Annals of Anatomy 225 (2019) 54–56

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.com/locate/aanat

SHORT COMMUNICATION

The vasa vasorum reach deep into the human thoracic aorta

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ARTICLE INFO

Article history:
Received 28 May 2019
Received in revised form 13 June 2019
Accepted 17 June 2019

Keywords:
Human aorta
Vasa vasorum
Aneurysma
Pathophysiology
Aortic surgery
Tricuspid aortic valve
Unicuspid aortic valve

ABSTRACT

Only limited data are available on the extent of the vasa vasorum of the human thoracic aorta, although this could be important with regard to certain pathophysiological states, i.e. aortic aneurysm or atherosclerosis. A preliminary investigation shows that the vascularization of the human thoracic aorta reaches deeper layers than generally believed.

1. Human aortic vascularization — background

Many hypotheses exist on mechanisms of thoracic aortic aneurysm formation but only limited data is available on the vasa vasorum of the human thoracic aorta. The vascularization of the wall of the aorta could be a crucial point in pathophysiology (El-Hamamsy and Yacoub, 2009). According to Sousa et al. adventitial vessels fulfill nutritive functions for the inner parts of the arterial wall. Their publication discusses how deeply the vasa vasorum penetrates the vessel wall (de Sousa, 1960). Clarke found a penetration of the outer two thirds of the media in specimen of the human aortic arch (Clarke, 1965). Baikoussis et al. (2011) assumed that the vasa vasorum reach the outer layer of the aortic media. Most studies were performed in dogs and pigs. A recent investigation in the porcine thoracic aorta used immunohistochemistry (anti-von Willebrand factor antibody) and found that the vasa vasorum had reached the fourth of five virtual layers of the tunica media, layer 1 being the abluminal layer and 5 the adluminal one (Tonar et al., 2016). The density in layer 4 was low compared to the outer layers, and generally the density decreased with the age of the animals. In this preliminary investigation we show that the vascularization of the human thoracic aorta reaches deeper layers than generally believed.

2. Materials and methods

20 samples of the anterior circumference of the ascending aorta slightly above the sino-tubular junction were harvested. The descriptive data with respect to age, gender, valve morphology and status of dilatation are given in Table 1. Fixation was performed using 4% phosphate-buffered formalin (Roti-Histofix 4%, Carl Roth, Karlsruhe, Germany). The specimens were embedded in paraffin for routine-procedure microtomy. Two slices per case were mounted on poly-l-lysine-coated glass slides. The sections were dewaxed, rehydrated and underwent a microwave demasking technique. Immunohistochemistry was performed using primary monoclonal antibodies against Cluster of Differentiation 31 (CD31) (mab from rabbit, clone EPR3094, IgG, anti-human CD31, Abcam, Cambridge, UK, dilution 1:100) and endothelial nitric oxide synthetase (eNOS) (mouse mab, clone 6H2, IgG1, anti-human eNOS, Cell Signalling Technology, Danvers, MA, USA, dilution 1:50) and

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https://doi.org/10.1016/j.aanat.2019.06.001
0940-9602/

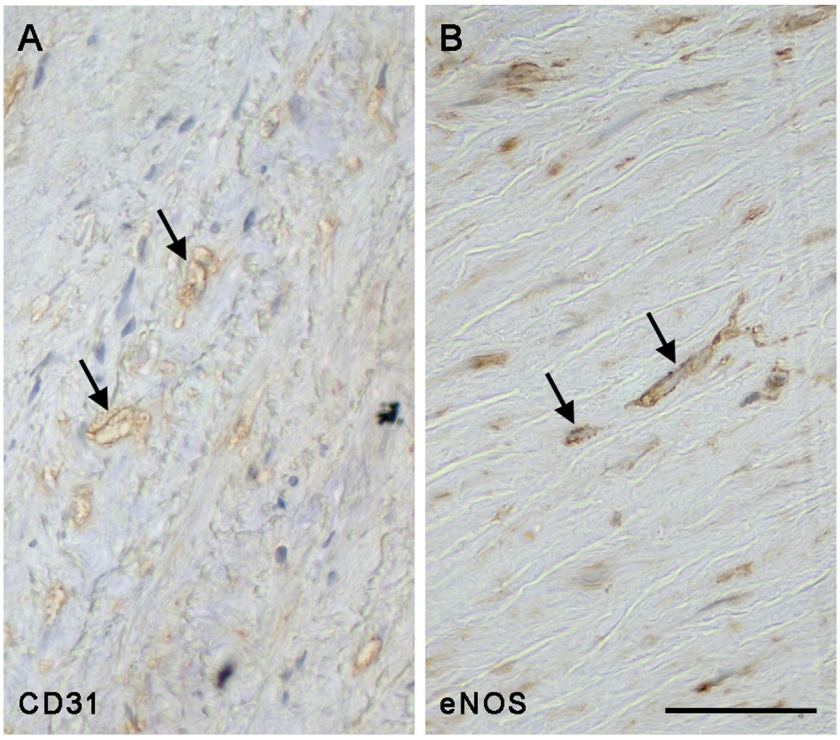

Fig. 1. AB: Representative immunostaining of CD31 (A, left) and eNOS (B, right) in a section of specimen of the human thoracic aorta is shown. The specific labels are in brown color. Vasa vasorum are located within the tunica media close to the tunica intima as indicated by arrows. The magnification bar represents 50 μm in both parts of the figure. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Assignment of distinct parameters to the respective patients.

Patient/age		Gender	Dilatation/valve	CD31	eNOS
1.	70	m	ND/TAV	185	120
2.	66	m	ND/TAV	111	210
3.	48	m	ND/UAV	213	318
4.	38	m	D/UAV	138	173
5.	50	w	ND/TAV	142	101
6.	65	w	D/TAV	123	49
7.	23	m	ND/UAV	133	149
8.	50	m	ND/UAV	167	110
9.	32	m	D/UAV	125	96
10.	36	m	D/UAV	121	142
11.	48	m	D/TAV	96	87
12.	65	m	D/TAV	175	125
13.	70	m	ND/TAV	116	130
14.	36	m	ND/UAV	169	148
15.	63	m	D/TAV	168	125
16.	66	m	D/TAV	70	98
17.	61	m	D/UAV	76	51
18.	71	m	ND/TAV	98	99
19.	66	m	ND/TAV	271	175
20.	64	w	D/TAV	106	197

Abbreviations: age = age in years of life, m = male, w = female, ND = non dilatated, D = dilatated, valve = aortic valve morphology, TAV = tricuspid aortic valve, UAV = unicuspid aortic valve, CD31 = mean of five distances located nearest the aortic lumen in μm, eNOS = mean of five distances located nearest the aortic lumen in μm.

overnight incubation. The secondary antibody was then added (CD31: goat-anti-rabbit POD 1:100; eNOS: biotinylated goat anti-mouse 1:100 and streptavidin-peroxidase) and DAB used for development. A haemalaun counterstaining was performed and the slides were mounted with cover slips using a mounting medium (Roti-Histokitt II, Carl Roth, Karlsruhe, Germany). Light microscopy (Olympus DX60, Olympus, Tokyo, Japan) with 200-fold magnification was used for evaluation. The mean length of the evaluated luminal border was more than 10 mm. From each sample two separate sections were evaluated for both markers. The five signals located nearest the lumen were identified. Then the shortest distance to the luminal border (endothelium) was determined (software CellSens, Olympus). A linear Generalized Estimation Equation (GEE) analysis with random intercept was used to investigate effects of sex, dilatation status and the morphology of the cardiac valve on quantitative CD31 and eNOS values (IBM-SPSS, version 23). The GEE procedure extends the generalized linear model to allow for analysis of correlated observations, such as clustered data.

3. Results

The thickness of the aortic wall was evaluable in 19 cases (mean 1922 μm + SD 547). In one case an accurate measurement of wall thickness was not possible due to processing artefacts. The immuno-stainings against CD31 (Fig. 1A) and eNOS (Fig. 1B) revealed labelling of the endothelium and labelled spots indicating the presence of capillaries of the vasa vasorum. The minimal distance of CD31 labelled spots from the luminal border was 81 μm + 35 (mean + SD) (Fig. 1A). The minimal distance of eNOS from the luminal border was 75 μm + 32 (mean + SD) (Fig. 1B). The average distances of all measured data were 142 μm + 73 μm (mean + SD) for CD31 and 135 μm + 78 μm (mean + SD) for eNOS. A significant influence of gender, dilatation status and valve morphology was not calculated but a tendency was observed: the distances of CD31 and eNOS stainings were lower in the group with a dilatated aorta.

4. Discussion and conclusion

The data presented here must be regarded as preliminary in view of the small number of examined specimens and in view of

specimens of aorta from patients with a specific valve morphology which may have implications for the aortic perfusion. Some questions remain, such as the influence of pathology, e.g. proliferation of the tunica intima (neointima). Although the detection of CD31 and eNOS in combination with morphology is a strong indication for the perfusion of adluminal layers of the tunica media, it cannot be regarded as proof thereof. Open questions are also the exact localization of initial aortic lymphatic vessels and different nerve fibers.

However, the data corresponds well to the findings in the report of Tonar et al. (2016) and it seems most likely the perfusion by vasa vasorum reaches not only the outer part of the aortic wall but also its inner parts.

Author contributions

HJS, PS and ML conducted the study, SW and TT were involved in writing and statistical work and JMF performed the evaluations and wrote the manuscript. All authors approved the manuscript.

Funding

This research received no external funding.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of regional ethics committee (proposal 47/14) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Acknowledgments

The authors thank Tanja Schwab for the stainings, Ingrid Lang for help with the figures and Ann Söther for language editing.

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