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SPECIFIC FEATURES OF AORTIC WALL CALCIFICATION IN PATIENTS WITH THORACIC AORTIC ANEURYSM

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Histological assessment of calcification patterns in different sites of ascending aorta in patients with thoracic aortic aneurysm has been performed using alizarin red S staining. Biopsy specimens from 67 patients (47 males, 20 females, mean age 56,9±11,1 years) with thoracic aortic aneurysm from three different sites of ascending aorta were taken intraoperatively. Calcium content in aorta was calculated as the percentage of positively stained area using FiJi. Statistical data processing was performed using JASP 0.17.2.1. Calcific precipitates were diffusely distributed mainly in the *tunica media* of the vessel. The mean calcium percentage was: for the sinotubular junction (zone 1) - 7.85 [2.2; 15.4]%, mid-ascending aorta (zone 2) – 10.45 [3.83; 22.35]%, proximal aortic arch (zone 3) – 10.95 [4.65; 18.63]% (p=0.216). Patients with maximum calcification level in zone 3 were younger (53 [41; 62] years) than patients in whom the maximum calcification was detected in zone 2 (60 [55.25; 65] years) and zone 1 (64 [58; 68] years, p=0.035).

Keywords: thoracic aortic aneurysm, vascular calcification, alizarin red S.

Introduction. Vascular calcification is a process characterized by the accumulation of calcium salts in the vessel wall. Although calcification is thought to be a part of normal aging, it is associated with a high risk of morbidity and mortality [9].

While the blood vessel wall has three layers - *tunica intima, tunica media, tunica adventitia* - the phenomenon of calcification is described for the first two. Intimal calcification is associated with atherosclerotic disease; medial calcification is often, but not always, associated with diabetes, chronic kidney disease and aging. [16].

Atherosclerotic calcification is the most common form of vasculopathy. It occurs at a young age (already in the second decade of life) immediately after the stage of fatty streaks. Medial calcification is less common and develops along the elastic fibers. Calcium deposits are observed throughout *t. media*, and at later stages become circular, covering the entire circumference of the vessel. In some cases, maturation of osteocytes and bone trabeculae is observed [1].

Thoracic aortic aneurysm (TAA) – a progressive disease that is more likely to occur in older patients (>60 years) [2]. It is supposed that smooth muscle cells (SMCs) play a central role in the development of aortic aneurysms. Normally, they have a contractile phenotype that sometimes may switch to a synthetic. In response to various stress signals, SMCs can induce and enhance calcification through several mechanisms: increased apoptosis, release of extracellular vesicles, loss of natural inhibitors of calcification, such as matrix protein Gla, etc. [23].

Data on calcification of the main vascular beds (coronary/carotid arteries) and aorta is used in clinical practice to predict adverse cardiovascular events [22]. The accumulation of calcium in the coronary arteries correlates with that in the ascending aorta [19] which indicates the systemic nature of the pathological process. TAA calcification may also be a predictor of adverse cardiovascular events, so preoperative assessment of the intensity and volume of calcium deposits in the aortic wall may play a role in identifying risk groups among patients. [13].

Most studies describe a direct correlation between coronary artery calcification index and abdominal aortic diameter [3], however for the ascending aorta data on the association of its diameter with coronary calcium remains inconclusive and require further investigation [4, 8, 12]. Abovementioned studies utilized non-invasive diagnostic methods, and no histological assessment of calcium deposits in patients with TAA considering biopsy site has previously been performed. Thus, the aim of this study was to assess content and distribution of calcium in different sites of ascending aorta in patients with its aneurysm using alizarin red staining.

Materials and methods. The study included 67 individuals (47 males, 20 females) diagnosed with thoracic aortic aneurysm from Department of Cardiovascular Surgery, Cardiology Research Institute. All patients underwent surgical intervention in the period from 2013-2023. The inclusion criteria were: signed informed consent and age over 18. The mean age of the patients was 56.9 ± 11.1 years (minimum and maximum ages were 23 and 76 years, respectively). The mean diameter of the ascending aorta was 51.5 mm (minimum and maximum diameters were 42 mm and 65 mm, respectively).

Biopsy specimens from three different sites of ascending aorta (1 – sinotubular junction; 2 – mid-ascending aorta; 3 – proximal aortic arch, Figure 1) were taken intraoperatively. Fixation, processing, embedding were performed according to standard method. Histological sections

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4-5 μm thick were obtained using an automatic rotary microtome HM 355 S (Thermo Scientific, USA).

The sections were stained with alizarin red S (Dahl's method, 1952) and covered with Vitrogel mounting medium (BioVitrum, St. Petersburg). Light microscopy was utilized to examine the results (Axioskop 40, Carl Zeiss, Germany). Microphotographs of samples were obtained using a Canon G10 camera (Japan).

To assess the amount of calcium deposits in the aorta we measured the percentage of positively stained area using the FiJi program [15]. Up to 7 images (400x) were obtained from each slide, followed by calculation of mean value of the positively stained area (mean percentage of calcification). Statistical analysis was performed using JASP 0.17.2.1 software package. The normality of distribution was checked using Shapiro-Wilk test. Group comparison was performed using non-parametric tests. Results are presented as median, 1st and 3rd quartiles (Me [Q1; Q3]). Differences were considered significant at $p<0.05$.

Results and discussion. Calcific precipitates were diffusely distributed mainly in the *tunica media* of the vessel (Figure 2). Such localization may indicate an isolated process of calcium salt deposition, that is not associated with atherosclerosis, in which salts are located mainly in the subendothelial layer of the *tunica intima* [16]. Calcium density in the ascending aorta has previously been shown to be inversely correlated with the risk of cardiovascular events, as supported by several independent studies. [5, 18].

Medial calcification in TAA may be due to increased degradation of elastin, which is one of the main causes of pathological vascular dilation [20]. One of the possible mechanisms is that transforming growth factor-b (TGFb) in combination with products of elastin degradation stimulates osteogenic transformation of fibroblasts and accumulation of calcium ions in the culture of vascular SMC [21]. In addition, TGFb is known to be one of the key factors in vascular wall remodeling in TAA by inhibiting the proliferation of SMCs in the vessel wall and stimulating the formation of extracellular matrix, which subsequently leads to aortic dilatation [7].

In the present study, we compared the percentage of calcification in different sites of ascending aorta to assess the relationship of their embryonic origin with the degree of vascular calcification. It is known that the most proximal region of the aorta - the root - consists of pharyngeal mesoderm cells [11], aortic arch originates from neural crest cells [14], and the descending part (including both the thoracic and abdominal regions) comes from the somite mesoderm [10].

The mean calcium percentage was: for the sinotubular junction (zone 1) - 7.85 [2.2; 15.4]%, mid-ascending aorta (zone 2) – 10.45 [3.83; 22.35]%, proximal aortic arch (zone 3) – 10.95 [4.65; 18.63]% ($p=0.216$; Figure 3).

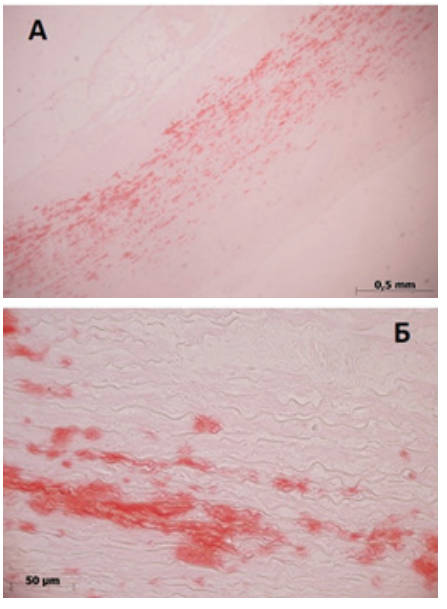

Fig. 2. Calcium precipitates in tunica media of aorta. А – 50x, Б – 400x

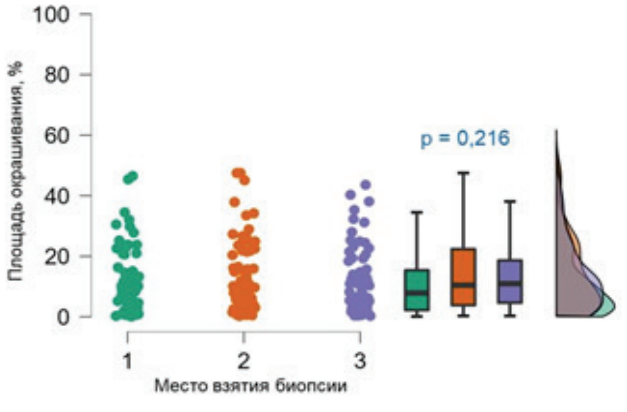

Fig. 3. Comparison of calcium percentage in different biopsy sites. 1 – sinotubular junction, 2 – mid-ascending aorta, 3 – proximal aortic arch

Fig. 1. Biopsy sites of aorta for histological examination. 1 – sinotubular junction, 2 – mid-ascending aorta, 3 – proximal aortic arch

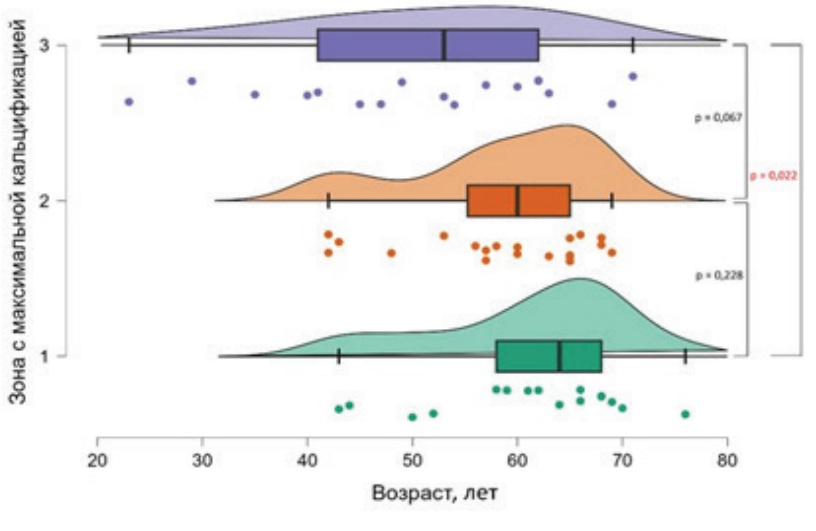

Fig. 4. Age difference in patients with different sites of maximal aortic calcification. 1 – sinotubular junction, 2 – mid-ascending aorta, 3 – proximal aortic arch

Based on mean calcium percentage among 3 zones of ascending aorta, we divided all patients with TAA into two groups with high (17.20 [13.78; 25.28]%) or low (2.2 [4.9; 6.3]%) calcium salts in *tunica media* of the aorta. The median value of calcification (10.3%) in the series of all observations was set as the threshold. Patients with a high percentage of calcification were older compared with patients with a low percentage of calcium deposits in the aorta (63 [56; 66] vs 57 [43;22], p=0.010). As previously shown, risk factors for calcification of the ascending aorta include, among others, older age and male gender [6]. Our results confirm the effect of age, but do not confirm the effect of gender on TAA calcification.

Patients with the maximum calcification in zone 3 were younger (53 [41; 62]) than those with maximum calcification in zone 2 (60 [55.25; 65]) or zone 1 (64 [58; 68]; p=0.035; Figure 4).

The fact that the youngest patients have a greater percentage of calcification in the most distal part of the ascending aorta may be the evidence that the first calcification of the ascending aorta occurs distally in the zone adjacent to the brachiocephalic trunk, and with age "descends" closer to the root. This is confirmed by experimental data: Leroux-Berger et al. in a series of *ex vivo* and *in vitro* experiments showed that the embryonic origin of SMCs affects their ability to mineralize. According to their results, the *tunica media* of the aortic arch, where SMCs are descendants of neural crest cells - calcifies much earlier than the descending aorta, where SMCs are of mesodermal origin [17].

Conclusion. In patients with thoracic aortic aneurysm calcium salt deposits are diffusely localized in the media of the vessel and distributed equally throughout the ascending aorta. Age is one of the factors affecting the calcification of media in patients with TAA. Younger patients with TAA have a greater percentage of calcification in the proximal aortic arch. Further study of calcification of the ascending aorta in patients with an TAA will complete our understanding of the molecular mechanisms of this pathology.

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