MICROSCOPIC IMAGE ANALYSIS OF ELASTIN AND MORPHOMETRY OF WALL OF THORACIC AND ABDOMINAL PORCINE AORTA

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Abstract: We analyzed tissue samples of the ventral and dorsal wall of normal thoracic and abdominal porcine aorta. The results of the two-dimensional Fourier analysis of micrographs of tunica media proved that the Fourier transform provided an efficient method for evaluating transversal and longitudinal cross sections of the elastin membranes and fibres. The shape of the power spectrum of elastin was a simple pattern, whose description was quantified by the shape factor of its polar coordinates histogram. We found no significant differences between paired shape factor values of thoracic and abdominal aorta. There were no significant differences between samples from ventral and dorsal wall of aorta. The FFT-based analysis provided us with reasonable results, therefore its further use can be recommended being aware of its limitations. We suggested several advisable applications of this method for elastin-related research. Lamellar unit thickness was higher in abdominal than in thoracic aorta, unlike the relative elastin content, which was the same in the thoracic as in the abdominal segment.

Introduction

Elastic fibres represent a crucial constituent of the tunica media of large arteries. They are arranged into a complex of branching and anastomosing concentric fenestrated membranes responsible for elasticity and resilience of large blood vessels. The predominant component of these fibres is elastin, an extracellular matrix protein synthesized as a precursor, tropoelastin, mostly by smooth muscle cells and fibroblasts. Elastin itself is an insoluble, hydrophobic and extensively cross-linked protein with considerable degree of branching and interconnections.

Lamellar unit: The basic architecture of the fibrous and cellular components of tunica media of large arteries consists of repeating concentric layers of smooth muscle cells (SMC) separated by collagen and elastic fibres, the latter forming interconnected fenestrated sheets, or lamellae. The number of lamellar units in a vascular segment is related linearly to tensional forces within the wall, with the greatest number of elastic layers occurring in the larger, more proximal vessels that experience the highest wall stress [1]. When the vessel wall is forming, SMC differentiation, lamellar number, and elastin content increase coordinately with the gradual rise in blood pressure until the proper number of lamellar units are organized. It has been suggested that the artery wall is designed to distribute uniformly the tensile stresses to which it is subjected. The basic morphological plan occurs in various modifications. Larger mammals have larger arteries with proportionately more elastin layers, as proved by morphometric studies discussed by Shadwick [2]. Diameter and wall thickness increase in nearly constant proportion and the lamellar thickness remains constant at approximately 15 mm. The number of lamellar units increases in direct proportion to the radius and wall thickness. This led to the conclusion that the elastin-muscle-collagen lamella is the basic structural and functional unit of the aorta. Further refinement of this model based on scanning electron microscopy showed that the elastic tissue between concentric layers of circumferentially oriented smooth muscle cells actually consists of two layers of elastin fibres, each associated with adjacent muscle layers and containing interposed bundles of wavy collagen fibres. While there are no apparent connections between fibres of elastin and collagen, both appear to be linked to the membranes of adjacent muscle cells, from which they are synthesized [2]. Abdominal aorta is more susceptible to atherosclerosis than the thoracic aorta. In abdominal aorta of minipigs [3], medial elastic structure was found to be altered in proximity of atherosclerotic plaques. Research on dissecting aneurysms splitting the wall of the thoracic and abdominal aorta suggested that the difference in propagation might be tied to the difference in elastin structure [4]. The elastin pattern along the whole path of the dissection has not been compared yet. In thoracic aorta of pig, the elastin was reported to be arranged as interconnected parallel sheets with fenestration. In abdominal porcine aorta, the sheets of elastin were described as fused, creating a honeycomb appearance.

Should the morphology of the elastin network be assessed, several parameters could be measured and quantified, e.g. the interlamellar distance or the number of the elastin membranes across the tunica media. Increase

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of the interlamellar distance and microscopically apparent loss of the elastin membranes occurs e.g. in cystic medionecrosis, atherosclerosis, aortic aneurysm and/or inflammatory infiltration of the aortic wall. However, for assessing early and inconspicuous pathological changes of the elastin network complexity, or evaluating its regional differences in normal aorta, these parameters may not be sensitive enough. For the latter purpose, two-dimensional (2-D) fast Fourier transform (FFT) has been introduced [5]. It has been used for the evaluation of degradation of the elastin network in selected images of the tunica media of the AAA, when compared to the samples of normal and atherosclerotic non-aneurysmatic abdominal aorta. Although this approach proved itself to be useful when searching for a universal method of elastin network description, it required further evaluation. The regional differences in pattern of aortic elastin described in the above cited references led us to the decision to perform a further test of the FFT analysis of elastin morphology by comparing the thoracic and abdominal porcine aorta.

Conventional image processing techniques operate within a "real" space. We introduced another, mathematical space representing spatial frequencies of the periodic components of the patterns observed in the real structure. Spatial frequency means the number of intersections of the elastin network with an overlaid straight line per unit length, therefore its dimension is m^{-1}. The image, as observed in real space, is coded as grey level $F(x;y)$ at pixel position $(x;y)$. The function is expanded into its harmonic components by the Fourier transform. The image is first transformed from the spatial domain to the frequency domain (Fourier space) where a visual representation of the frequency content of the image can be examined. Therefore, the image data displayed in the frequency domain can reveal features not immediately apparent in the spatial domain, where our visual system is often distracted by bright objects, other strong features or apparent structural uniformity. Mechanical deformation of the arterial wall, as well as biochemical processes, may result in disintegration of the fine interconnecting elastin fibres in one direction and in straightening of the persistent elastic membranes in the other direction. That is why the spatial frequencies diverge in different directions: they are lower in the direction in which the fibres have been relatively elongated and higher in the direction in which they have been compressed. As a consequence of this, the Fourier transform of the micrograph becomes anisotropic and elliptical. This contrasts with the case of the equiaxial elastin network, where the spatial frequencies are the same in all directions and the Fourier transform of the micrograph of such a structure is isotropic, i.e. circular. The anisotropy of the Fourier transform $F(u;v)$ of the micrograph under analysis reflects the anisotropy of the structure as perceived directly on the micrograph of the structure $f(x;y)$. But the Fourier spectrum $F(u;v)$ is much simpler than the image $f(x;y)$, and therefore the evaluation of the FFT of images is easier and gives more detailed and exact information on deformed structures than the evaluation of the image of the micrograph. Because the Fourier and power spectra are not easily understood, a shape factor of their circular histogram may be used as a single quantitative parameter.

Aim of the study was to test the following hypotheses

H_A – In an individual, there are no significant differences between paired average **shape factor values of circular histograms of 2-D FFT** (fast Fourier transform) **spectra** in both transversal and longitudinal sections of thoracic and abdominal aorta.

H_B – In an individual, the results of FFT of transversal sections do not correlate to those of longitudinal sections through neighbouring samples of the same part in the same individual.

H_C – FFT results of transversal sections have the same variability (standard deviation) as those of longitudinal sections.

H_D – There are no significant differences between average values of shape factor in samples taken from ventral wall and dorsal wall of aorta.

H_E – There are no significant differences in **relative elastin content** between samples of thoracic and abdominal aorta.

H_F – There are no significant differences in **lamellar unit thickness** between samples of thoracic and abdominal aorta.

Materials and Methods

We analyzed samples of the ascendent thoracic aorta $(n = 6)$ and abdominal suprarenal aorta $(n = 6)$ removed from five pigs (weighing between 100–120 kg) freshly slaughtered in the Jatky Plzeň butchery. After removing loose connective tissue, segments of the aortas were processed by common paraffin technique and cut into 5–7 μm thick histological sections stained with Verhoeff's hematoxylin and green trichrome [6]. In both the thoracic and abdominal aorta, closely neigbouring tissue samples were processed in a parallel way – one with cutting plane oriented transversally, the other with cutting plane oriented longitudinally.

In each section, two micrographs represented ventral wall and two images represented dorsal wall, labelled *a*, *b*, and *c*, *d*, respectively (Fig. 1) so that the total number of micrographs under study was $n = 48$ both in group of thoracic and abdominal aorta samples. The captured areas were free of any observable lesions. The micrographs were converted to greyscale (Fig. 2) and resampled to resolution of 682×512 pixels in order to get images suitable as an input for the FFT analysis.

We developed two programs in the software system MATLAB (The MathWorks, Natrick MA, USA). The first one read the 512×512 pixels matrix from the input and performed a 2-D FFT on this data [7]. The second program [8] used the image of the Fourier spectra as an input image and applied the methods for recognition of

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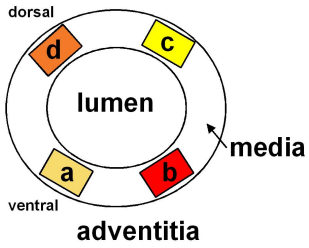

Figure 1: Location of micrographs.

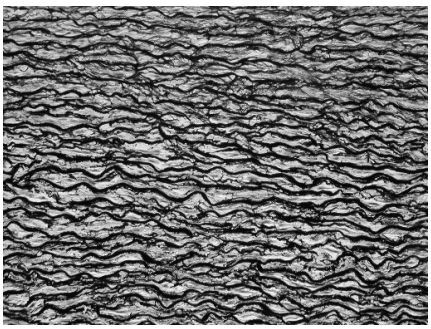

Figure 2: Image converted to greyscale.

the degree of elastin complexity. These methods used calculated histograms in polar coordinates (Fig. 2b), i.e. they summarized the number of white pixels in each direction (0 to 360) from the centre of the image. Then an enclosed rectangle was constructed and the shape factor was calculated as a ratio of its sides (Feret's ratio). A minimum of the shape factor was 0, values near 1 meant small or no deformation of the polar histogram. The methods assume that the deformation of the elastin network may differ among samples under study in the direction of the vertical axis of the micrograph, i.e. perpendicular to the direction of the arterial circumference.

We used the *Oclass* learning classifier module in Lucia software (Laboratory Imaging, Prague) in order to perform a phase segmentation of elastin, collagen and smooth muscle pixels (Fig. 11–12) in the RGB colour space (Fig. 13). After a visual verification, the area proportions of individual phases of micrographs were assessed (Fig. 14).

For assessment of average lamellar unit thickness we

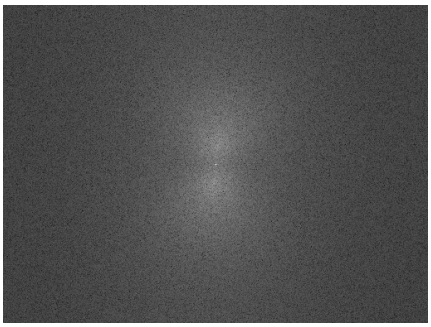

Figure 3: FFT of the Fig. 2.

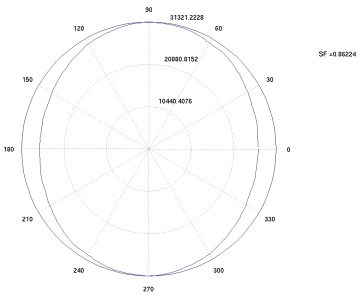

Figure 4: Histogram of the FFT, $SF = 0.86$.

used the *Plot Profile* function in software ImageJ (W. Rasband, NIH, Bethesda, MD, USA) [9]. The testing line was perpendicular to elastin lamellae. In each cross section we assessed three profiles $(n = 72)$. The lamellar thickness was calculated as e/f, where e stood for the length of testing line and f for the number of intersections between lines and elastin lamellae.

Results

Both in the thoracic and abdominal aorta, the tunica media formed a 550–750 μm thick layer composed of 40–60 concentrically arranged anastomosing spiral elastic laminae, which appeared as branched wavy membranes surrounding the 6–26 μm wide spaces containing the fine connective tissue with fibroblasts, collagen, and smooth muscle cells. The elastin system appeared as a network-like complex of bundles with many sections through the branched elastin bridges interconnecting the neighbouring elastic membranes. The Fourier transform of most of these micrographs showed regularity and rotary symmetry. The parallel system of intact elastin membranes with periodicity in the y axis contributed to the high frequencies. This was balanced also in other directions by uniform wavy appearance of highly branched elastin network without elastin loss or media disintegration. These features caused the circular histogram to have a nearly circular shape, resulting in relatively high values of shape factor. An overall morphology of tunica media of thoracic and abdominal aorta is demonstrated in Fig. 5–6, respectively.

(A) The results of paired t-test do not allow us to reject the hypothesis H_A. In an individual, no significant differences between SF values of thoracic and abdominal aorta were found. The results of FFT were not biased by varying values of SF (see the Bland-Altman graph, Fig. 7). The SF values of thoracic aorta were highly correlated to those of abdominal aorta (Fig. 8), $R(thor, abdom) = 0.90$.

(B) Unlike the SF values of transversal sections, the values of SF of longitudinal sections were far from normal distribution, having two peaks (Fig. 9). Although the confidence intervals overlapped, there was no significant correlation between the groups (Fig. 10). We

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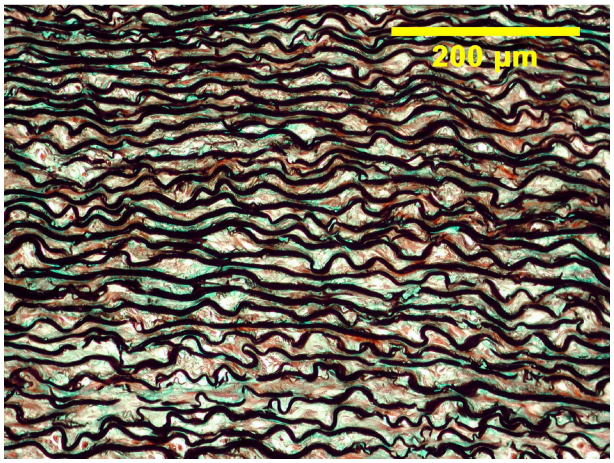

Figure 5: Ascendent thoracic aorta.

Figure 6: Abdominal aorta.

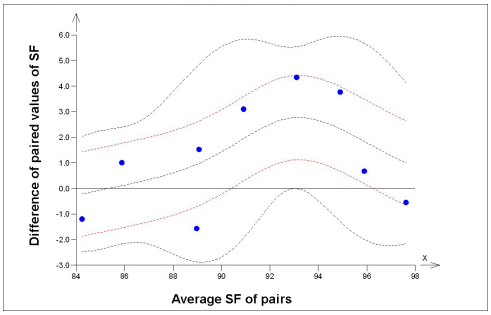

Figure 7: Difference plot (A).

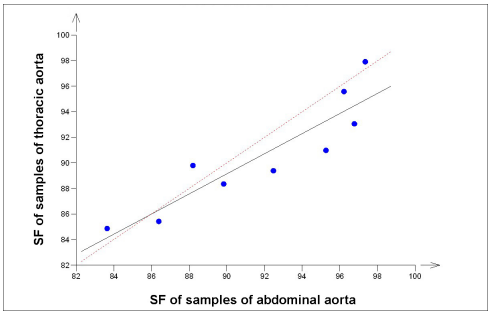

Figure 8: Bivariate scatterplot (A).

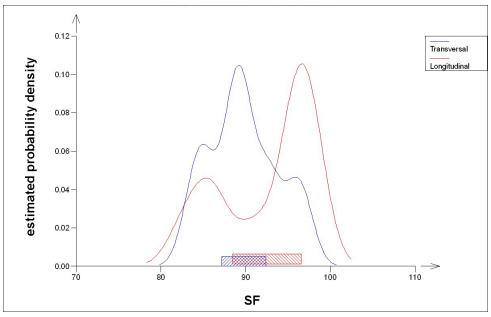

Figure 9: Probability density plot (B).

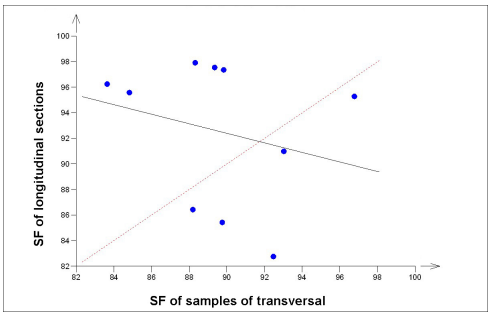

Figure 10: Bivariate scatterplot (B).

are not allowed to reject the hypothesis H_B. Transversal sections represent a different viewpoint than longitudinal sections and their contributions to assessment of the elastin network complexity is not interchangeable.

(C) The test result does not allow us to reject the hypothesis H_C and the differences of SD in transversal and longitudinal sections should be regarded as not significant.

(D) We do not reject the hypothesis H_D. There are no significant differences between mean SF values from ventral and dorsal wall. They are correlated, $R = 0.86$

(E) We do not reject the hypothesis H_E. Relative proportion of elastin does not differ between thoracic (37.59% in average) and abdominal (37.41% in average) aorta.

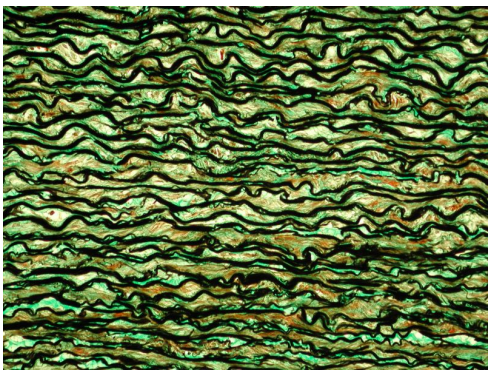

Figure 11: Elastin (black), collagen (green), smooth muscle (reddish to brown) in thoracic aorta.

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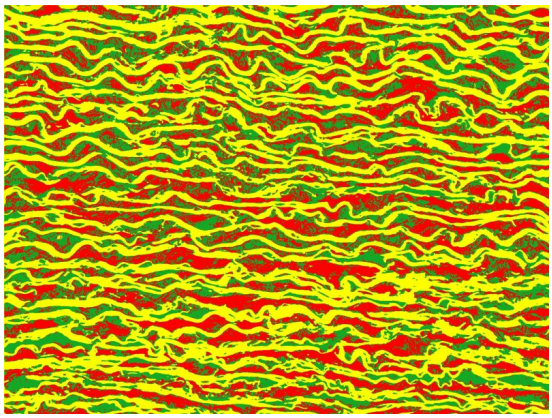

Figure 12: Pixels classified into three phases.

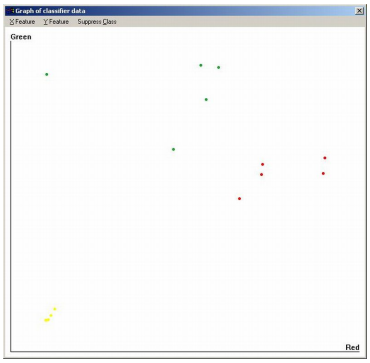

Figure 13: Classifier learning data in RGB space.

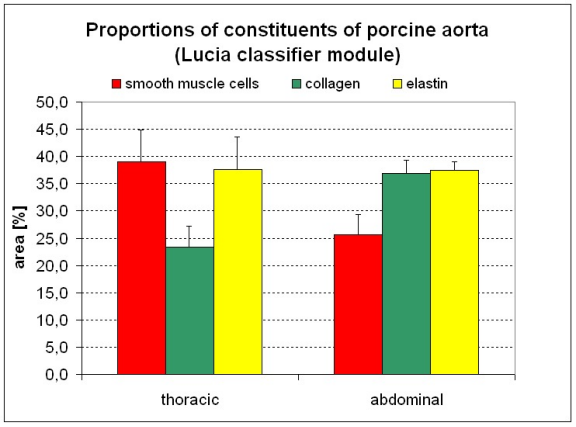

Figure 14: Results of segmentation.

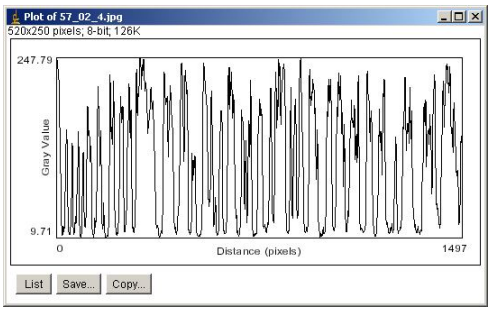

Figure 15: Intensity profile plot. The periodic minima of gray value correspond to intersections of the testing line with elastin.

(F) We reject the hypothesis H_F. The lamellar unit is more thick $(p = 0.03)$ in abdominal aorta (16.70 μm in average) than in thoracic aorta (16.10 μm in average).

Table 1: Results of morphometry of thoracic (TA) and abdominal (AA) porcine aorta. (SD – Standard Deviation, n – number of samples, cross sections or micrographs, SMC – smooth muscle cells, EVD – external vessel diameter, IVD – internal vessel diameter, WT – wall thickness, LUT – lamellar unit thickness.)

Quantity	**AA**		**TA**	
	Mean±SD	**n**	**Mean±SD**	**n**
matrix [%]	2.0±1.0	24	2.0±1.0	24
SMC [%]	38.2±5.94	24	25.2±3.62	24
collagen [%]	23.0±3.77	24	36.2±2.42	24
elastin [%]	36.8±5.92	24	36.6±1.62	24
EVD [mm]	20.2±0.90	6	13.0±0.68	6
IVD [mm]	13.7±0.70	6	10.0±0.60	6
WT [mm]	2.10±0.21	6	1.40±0.15	6
LUT [μm]	16.1±1.40	72	16.8±1.48	72

Discussion

The FFT was not able to discriminate between thoracic and abdominal aorta (HA), which was rather surprising [4]. However, this could be explained by the fact the samples came from young and healthy animals having no obvious reason for different morphology of elastin in thoracic and abdominal aorta. The FFT helps to assess the morphology of elastin network in porcine aorta morphology rather than different amounts of elastin.

Although this paper deals with normal aorta only, it implies several possible ways to contribute to clinical-related research. We suggest that 2-D FFT-based assessment of elastin morphology could be applied to study of following problems.

There could be compared the morphology of elastin between aortas fixed under diastolic pressure and systolic pressure-fixed aortas. Fourier transform is a suitable tool for describing varying degrees of complexity of

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oriented and network-like structures. It is quite sensitive to straightening of elastin which is known to cause the power spectrum to become stretched in the vertical direction and squeezed in the horizontal direction. As a result, the high frequencies in the direction of the arterial circumference (y axis) decreased as did the shape factor [5]. When unpressurised, the elastin lamellae appear wavy and disorganized in longitudinal and transversal sections. With increasing pressure and distention, there is a progressive straightening of these lamellae and a decrease in the interlamellar distances. At the low end of the physiological pressure range, the lamellae are straight and give the appearance of regular concentric cylinders with uniform thickness and radial spacing [2].

In vitro elasticity and residual strain measurements were performed separately on the inner and outer half of the pig aortic media [10]. The reported possible an/isotropy of elastin morphology together with a histomorphometric assessment of the radial distribution of elastin could be verified. In a similar way, evaluation of rotational asymmetry of the elastin morphology in the abdominal aortic aneurysm [11] might contribute to the understanding of the location of preferential sites of aneurysm rupture.

The FFT-based analysis could be used together with other microanalytical techniques as a complementary method in to maximize the information available from the specimen. The structure of elastin could be characterized in terms of textural features, using other stereological methods for quantitation of elastin alteration [12].

The technique described in this investigation requires highly standardized processing of tissue samples and hence is not readily applicable to routine use. Nevertheless, it is suitable for research purposes to study the effects of ageing, hypertension, atherosclerosis, and other conditions associated with changes of elastin. We presented an approach for description of the complexity and regional differences of morphology of elastin network in samples of thoracic and abdominal porcine aorta.

Conclusions

We presented an approach for describing the complexity and regional differences of morphology of elastin network in samples of thoracic and abdominal porcine aorta. Using the Fourier transform of the micrographs, we found no significant differences between paired shape factor values of thoracic and abdominal aorta. We found no significant differences between samples from ventral wall and dorsal wall of aorta. The FFT-based analysis provided us with reasonable results, therefore its further use can be recommended in case of respecting its limitations. We suggested several advisable applications of this method for elastin-related research. We assessed lamellar unit thickness and relative elastin content.

Acknowledgements

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