therefore the biggest narrowing of the aortic lumen in all observed samples in present study were found proximally from the ductal orifice to the aortic wall. Maximum thickness of the coarctational shelf was found at different distances from the level of ductal orifice in the observed group of samples. The coarctational shelf is more prominent on the posterior aortic wall.

The observed changes cover the entire vascular wall and are most prominent in the intima. There are also differences in the distribution of changes along the aorta wall, from isthmus to below ductal orifice in the aorta wall (Figure 1).

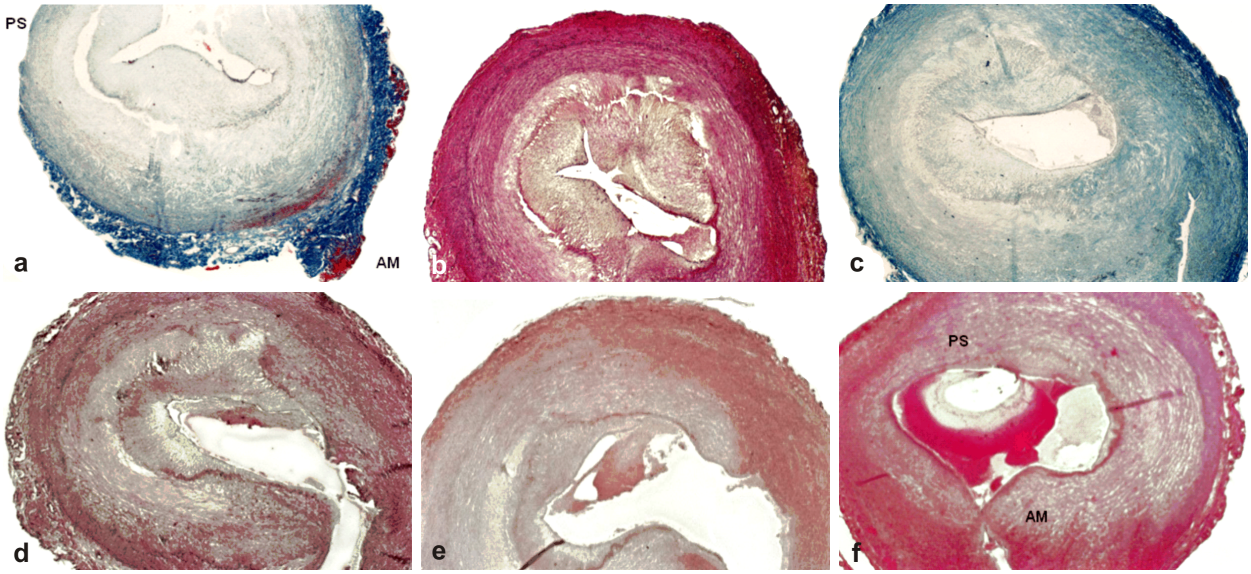

Figure 1 – ***(a–f) The intimal thickening "the shelf" is most prominent proximally to ductus orifice (preductal position of the shelf) and decreases in proximal–distal direction (from a–f), being completely in the line with the wall in several segments above ductal orifice: (a–c) Segment proximally to the ductus orifice; (d) On the level of the ductal orifice; (e and f) Segment distally to the ductus orifice. Azzan–Heidenhain histochemical staining: (a) ×40; (c) ×100. PAS histochemical staining: (b) ×150; (f) ×200. Orcein histochemical staining: (d) ×80; (e) ×100. AM: Anteromedial; PS: Posterior; PAS: Periodic Acid–Schiff.***

Tunica intima at the level of ductal orifice

By comparing the part of the aortic wall at the point of ductal orifice with proximal parts of the wall, we can observe that at this point, the wall is less thick, but subendothelial area is wider. Still, there is no considerable prominence of this part of the wall in the lumen, and therefore lumen obstruction is smaller (Figure 1).

Endothelial cells in all samples and in all segments express vWF and vimentin (Figure 2c).

Basal membrane is not stained on collagen IV; however, it has established its diffuse presence below basal membrane (Figure 3). Generally, basal membrane is well differentiated and can be visualized by the IHC staining on laminin and fibronectin, and also by PAS and Reticulin techniques. This characteristic is also found both, in the posterior and anteromedial aortic wall.

Subendothelial layer at the level of ductal orifice

On the posterior aortic wall, in all samples of this group was noticed focal intimal thickening, with accumulation of SMCs, which show α-SMA- and vimentin-immunoreactivity, while the expression of desmin or MHC is missing (Figure 2, a–c). Those cells also expressed PCNA. The biggest number of PCNA-immunoreactive cells was found in the internal part of intima. At TEM analysis, those SMCs showed a fibroblast-like morphology, *i.e.*, synthetic phenotype (Figure 4).

The analysis of the distribution of the S-100 protein-immunoreactive cells showed a reaction in the subendothelial layer and on the border with media (Figure 5c).

Intimal thickening is separated from media by a gap at the level of internal elastic membrane; it is made of radial, very thin, discontinuous fibers (the weak reaction to Orcein; most intensely staining by Reticulin) and SMCs between them. Extracellular matrix (ECM) among thin fibers is stained with the PAS technique and the Alcian Blue–PAS technique, at pH 2.5, as well. If this reaction happens at pH 1, only the surface layer of the ECM is stained intensely.

On the anteromedial wall, the results of IHC staining in subendothelial layer showed the presence of SMCs with α-SMA and vimentin expression (Figure 6a). The cell population of SMCs in the external part of intima showed a weak reaction to MHC and there was no reaction to desmin. Proliferating SMCs have not been identified in intima. The results of our study showed the presence of rare S-100 protein immunoreactive cells only on the border with media. Research by TEM indicates the variability of the SMCs phenotype: in surface layers, SMCs expressed the synthetic phenotype, while in deeper layers they most probably expressed both, the synthetic and contractile phenotype.

The IHC methods for the demonstration of the expression of the CD45 and CD3 antigens in intima of both sides of the wall did not indicate inflammatory cells. Also, the analysis of the CD68 expression did not show the presence of macrophages.

Tunica media at the level of ductal orifice

Intima is separated from media by the internal elastic membrane which is damaged both, on the posterior and anteromedial side. Internal elastic membrane is differentiated by the Weigert–van Gieson, Orcein, PAS and Reticulin methods, in the places where it was found. There are large fragmentations all across on this side of the wall and below the biggest thickening on the posterior wall was completely missing (Figure 1, a–f).

The appearance and composition of tunica media differed on the ductal side and the side opposite ductus. On the lateral sides and the posterior side (opposite ductus), tunica media had two layers: the internal and external layer. On the side opposite ductus, tunica media was thinner than on the ductal side. The anteromedial aortic wall was thickened due to the increase in the number of cells in media, while subendothelium was much thinner in comparison with the opposite side.

On the posterior wall, the internal layer of media was comprised of a considerable number of elastic lamellae and SMCs among them. Staining of medial elastic fibers by the Weigert–van Gieson method, showed bundled up, but disorganized and straightened thick fibers in the internal part of media. These fibers were intensely stained by the PAS reaction.

SMCs in this part of media showed the reaction to α-SMA and vimentin, while rare cells reacted to MHC as well. The cell population in the internal part of media did not react to desmin. There was an intense PCNA immunoreactivity and a large number of S-100-immunoreactive cells in the internal part of media (Figure 2).

The external part of media on the posterior wall was characterized by more preserved elastic lamellae and here they were completely straightened, thickened, sporadically duplicated and fragmented as well. SMCs among lamellae expressed α-SMA and MHC. Only rare SMCs on the border of the external part of media and adventitia expressed desmin.

The analysis of the PCNA immunoreactivity indicated weaker expression of this antigen than in the internal part of media. A large number of cells reacted positively to the S-100 protein (Figure 5). There was no visible reaction to CD3 and CD45.

On the anteromedial, ductal aortic wall, in all samples of this group, the dominant change was the thickening at the point of ductal orifice. This thickening makes the impression of ductal media spreading into aortic media. Besides this thickening, in all samples of isolated coarctation there were mucoid changes in the ductal wall, which indicated the process of closing and obliteration (Figure 6).

The analysis of the composition of ductal medial thickenings indicated that SMCs here were extremely α-SMA-immunoreactive and showed the intense expression of MHC. In inner media on the anteromedial wall it was noticed a large number of SMCs, which expressed desmin and MHC. The most intense reaction to both markers was observed in the SMCs around the cavities in the ductus wall (Figure 6b). Cells of the ductal thickening did not express the S-100 protein or PCNA. At TEM analysis, those SMCs showed a usual morphology of SMCs of the contractile phenotype (Figure 7). The analysis of the distribution of the CD3, CD45 and CD68 markers showed the lack of reaction.

Tunica adventitia

Tunica adventitia was made of a developed network of elastic and collagen fibers, *vasa vasorum*, nervous fibers and fibroblasts. Adventitial layer was thicker on the ductal side. It was mildly stained by the PAS and Alcian Blue–PAS reactions, and most intensely stained (compared to other parts of the wall) by Reticulin and trichrome techniques. Adventitial fibroblasts showed immunoreactivity on PCNA, S-100 protein and CD34. A number of adventitial fibroblasts showed immunoreactivity on α-SMA and MHC.

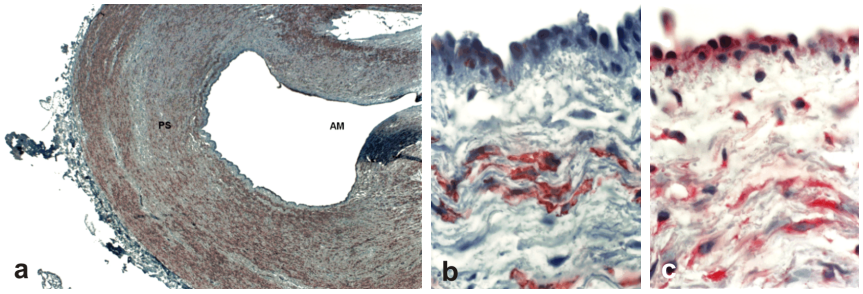

Figure 2 – ***(a–c) Neonatal aortic coarctation, posterior aortic wall (opposite ductus), endothelial and subendothelial layer. IHC staining on vimentin: (a) ×40; (b) ×256. IHC staining on α-SMA: (c) ×256. IHC: Immunohistochemical; α-SMA: Alpha-smooth muscle actin; AM: Anteromedial; PS: Posterior.***

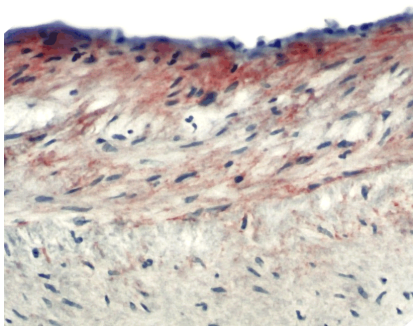

Figure 3 – *Neonatal aortic coarctation. The diffuse presence of the collagen IV below basal membrane. IHC staining on collagen IV, ×256.*

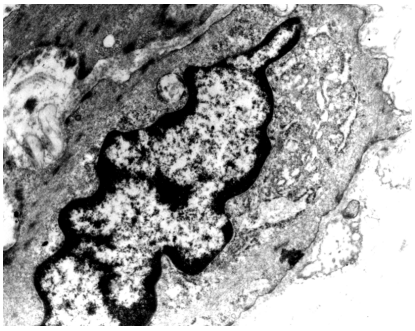

Figure 4 – *Posterior aortic wall. SMC of the synthetic phenotype in the shelf. TEM, ×17 500. SMC: Smooth muscle cell; TEM: Transmission electron microscopy.*

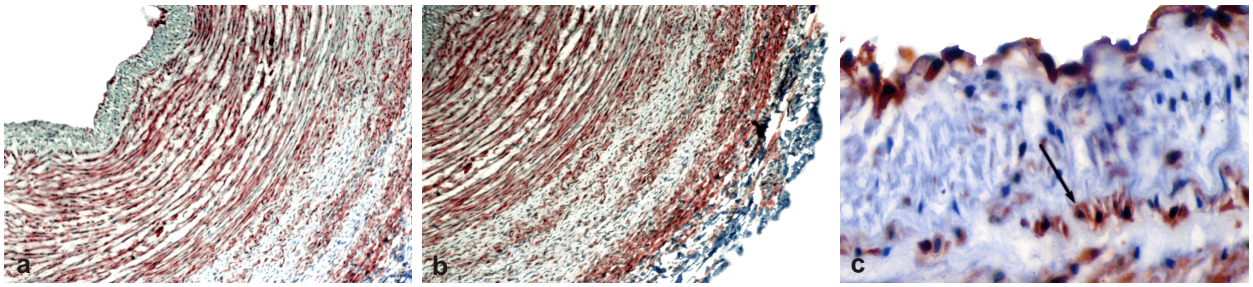

Figure 5 – *(a–c) Neonatal aortic coarctation, posterior aortic wall: (c) Arrow shows VDCs on the border with media. IHC staining on S-100 protein: (a and b) ×320; (c) ×256. VDCs: Vascular dendritic cells; IHC: Immunohistochemical.*

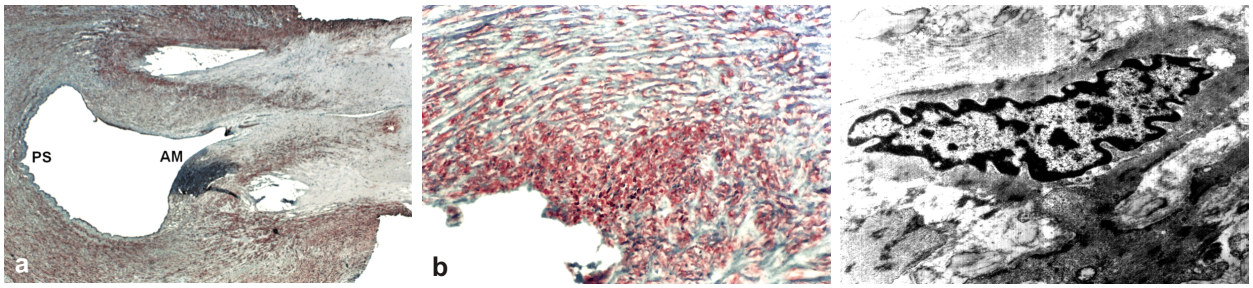

Figure 6 – *(a and b) Neonatal aortic coarctation, anteromedial (ductal) wall. IHC staining on α-SMA: (a) ×80. IHC staining on MHC: (b) ×256. IHC: Immunohistochemical; α-SMA: Alpha-smooth muscle actin; MHC: Myosin heavy chain; AM: Anteromedial; PS: Posterior.*

Figure 7 – *Neonatal aortic coarctation: SMC in the anteromedial aortic wall. TEM, ×17 500. SMC: Smooth muscle cell; TEM: Transmission electron microscopy.*

Discussions

The analysis of the results of neonatal coarctation indicated that dominant change in the aorta wall is the thickening of the posterior part of the wall, which is most prominent proximally to ductus orifice. In this part, the thickening is not limited only to the posterior wall, but spreads along the entire aorta circumference. According to literature, characteristic neointimal thickening is described as the coarctational shelf [3, 6]. The presence of the coarctational shelf in the samples analyzed in this study can be seen in the short segment of the aorta wall proximally to ductus orifice. This observation corresponds to the studies of other authors, which show proximal localization of the shelf (preductal position of the shelf), in the cases of neonatal coarctation [3, 6]. The results presented in this study have shown that the intimal thickness decreases in proximal–distal direction, being completely in the line with the wall in several segments above ductal orifice. The available literature does not provide data on the studies of other authors on the regional distribution of thickenings analyzed from a histological aspect.

The results of our study showed that endothelial layer was preserved in all analyzed samples. The endothelial cells expressed vWF and vimentin. These results correspond to the literature [7].

The results obtained from all analyzed samples showed the absence of collagen IV in the basal membrane, with its simultaneous deposition below it. At the same time, the basal membrane showed intensive immunoreactivity to laminin and fibronectin. It was also selectively stained with the PAS technique.

According to the existing literature, the collagen IV fibers go together with the type V and a great quantity of structural glycoproteins, mostly laminin and fibronectin and a small quantity of proteoglycans, mostly heparan sulphate [8, 9]. This finding suggests that in the samples of neonatal coarctation, *i.e.*, in the early months after birth, there is not collagen IV built in the structures of basal

membrane yet, meaning that in the initial phases of formation it is made of polysaccharide components, laminin and fibronectin, indicating in turn its glycoprotein structure in neonates.

On the posterior aortic wall, in all samples of this group is noticed focal intimal thickening. According to the literature, this finding could correspond to the described "coarctation shelf" [3]. The results of the present study showed that the coarctation shelf is characterized by the presence of a number of proliferating SMCs of the synthetic phenotype (they expressed only α-SMA and vimentin). The loss of desmin expression with concurrent vimentin expression is the first sign in the process of the switching from the contractile to the synthetic phenotype [10–12]. According to the existing literature, vimentin is an intermediary filament that can be found in SMCs with contractile phenotype as well, but it is coexpressed there with desmin. With the loss of the contractile phenotype and characteristic desmin expression (with the switching of cells to the synthetic phenotype), the expression of vimentin filaments can be noticed [10, 11]. The process of losing of contractile characteristics considerably covers the side opposite ductus, where SMCs express only α-SMA and vimentin. At the same time, a large number of cells express PCNA and S-100 protein.

However, it is uncertain to what extent the described process is losing of contractile characteristics and to what extent it is the prevention of SMCs differentiation under the invasive influence of hemodynamic influences. It is known that in the early months after birth, 50% of SMCs still do not express α-SMA, while there are less than 1% of these cells in a grown up organism. Since α-SMA is a proven marker of SMCs differentiation, it is obvious that differentiation of this phenotype is completed in the first months after birth [13, 14].

Literature states that the reactive–adaptive intimal proliferation (the "early response" of the wall) does not have the mechanism of negative feedback and that it can be continued in the form of intimal hyperplasia. According to the existing literature, in the primary response to the conditions of increased pressure, in the first 4–5 weeks (in experimental conditions), there is a proliferation of SMCs in vascular wall [10, 12]. The other studies also confirm that the proliferation of endothelial cells is increased during the earliest stage of remodeling of the vascular wall in the conditions of increased pressure. After endothelial proliferation, during a rather long period of time it is characterized by the SMCs proliferation accompanied by vascular wall thickening [15, 16]. The increased synthesis of collagen and elastin, which is deterioration of gene expression for the synthesis of these proteins, is the explanation for rapid enlargement and irreversible thickening in stress-induced vascular remodeling, in spite of apoptosis, which is activated after the fourth week [16, 17]. The results of our study showed the cell proliferation, which can be observed in the media, but also in the subendothelial layer and adventitia, corresponds to the above-described results of other authors.

By comparison of the results from this study with the results of other authors, where the mechanisms of vascular remodeling have been analyzed [16–18], we could conclude that in the first month after birth, this process has already undergone the stage of endothelial proliferation, intimal hyperplasia (and compensatory vascular dilatation), and that it is in the stage of wall hypertrophy with intensive proliferating activity of cells in all layers.

According to these results, we could conclude that the migrations of medial SMCs in the subendothelial layer, their proliferation and synthetic activity are continual processes, which create neointima [15, 19]. Besides, the participation of ductal medial SMCs in these processes cannot be excluded.

The results obtained from the analysis of all samples on posterior wall, also show a large number of S-100-immunoreactive cells in all parts of the vascular wall. Some of those cells were mainly localized in the subendothelial layer and in the internal part of the media and on the border with intima and were characterized by several processes by which they contacted both endothelial and SMCs. A substantial number of circularly oriented S-100-immunoreactive cells were localized in media and adventitia, and they look similar to the medial SMCs.

The results of this study, which indicate the localization of S-100-immunoreactive cells in the intima and in the internal part of the media and on the border with intima, could correspond to the described usual distribution of vascular dendritic cells (VDCs) [20]. According to existing literature, the antigen-presenting VDCs are described in large arteries intima and to a less degree in normal intima, while they can be found in the incomparably bigger number in the intima of atherosclerotic lesions [20, 21].

The observed S-100-imunoreactive cells in media of the analyzed samples are most likely modified SMCs. Based on the available literature, it has been established that some vascular SMCs can express S-100A6, one of 19 subtypes of the S-100 protein [22]. The fact that only some SMCs express S-100 protein is possibly related to the different embryonic origins of SMCs in different parts of the circulatory system. SMCs of large arteries in the upper parts of the body can have a neuroectodermal origin (S-100-immunoreactive); in the arteries of the lower parts of the body, they mainly originate from the mesoderm; and in coronary arteries, they originate from a proepicardial organ [23]. It has been assumed that the SMCs of the aorta, due to their neuroectodermal origin, can express some types of S-100 protein. Furthermore, it is also known that S-100 protein expression is increased in cells that are in the process of differentiation, protein phosphorylation and what is particularly important here, proliferation – events that are Ca^{2+}-mediated [22, 24, 25]. According to the same literature, proliferated vascular myofibroblasts can express S-100 protein as well [22].

The finding of CD34-, S-100, PCNA- and α-SMA-immunoreactive fibroblasts in the adventitia of analyzed samples could indicate that in the mechanism of adaptive response this layer of the wall is also included. Moreover, fibroblast proliferation is observed in this place. The proliferation of the myofibroblast is the phenomenon of the "early vascular response" to the conditions of increased

pressure [25, 26]. According to the same data, the increased expression of the transforming growth factor (TGF) in the adventitia (and other layers of the wall as well) is the signal for differentiation of fibroblasts which start to express α-SMA and then to migrate. Once activated, fibroblasts express not only α-SMA, but other markers of SMC differentiation as well, which makes it hard to differentiate of medial SMCs [25–27]. At the same time, arterial media shows a considerably lower level of proliferation with high synthetic activity of the resident SMCs [26, 27].

The lack of CD3- and CD45-immunoreactive leukocytes in the aorta wall complies with current literature, according to which the mechanism of thickening occurrence in neonatal coarctation is non-inflammable in nature [28].

On the ductal side, there are more differentiated SMCs, which express α-SMA and MHC (*i.e.*, the contractile characteristics of SMCs are more preserved). A small number of cells proliferate; they express vimentin substantially less. TEM analysis confirms that in the surface layers, there are SMCs of the synthetic phenotype and in deeper layers, there are those of synthetic and contractile phenotype. On this part of the wall, in all samples of this group, at the point of ductal estuary (on both sides), the dominant change is the thickening in inner media of the aortic wall consisted of SMCs of contractile phenotype (they expressed α-SMA, MHC and desmin) and their origin could be from *ductus arteriosus*. This medial thickening on the anteromedial wall is especially prominent at the point of the transformation of ductal media (characterized by the presence of SMCs with contractile phenotype) into aortic media (characterized by the presence of SMCs of the synthetic phenotype). According to existing literature, this finding could correspond with the "ductal sling" that was mentioned in literature [3].

The results of the present study, which indicate the hypertrophy in the media of the anteromedial wall on the point of ductal orifice (the ductal sling) and hypertrophy in the subendothelial layer of the posterior wall (coarctation shelf), corresponds with two existing hypothesis of the coarctation developing, derived from the clinical studies of other authors: hemodynamic hypothesis and hypothesis of ductal sling.

Those results could comply with the existing hemodynamic hypothesis, under which coarctation is developed in the conditions of increased ductal flow, which causes the hypertrophy of ductus and its remodeling [29]. According to this hypothesis, in the conditions of isolated coarctation, while ductus is wide-open, blood flow has usual hemodynamic distribution (for fetal circulation) and there is no obstruction in the aorta. Blood from *aorta ascendens* (about 20%) arrives to *aorta descendens* through isthmus. However, closing of ductus (which starts from the pulmonary side and lasts for several days/weeks until the aorta side is closed) causes all its flow to be redirected through the narrowed isthmus, causing increased pressure, which produces remodeling of aorta wall by recorded intimal hypertrophy, the creation of shelf and changes in the media and adventitia [29], which corresponds to the results obtained from the samples analyzed in our study.

The presence of shelf in the conditions of isolated coarctation could also be explained by the theory of ductal SMCs propagation in the aorta media, which is by the "ductal sling hypothesis" [30]. Some researches have assumed the propagation of ductal sling (ductal SMCs) to proximal and distal parts of the aorta wall [3]. Constriction and fibrosis at the time of ductus closing (which are evident in the samples of isolated coarctation of present study), cover ductal tissue, including the tissue in the aorta wall (in the media) and this causes the creation of localized thickening, shelf and consequential narrowing of lumen [3].

The presence of the shelf in the cases of coarctation joint with the obstruction of left ventricular outflow tract and open ductus, also could comply with the hemodynamic hypothesis [31]. According to this hypothesis, in normal fetal hemodynamic distribution, as it was mentioned above, there is big blood flow in *aorta ascendens* and *descendens* and in ductus as well, but it is reduced in isthmus. In the lesions of the obstruction of left ventricle exit tract, the flow through *aorta ascendens* is additionally decreased and ductal flow is increased [32, 33]. According to this theory, intraluminal shelf can appear as the result of increased ductal flow in the direction of the posterior aorta wall [33]. The posterior wall responds to the conditions of increased pressure with remodeling, by compensatory dilatation and then hypertrophy, which can be also found in all observed samples in the present study.

According to the "hypothesis of ductal sling", in the cases of samples where coarctation is jointed with the obstruction of the left ventricular outflow tract, ductal orifice is wide-open, without the signs of obliteration in the aorta wall (*ductus arteriosus persistens*). In those conditions (when ductus is wide-open), ductal SMCs can easily migrate to the aortic media [3]. The results of the present study showed the presence of well-differentiated SMCs of the contractile phenotype in the form of a "pad", in the place of ductal and aortic media connection, directed normally to SMCs of the synthetic phenotype in the aortic media. This result could comply with hypothesis of ductal sling. Also, in those conditions, when ductus is wide open, we could conclude that the posterior aortic wall is exposed to increased blood pressure and shear stress, which initiate intensive remodeling and formation of intimal thickening, found in all samples analyzed in the present study.

Conclusions

On the posterior aortic wall, in all samples of this group was noticed a focal intimal thickening, with accumulation of proliferating SMCs of the synthetic phenotype. At TEM analysis, those SMCs show a fibroblast-like morphology, so their functions could be to proliferate and secrete ECM components as SMCs of the synthetic phenotype. In all studied samples of the neonatal coarctation, on the posterior aortic wall, the immunocytochemical and TEM examination revealed the presence of SMCs of the synthetic phenotype in intima, media and adventitia. Results also showed an increase of the cell number in intima of this part of aortic

wall, followed by proliferated SMCs in inner media and absence of inflammatory cells. This finding suggests that proliferation of the SMCs, their synthetic activity and increase of the cell number could lead to intimal thickening – a "coarctation shelf" on the posterior wall.

On the anteromedial wall, in all samples of this group, at the point of ductal orifice (on both sides), the dominant change was the thickening in inner media of the aortic wall – a "ductal sling". It was especially prominent at the point of the transformation of ductal media into aortic media. This thickening makes the impression of ductal media spreading into aortic media. The IHC and TEM analysis of the medial thickening indicated SMCs with contractile phenotype and their origin could be from *ductus arteriosus*.

According to these results, we could conclude that the migrations of SMCs from the media to the intimal subendothelium of the posterior aortic wall, their proliferation and synthetic activity are continual processes, which create neointima. Besides, the participation of SMCs from ductal media in these processes cannot be excluded. This process could be caused by specific hemodynamic circumstances in neonatal coarctation, which is, in some cases associated with wide-open *ductus arteriosus persistens* (as it is the case in 10 samples of this study), or in the conditions of isolated coarctation (as it is the case in 20 samples of this study). An increased flow through *ductus arteriosus persistens* at the level of ductal orifice (located on anteromedial aortic wall) leads to vascular remodeling of the opposite side, that is, posterior aortic wall and to formation of intimal thickening on this part of wall or in the conditions of isolated coarctation, when closing of ductus cause increased pressure through the narrowed isthmus, which also produces remodeling of aortic wall. By comparing the results from this study with the results of a great number of contemporary studies, where the mechanisms of vascular remodeling have been analyzed, we could conclude that in the first month after birth, this process on the posterior aortic wall has already undergone the stage of endothelial proliferation and intimal hyperplasia, with compensatory vascular dilatation and that it is in the stage of wall hypertrophy, with prominent proliferating activity of cells in all layers.

Conflict of interests

The authors declare that they have no conflict of interests.

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