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Morphometric study of aortic wall parameters evolution in newborn and child

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Abstract

The largest artery in the human body, intimately connected to the heart, aorta is usually regarded as the major source of oxygenated blood for the circulatory system. The three concentric layers, which surround the aortic lumen-the tunics intima, media and adventitia, transform the aorta in a large elastic duct, which is irregular calibrated according to its segments. The special aortic distensibility is facilitated by its elastic circumferential lamellar complex. Any disturbance of its structural components is able to interfere with its normal and vital activity. Our study intends to reveal that the development of elastic lamellae should be regarded not only as an indispensable step for the aortic wall configuration, but also like a process in a firm connection with the rest of aortic wall components. The transition from intrauterine life to a new stage of life, childhood, has to determine an adequate adaptation of almost all the components of aortic wall, in order to sustain a consistent pulsatile blood flow. Stereological quantitative analysis of thoracic aortic fragments prelevated from newborns and children was performed in order to estimate the dynamic of vascular wall increase. We first estimated the general configuration of the thoracic aortic wall, quantifying the principal constituents; the connective tissue profile, investigated through its main elements, collagen and elastic fibers, supports the idea that each type of fiber has a distinct evolution in different groups of ages and has to be correlated with their involvement in maintaining of the aortic wall mechanical properties. Elastic fibers percentage volume was increased in both examined groups, with a small difference reported in children aorta, while collagen fibers exhibit a slow increase in children aorta. Our morphometric quantitative assessment suggests that further studies have to draw of in a precisely manner the outline of the secretory well defined function of vascular smooth muscle cells; the elucidation of the manner in which the secretory pathway for each type of fiber becomes fully adapted to every stage of aortic development will allow a new perspective in aortic pathology.

Keywords: aortic configuration, morphometric, dynamic, newborn, childhood, quantitative.

Introduction

The approach of the aortic pathology has to be concerned with a correct age related comprehension of aortic structure. Aorta is one of the most suggestive examples of the direct relationship between biomechanical properties and vascular structure. Its own dynamic regarding compliance is generated by the components of the aortic wall, which are able to define an extremely large and extensible artery. In this way, the aortic structure with its closest position to the heart and its significant caliber sustains a high systemic blood pressure.

The aortic wall contains three layers or tunics. The tunica intima, the innermost layer surrounding the aortic lumen, is composed of a flat endothelium sustained by a subendothelial layer (which ensures a variable degree of local mobility). Internal elastic lamina separate the tunica intima from the tunica media, which is responsible for the distensibility of the aortic wall and also is the most representative component in every large artery; tunica media usually consists of a variable number of concentric fenestrated lamellae of elastic fibers, together with dispersed collagen fibers and smooth muscle cells. The tunica adventitia attaches the aorta to the adjacent connective tissue; external elastic lamina separates it from the tunica media. It is argued that the elastic texture from the middle aortic layer is essential for its expanding during maintaining a continuous blood flow [1, 2].

After birth, aorta is confronted with a new circulatory status and it has to be adapted – in this case, the connective tissue elements have to determine a proper configuration for the aortic wall. Vascular smooth muscle cells (VSMC) from medium layer are responsible for the new aortic architecture, because they are the source for both elastic and collagen fibers; if the new findings accurately certify that VSMC can differentiate into the tunica adventitia in stressed aorta, it is also demonstrated the fact that they display an extremely reversible potential [3]. The term of VSMC phenotypic switching, frequently used today, reflect the ability of VSMC to change their phenotype according to a great number of factors, without loosing their secretory function and transforming them in a perfect choice for a therapeutic approach [4].

Despite the complete absence of their implication in a conventional muscular support of the aortic wall, VSMC are really performant as redoubtable "centers" of extracellular matrix synthesis. An adequate secretion of extracellular matrix components – elastic and collagen fibers – will be able to confer to the aorta the essential structural features, congruent with its mechanical characteristics. Signals directed to the VSMC control the synthesis; every vascular component has its significance in the aortic edifice, whatever it is or it is not directly implicated in aortic distensibility [5, 6].

Collagen fibers, often disposed in dispersed bundles between medial elastic lamellae, do not interfere with elastic fibers action, except their overdistension, when collagens prevent vascular rupture. Aortic integrity and tensile strength are considerably diminished in altered collagen synthesis [7, 8].

The elastic configuration represents an essential factor, which individualizes the circulatory system in vertebrates [3]. Elastic structure is indispensable in aortic wall architecture. The lamellar distribution of elastin in the tunica media, keeping a circumferentially arrangement, will transform the aorta in a distensible vessel, as long as blood has to be carried in the whole body [9]. It seems that the elastic fibers are precisely inserted in a lammelar manner, a so-called lamellar unit containing an elastic lamella together with interlamellar fibers and cells. Aortic reversible stretch is generated by the high level of elastin. Aortic circumference is surrounded by these lamellar units, which form a framework for the VSMC [10, 11].

Extremely varied and frequently according to age, aortic pathology opens a broad debate field in which the most significant questions arise from the still uncertain aspects regarding aortic structural characteristics. There is strong evidence that these elements are highly responsive for the vascular mechanical properties and for the aortic damage. Quantitative analysis of aortic wall components in normal and in pathological status, for different stages of life, can be useful for a better understanding of the evolutive dynamics of aortic wall [12, 13].

Materials and Methods

Samples of thoracic aorta prelevated from 12 newborns and 12 children (who did not die of any cardiovascular disease) were used for morphometric assay. The age of children ranged from two to 12 years. The study was performed in accordance with guidelines and all imposed ethical criteria of "St. Mary" Children's Hospital, Iassy, Romania.

Histological exam

Paraffin-embedded thoracic aorta fragments were specifically processed; sections were cut at 5 μm and stained with trichromic Szekely and Verhoeff's iron Hematoxylin.

Light microscopy and image acquisition

Aortic samples were examined with light microscopy (Nikon Eclipse 50i). Digital images were obtained with DS Camera Control Unit DS – L2 connected to PC. We finally applied the interactive digital programme PRODIT 5.2. This professional programme allowed us to identify and also to count the structural elements that are distinctive for the aortic wall configuration. Morphometric determination was realized at 40×; after a proper selection from the menu of the desired morphometric method, the results were automatically calculated.

The accuracy of these techniques was dependent on an initial standardization, performed in order to remove any disturbance produced by specific tissular preparation or technical variability.

Stereological analysis

Standard grill with Weibel parallels was used to quantify percentage volumes of the significant aortic components; a combined test-line and test-point in a standard surface will allow the estimation of the percentage volume of the component structures (Figure 1).

Digital overlapping of standard grill on the aquisited images has to be preceded by a distinct grill adaptation for the specific nature of investigated parameters. The sequence of stereological events is formed by the following stages:

- identification of investigated structure (collagen fibers, elastic fibers, muscular cells, interstitium);
- overlapping of Weibel grill on the aquisited microscopic image;
- determination of total point number which has to be count;
- adjustment of lines position in their random intercrossing of randomized structures;
- automatically assay of stereological account;
- statistical assessment of investigated parameters.

The exam was realized at 40×, on a test surface corresponding to 540 sampling points on superimposed Weibel standard grill, the distance between two points totaling 15.07 μm.

Statistical analysis

The resulted data was statistically analyzed. The programme automatically realizes the statistic evaluation for all the measured fields from every group. All comparisons between the two explored groups were realized with χ^2-test and Student's t-test (for differences between investigated groups). Differences were considered statistically significant if $p<0.05$.

Results

The quantification of volume percentage of connective tissue, muscular cells and interstitium was realized on trichromic Szekely stained aortic samples prelevated from newborns (group 1) and from children (group 2) (Figures 1 and 2). For a better approach of investigated structures, we had examined the tunica media from each aortic sample.

The stereological assay for newborn medial aortic structures obtained after morphometric determination revealed the specific percentage volumetric values: connective tissue 79.07%, smooth muscle cells 1.11%, interstitium 19.81% (Table 1).

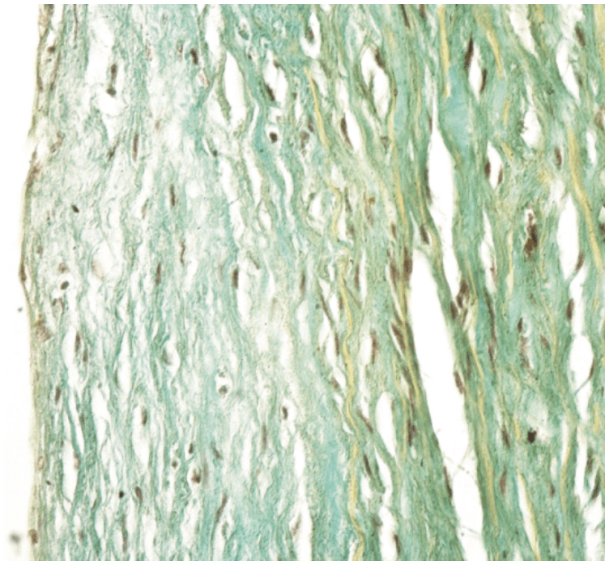

Figure 1 – ***Group 1: Aortic medial layer, trichromic Szekely stain (ob. ×40).***

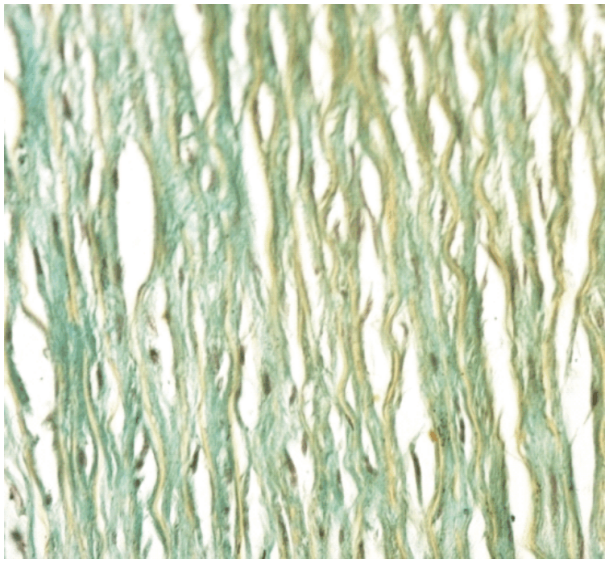

Figure 2 – ***Group 2: Aortic medial layer, trichromic Szekely stain (ob. ×40).***

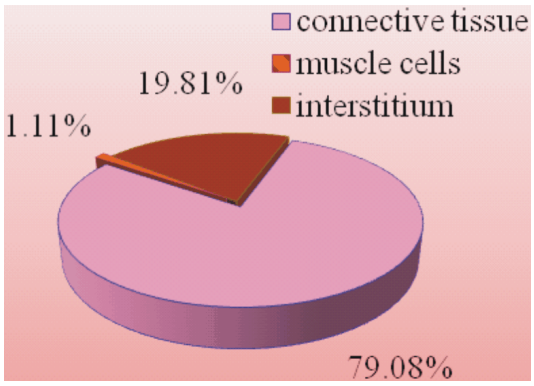

Figure 3 – ***Group 1: Stereological estimation of structural components of the aortic media.***

The stereological assay for children medial aortic structures obtained after morphological determination revealed the specific percentage volumetric values: connective tissue 67.59%, smooth muscle cells 16.11%, interstitium 16.3% (Table 1).

Table 1 – ***Quantification of aortic parameters***

Group	Quantified aortic parameters [%]		
	Connective tissue	*Muscle cells*	*Interstitium*
1	79.07	1.11	19.81
2	67.59	16.11	16.3

Distribution of the specific components of the aortic media pointed to the different values in newborns compared with children (Figures 3 and 4). The dynamic changes in the aortic structure observed during this study are summarized in order to reflect the differences between the investigated groups (Figure 5).

The collagen and elastic fibers, together with other structures dispersed in aortic media were stereologically analyzed on Verhoeff's stained aortic samples (Figures 6 and 7).

The stereological assay for newborn aortic fibrillar content indicated the specific percentage volumetric values: collagen fibers 16.26%, elastic fibers 39.97%, interstitium 16.18% and other structures 27.6% (Table 2).

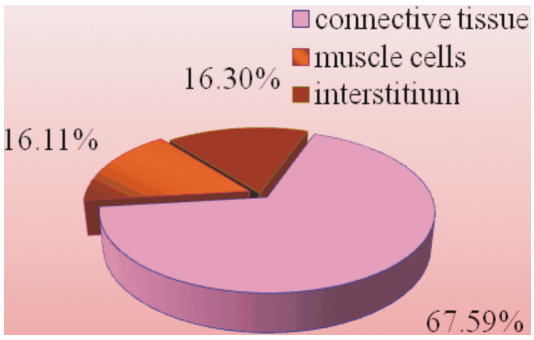

Figure 4 – ***Group 2: Stereological estimation of structural components of the aortic media.***

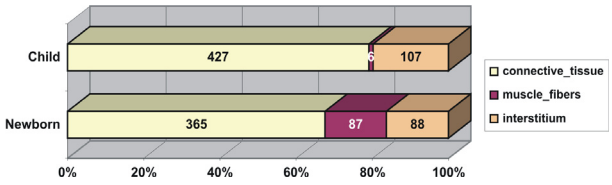

Figure 5 – ***Quantification of percentage volumes of main aortic structures.***

Figure 6 – ***Group 1: Aortic medial layer, Verhoeff's stain (ob.×40).***

Figure 7 – ***Group 2: Aortic medial layer, Verhoeff's stain (ob×40).***

Table 2 – ***Quantification of aortic parietal elements***

Group	Quantified structural parietal elements [%]			
	Collagen fibers	*Elastic fibers*	*Interstitium*	*Other structures*
1	16.26	39.97	16.18	27.6
2	25	34.26	14.63	26.11

The stereological assay for child aortic fibrillar content indicated the specific percentage volumetric values: collagen fibers 25%, elastic fibers 34.26%, interstitium 14.63% and other structures 26.11% (Table 2).

Medial distribution of aortic fibrillar content indicated the differences between the two-investigated groups (Figures 8 and 9). The evolution of aortic fibrillar medial content was summarized in order to allow a correct evaluation for the two investigated groups (Figure 10).

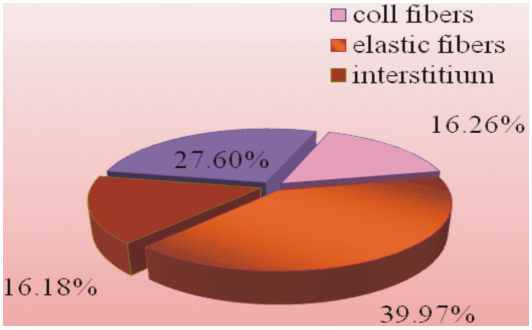

Figure 8 – ***Group 1: Stereological estimation of the aortic fibers.***

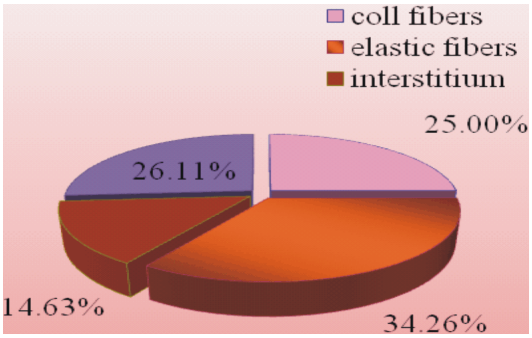

Figure 9 – ***Group 2: Stereological estimation of the aortic fibers.***

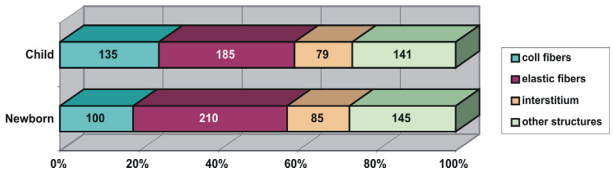

Figure 10 – ***Quantification of percentage volumes of main aortic structures.***

Discussion

Previous studies have demonstrated that there are notable differences between time of birth, infancy and childhood, regarding aortic hemodynamic mechanism, with significant consequences on structure and diameter of vascular wall [14, 15]. Under normal conditions, the tendency of the aortic wall to adapt to gradual changes in blood pressure has been shown to be in a close relation with the evolution of its structural components. Because medial layer of the aorta is considered to be the determinant factor modulating the functions of the largest elastic artery from the human body, it is considered that the elements which form this tunic are responsible for the whole vessel integrity and also reactivity to tensional forces [16].

Medial thickness increases with age, from the moment of birth to adult stage, because of a complex synthesis of well-defined elements with precise roles. Any change in aortic wall composition will be reflected in disturbances of aortic responsiveness to different vascular conditions, especially those due to longitudinal stretch. Elastin, VSMC and collagen fibers form the main tripod that ensures the aortic stability [17–19].

Elastin has to be regarded not only as a sign of evolution of the cardiovascular system (appears only in vertebrates aorta), but also as the essential component of the aortic wall, responsible for its large viscoelasticity [7]. The particular distribution – as fenestrated lamellae –, is the ideal form that allows aorta withstanding to a great blood pressure. The lamellar unit remains the structural and functional aortic key element. The existence and the particular distribution of the elastin pores offer the possibility of a better understanding of elastic design [20]. On the other hand, elastin and collagen are the critical elements in sustaining aortic microarchitecture, the two-interrelated types of fibers displaying a close relation in their way of assembling in the tunica media [21, 22].

VSMC are disposed between elastic lamellae, together with individual fibers or bundles of collagen fibers. There are still discussions about the most adequate perception of the proper distribution of all these elements in the aortic media, referring to their prevailing orientation, their close relationship and their interplaying relations [23–25]. The secretory profile of VSMC continues to remain a real debate subject, because the entire extracellular matrix depends on it: the synthesis and the degradation of this matrix is assured by VSMC, which also maintains the necessary stability and force of vascular wall; there is increasing evidence that VSMC depend on mechanical stimuli, are age related and their secretion may interfere with gender determinants [26].

Our study intended to evidentiate, using a quantitative analysis, the way in which structural elements of the aorta tend to evaluate from the newborn to child. There are two distinct stages of life and it seems interesting to identify which from the constitutive aortic elements has the dominant connotation in determining the vascular new appearance. We had to consider that the different hemodynamic conditions are intimately correlated with an adequate remodeling of aortic structure. We also considered that responsible for the new age-related configuration of the aorta are VSMC through their differentiated secretory pattern of elastic and collagen fibers [27]. The results converge to the idea that the dynamic involvement of VSMC is essential for each stage of development [28].

The stereological estimation revealed that connective tissue is preponderant in both examined groups, but in the newborn it has a greater amount. We can hypothesize that this result is strictly determined by the reduced hemodynamic demand during prenatal stage.

In the same context, the volume percentage of VSMC appears as a significant factor in order to demonstrate a reduced implication in direct synthesis of elastic and collagen fibers immediately after birth, while in children, the volume percentage of VSMC becomes more prominent. The interstitium configuration remains at similar levels in both investigated groups.

The quantification of fibers from the aortic medial layer indicates that elastic fibers have a reduced degree of variability in both groups; since the process of vascular adaptation has to be accompanied with an augmentation of elastic fibers percentage volume, one could expect a clear increase in aortic samples from children. The result can be attributed to the wide age dispersion for the second group. Collagen fibers are moderately increased in second group.

Conclusions

Our study underlines the significance of a better understanding of age related aortic structure, with a direct and high impact on vascular associated pathology. The principal components of the aortic wall have their own progressive pattern of development – collagen and elastic fibers –, together with VSMC have a complex relationship and are the main structures involved in maintaining aortic configuration.

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