RESEARCH ARTICLE

Morphology of the vasa vasorum in coronary arteries of the porcine heart: A new insight

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ABSTRACT

Introduction: The vasa vasorum interna were described during the last decade as a special kind of vessels originating directly from the lumen of the paternal artery and participating in the nourishment of its wall, especially of the aorta and coronary arteries. At the same time, their existence was repeatedly denied/negated by many other authors.
Aim: The purpose of the actual study was the anatomical verification of the existence of the vasa vasorum interna in porcine coronary arteries.
Materials and methods: The vascular supply was studied on the wall of the anterior interventricular branch of the left coronary artery on 36 hearts taken from healthy pigs. Light microscopy, vascular injections and scanning electron microscopy were used for the analysis of 141 samples.
Results: In only two cases small arteries resembling vasa vasorum interna and originating directly from the lumen of the coronary artery were found. But, in both cases these vessels ran without branching, passed over the whole thickness of adventitia and branched in the wider periarterial space. In contrast to this all feeding arteries of the vasa vasorum arose from the larger branches of the paternal artery, branched entirely in its adventitia and did not enter the media.
Conclusion: Due to the very low incidence of these small arteries originating from the lumen of the paternal artery and the absence of their participation on the nourishment of the arterial wall we came to the conclusion that it is not suitable to use the term "vasa vasorum interna" for their designation.

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1. Introduction

While reviewing the literature we came across a large body of work, focused on the study of pathological angiogenesis of the vasa vasorum (VV) and its potential role in the bypass graft disease (review see Stingl et al., 2018) and in the etiology and pathophysiology of atherosclerosis of the coronary arteries (see Williams and Heistad, 1996; Kwon et al., 1998; Mulligan-Kehoe and Simons, 2014).

During this research we came across the work of Gössl et al. (2003a), who described three subtypes of VV in the walls of porcine coronary arteries by using micro-CT: vasa vasorum interna (VVI), vasa vasorum externa (VVE) and venous vasa vasorum (VVV). These results rapidly gained wide spread popularity and the work was highly cited. The most surprising part of their results for us was the description of the presence of the VVI as we had not detected them. In our previous work the VV of healthy vessels were detected only in the adventitia and in all cases arose from neighboring vessels. We did not notice any cases of the VV arising from the lumen of the same vessel it supplied.

The blood supply of the wall of the aorta was first described in 1678 by Thomas Willis and graphically depicted by Frederik Ruysch in 1695. However, it was not until 1739 that Christian G. Ludwig introduced the Latin term "vasa vasorum". After further terminological details were specified the term was used in vascular anatomy throughout the 19th century. In 1895 it was included in the first official anatomical terminology (His, 1895) and remained, unchanged, throughout all subsequent terminologies, including the most recent (FCAT, 1998).

The vasa vasorum are defined as a system of vessels supplying the walls of arteries and veins of predominantly medium and large caliber that arise from the adventitial side of the paternal vessel. From the second quarter of the 20th century interest began to arise in the potential role of the vasa vasorum in the development of atherosclerosis, predominantly in the aorta and coronary arteries. One aspect of these studies was devoted to the morphology of the VV in aortas of various different species of mammals. This led to the first detailed description of the structure of the VVI in a study led by Woodruff in 1926, who described in canine aortas multiple, but consistently present small arteries originating directly from the aortic lumen and branching within its media. Similar findings were subsequently reported in human adults, dogs and sheep by Robertson in 1929. Smetana also described these arteries in dogs in 1929, but did not find them in humans. In 1960, Schönenberger and Müller described similar vessels in cattle and Igarashi described them in pigs. However, it was only the last three authors who named these vessels "*vasa vasorum interna*" to distinguish them from the classic VV, which they named "*vasa vasorum externa*".

While on the one hand research focusing on the normal anatomical structure of the VV was being performed, an extensive body of work began to investigate the pathological neoangiogenesis of the VV in walls of atherosclerotic aortas and coronary arteries, beginning in 1938 with the work of Winternitz et al. These studies repeatedly mention a system of small arteries originating from the lumen, branching into aortic plaques and communicating with adventitial vessels (for an overview see Williams and Heistad, 1996; Ritman and Lerman, 2007; Siow and Churchman, 2007; Sun, 2014; Mulligan-Kehoe and Simon, 2014). Unfortunately, these pathological vessels were not given a term that distinguishes them from normal VVI. A detailed analysis of this clinicopathological topic, however, is not in the scope of our current study.

In 2003, Gössl et al. (2003a) were the first to note the existence of the vasa vasorum interna in the coronary arteries of healthy pigs. They described a system of small arteries originating from the lumen of the artery they supply and, along with the VVE, providing nutrition for the wall of coronary arteries of porcine hearts. It is important to note here that all the aforementioned authors state that the number of VVI is very small, and the nutrition of the vessel wall is predominantly provided by the adventitial VVE.

Finally, it is also necessary to emphasize the fact that, in parallel with occasional reports confirming the existence of the VVI, clinical and anatomical literature repeatedly states, particularly with respect to the coronary arteries, that the stems and ramifications of the VV are located exclusively in the adventitia (Geiringer, 1951; Johnson and Blake, 1968; McCune et al., 1952; Kwon et al., 1998; Subbotin, 2012; Nishimiya et al., 2015a,b; Aoki et al., 2015; Park et al., 2016).

We decided to attempt to resolve this current conflict of opinion over structure of the VV by performing a qualitative morphological study, using light microscopy, combined with two injection techniques and scanning electron microscopy.

2. Materials and methods

2.1. Laboratory animals

The Institute of Animal Sciences in Prague provided the porcine hearts, which were collected from 36 healthy pigs of both sexes (20 males, 16 females), 6–7 months of age and 110–120 kg of weight. All animals were hybrids, bred from the Landras line on the maternal side and the synthetic line H48 on the paternal side. The pigs were bred in natural conditions, without medication or dietary interference, for a complex ethological study — "Sustainable development of livestock in the European model of multifunctional
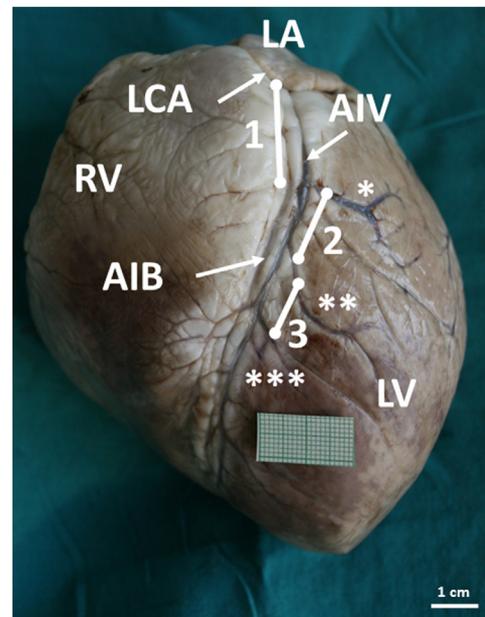
Fig. 1. The anterior surface of a heart after injection of India ink into the AIB. The segments of the AIB are labelled as followed: (1) upper segment (20 mm in length), (2) middle segment (12 mm in length) and (3) lower segment (15 mm in length). The three largest branches of the AIV are marked by asterisks (*, ** and ***) and were used to define the lengths of the three segments of the AIB. Bar = 1 cm.

agriculture" conducted from 2013 to 2015 with the support of grant 0002701404 from the Ministry of Agriculture of the Czech Republic.

After the end of the project the animals were euthanized in the Experimental Slaughter House of the Institute (No. of the official State Registration: CZ 11910002–2009/1412/KVSA/VHI/22.9.2009) by electrocution (the animals were not stunned before euthanasia) using the TBG 98 instrument (MASO.PROFIT in Prague, Czech Republic). The pigs were exposed to 230 V and 1.3 A at a frequency of 60 Hz for 12 s. All animals were euthanized according to the European Union Regulation on the Protection of Animals at the Time of Killing (1009/2009, L303/I, Chapter II, §4.1 and 4.2). The National Veterinary Board in Prague conducts regular inspections of the killing of animals in the Experimental Slaughter House of the Institute of Animal Sciences. After being killed, the pigs were eviscerated and subject to a detailed veterinary examination to ensure the animals were healthy and free from infection at the time of their death. Harvested hearts were transported in physiological solutions at temperatures between 4 and 8 °C to the Department of Anatomy of the Third Faculty of Medicine, Charles University, Prague, where they underwent examination.

2.2. Terminology

Similarly to the studies conducted by Gössl et al. (2003a,b) we focused on the upper, middle and lower segments of the anterior interventricular branch (AIB) of the left coronary artery (LCA) (Fig. 1). Despite being very frequently used in both theoretical and clinical research, this artery is referred to with different names in both porcine and human anatomy:

1 Porcine anatomy
   - Crick et al. (1998): *anterior interventricular artery*
   - Kwon et al. (1998): *left anterior descending artery*
   - Gössl et al. (2003a): *left anterior descending coronary artery*
   - Nomina anatomica veterinaria (WAVA, 2017): *ramus interventricularis paraconalis*
2 Human anatomy

Table 1
Summary of the measurements of the diameters of the lumen and the thickness of the media of the upper (A), middle (B) and lower (C) segments of the AIB. All values are given in micrometers (μm). Staining of the histological sections measured: Weigert's elastic stain with van Gieson counterstain.

	A (n = 21)		B (n = 26)		C (n = 31)	
	Diameter	Thickness	Diameter	Thickness	Diameter	Thickness
	2247	166	1374	250	927	185.5
	1975	177	1447.5	164.5	334	89.5
	1867	246	1306	183	930.5	135
	1901	274	1336	190.5	649	120.5
	1611	279	1020	161	945	194.5
	1708	268	1195	175.7	574.5	128.5
	1540	197.5	1047	177	525	133
	2254	327	1274	290	500	151
	2204	381	1080	153	317	77
	2024	229	1242	189	854	208
	1876	220	1252	191	576	76.5
	2270	165	1160	200	523	160
	1851	229	1018	185	745	186.5
	2176	234	1118	202	481	140
	1703	248	1125	166.5	971	253
	1690	257	1194	163.5	522	183
	1541	215.7	1280	182	223	119
	1922	291	1249.5	165.4	920	176.5
	1596	277.7	1449	225	835	99.5
	1500	248	1370	215.8	901	226
	1624	237.5	1092	318	989	114
			1295	163	651	197
			1145	154	560	220
			1062	153	860	121
			1049	168	740	190
			1463	212	553	200
					815	107
					820	192
					997.5	190.7
					999.5	152.3
					907	133
Total	1860.952± 258.3715	246.0667± 51.3636	1217.038± 137.9105	192.2269± 40.78497	714.3548± 222.9362	156.7581± 46.02797

- Terminologia Anatomica (FCAT, 1998): *ramus interventricularis anterior* and its English equivalent *anterior interventricular branch*
- English clinical term – *left anterior descending artery* (LAD; LADA).

In our work we follow the Terminologia Anatomica (FCAT, 1998) by using the term anterior interventricular branch and the term anterior interventricular vein (AIV) for its accompanying vein. All other terms for branches of the coronary arteries and veins are essentially the same in veterinary and human anatomy; we did not come across any further discrepancies.

2.3. Group A – histology

Thirteen porcine hearts of Group A were fixed "*in toto*" in 10% formalin for 10 days, after which we prepared tissue blocks from the upper, middle and lower segments of the AIB and AIV and the adjacent epicardium and myocardium. We used the three largest branches of the AIV, running subepicardially along the wall of the left ventricle, to define the position of the upper, middle and lower segments (Fig. 1). The lengths of the selected segments ranged from 15 to 20 mm. After being embedded in paraffin, the central areas of each of the three segments were cut transversely and longitudinally into sections of 5 μm thickness. The sections were stained with hematoxylin-eosin, Weigert's elastic stain with van Gieson counterstain, blue trichrome and the endothelial marker CD 34. The size of the lumen and the thickness of the media were measured – for the use in the following statistical evaluation above all – exclusively in sections stained with Weigert's elastic stain with van Gieson counterstain, by the software Quick Industrial 3.0 and the microscope Leica DMLB (resolving power 0.1 μm). Altogether 78

samples were analyzed and the results were statistically evaluated (Table 1).

2.4. Group B – India ink preparations

India ink was diluted at a ratio of 1:1: with normal saline at 26 °C and injected by hand pressure into the ostium of the left coronary artery (LCA) in seven intact hearts. After the entire circulatory bed had been filled with the dye, the hearts were fixed for 10 days in 10% formalin, after which they underwent histological examination, following the same procedure as the hearts of the previous Group A. Altogether 21 samples were examined.

2.5. Group C – Mercox corrosion casts

The LCA in 16 hearts was rinsed with saline heated to a temperature of 37 °C. Following this, 20 ml of prepolymerized resin (Mercox-Cl-2B; Ladd Research, Burlington, VA) was gently mixed with 5 ml of monomeric methylmethacrylic acid containing 8.5 mg paste MA (accelerator) and injected by hand pressure. The injection was stopped when the largest branches and stem of the AIV were filled. The specimens were then left at room temperature (20 °C) for 30 min to allow polymerization of the injected resin and then transferred into a water bath at 60 °C to temper over night. The following morning, the specimens were transferred to a solution of 7.5% potassium hydroxide at 40 °C to remove all organic material. After maceration, the remaining acrylate vascular casts were rinsed several times with distilled water and then submerged for 5–15 min in 5% formic acid. After further rinses, the specimens were frozen in distilled water. The ice-embedded casts were then freeze-dried and glued onto standard aluminium mounts using colloidal carbon. The mounted casts were then sputter-coated with 20 nm
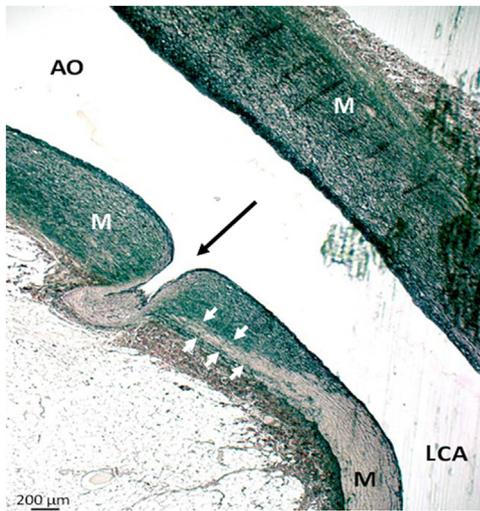
Fig. 2. Longitudinal section of the LCA at its origin on the ascending aorta. Above the well-delineated origin of the LCA (arrowheads) is a small arterial branch (arrow) with a luminal diameter of 70 μm. The thickness of the media of the aorta is 800 μm. The thickness of the media of the LCA is 430 μm. Hematoxylin–eosin. Bar = 200 μm.

of gold in an E5100 Polaron Sputter-Coater (Quorum Technologies Ltd., Ringmer, UK). Altogether 48 samples were examined at the Department of Microbiology, Academy of Sciences of the Czech Republic in Nova NanoSEM 4502 scanning electron microscope (FEI, The Netherlands) at 5 kV with a secondary electron detectorat WDS of 15 mm. In order to minimize charging artefacts, scanning rates of 500 ns and 128 frames or 1 μs and 64 frames integration were used. The 42 highest quality samples were used for the final examination.

List of abbreviations:

A — artery, Ad — adventitia, AIB — anterior interventricular branch, AIV — anterior interventricular vein, AO — aorta, EC — epicardium, L — lumen, LA — left auricle, LADA — left anterior descending artery, LCA — left coronary artery, LV — left ventricle, M — media, MC — myocardium, N — nerve fiber, RV — right ventricle, SEM — scanning electron microscopy, V — vein, VV — vasa vasorum, VVE — vasa vasorum externa, VVI — vasa vasorum interna, VVV — venous vasa vasorum,

All anatomical terms were used according to the Terminologia Anatomica (FCAT, 1998).

3. Results

3.1. Composition of the coronary artery wall

The walls of the coronary arteries differ from those of the aorta (Fig. 2). While the aortic wall has all the features of the elastic type of artery, the coronary arteries are typical muscular arteries, with a thick media containing a large amount of elastic fibres covered on the superficial surface by the adventitia. This morphology is preserved in the entire course of the AIB. Fig. 3 shows the relationships of the AIB to its surroundings. The lower surface of the artery lies close to the myocardium for most of its course while the rest of its surface is surrounded by a large amount of richly vascularized subepicardial adipose tissue.

3.2. AIB – diameter of the lumen and thickness of the media

We noted a successive narrowing of the lumen and a decrease in the thickness of the media of the AIB travelling in the direction of blood flow (Table 1). In the upper segments the average diameter of the lumen was 1860 μm and the average thickness of the media was
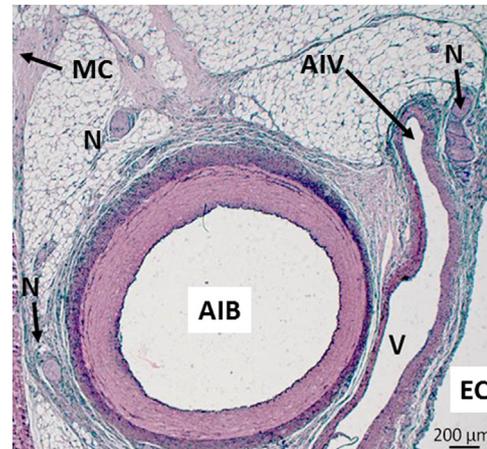
Fig. 3. Transverse section of the middle segment of the AIB and the adjacent AIV (collapsed). The diameter of the lumen of the AIB is 1300 μm. The thickness of the media of the AIB is 180 μm. Hematoxylin–eosin. Bar = 200 μm.
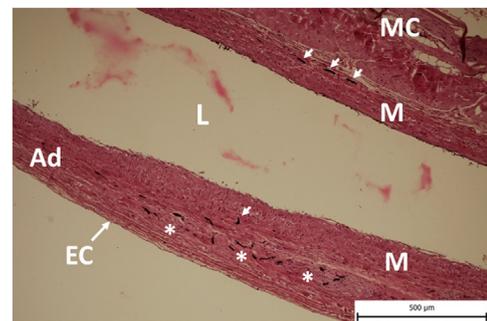
Fig. 4. Longitudinal section of the lower segment of the AIB after injection with India ink. The diameter of the lumen is 500 μm and the thickness of the media is 160 μm. Lumina of the VV filled with India ink (arrows) are visible at the border between the media and adventitia. Part of the subepicardial vasculature is marked with asterisks. Hematoxylin-eosin. Bar = 500 μm.

246 μm. In the middle segments the average diameter of the lumen was 1217 μm and the average thickness of the media was 192 μm. In the lower segment the average diameter of the lumen measured 714 μm and the average thickness of the media was 157 μm. The thickness of the media is the most important value here, as only after it has been measured can the relationship between the media and the vasa vasorum be ascertained.

### 3.3. Distribution of the vasa vasorum – histology

The small blood vessels surrounding the AIB are clearly seen in specimens that had been injected with India ink. This is shown in Fig. 4. In the upper part of the image the VV are located in a discrete border-zone between the external surface of the media, separated from the myocardium by a very thin layer of external adventitial connective tissue. On the side facing away from the heart, there is a rich network of vessels embedded in subepicardial adipose connective tissue. Histochemistry using the stain CD 34 confirmed an identical distribution of vessels in this region (Fig. 5). We did not find any small vessels that originated from the lumen of the AIB and travelling through the adventitia, thus representing the VVI, in any of the samples analyzed using these histological techniques.

3.4. Distribution of the vasa vasorum – Mercox corrosion casts

Mercox corrosion casts allow a significantly more complex depiction of the spatial organization of the microcirculatory bed of
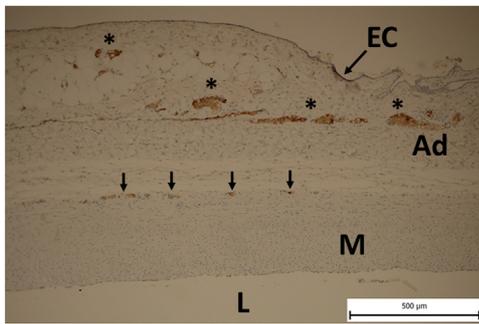
Fig. 5. Longitudinal section of the upper segment of the AIB using the stain CD 34. The thickness of the media is 240 μm. Small lumina of the VV are located in the external surface of the media (arrows). Above the surface of the adventitia, multiple lumina of the subepicardial vasculature are marked by asterisks, extending to a distance of 570 μm from the surface of the lumen of the AIB. Bar = 500 μm.
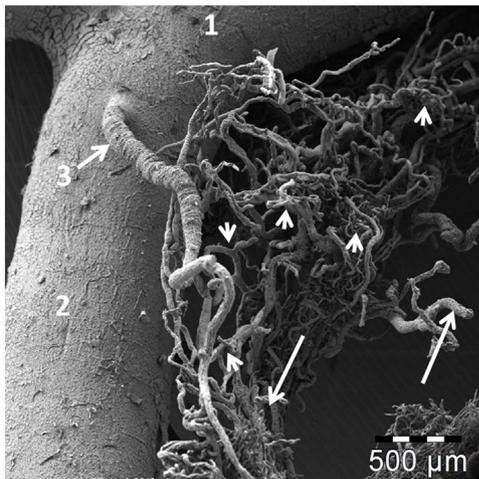
Fig. 6. Mercox corrosion cast of the upper segment of the AIB with some of its branches. A large side branch (2) with a diameter of 1 mm is seen leaving the AIB (1). At a distance of 300 μm this branch gives off a smaller arteriole (3) with a diameter of 160 μm. At a distance of 600 μm from its origin this arteriole starts giving off branches, which then subsequently branch within VV in the adventitia of the AIB (arrowheads), and other branches, which continue into the microcirculatory bed of the subepicardial space (arrows). Bar = 500 μm.

Fig. 7. Mercox corrosion cast of the middle segment of the AIB (1) with some its peripheral branches. Feeding vessels of the VV of the AIB are seen in the form of irregularly organized arterial and venous trunks (A, V). The diameters of the arterioles are around 40 μm and the diameters of the venules are around 50 μm. They are seen entering into the VV (2). Bar = 200 μm.

Fig. 8. Mercox corrosion cast of the VV in the adventitia of the middle segment of the AIB (1). The VV capillary bed is composed of irregularly organised network (2). To the right of this the capillaries are seen gradually collecting into the trunks of the postcapillary venules (arrowheads). The postcapillary venules have diameters of 50 μm. Bar = 500 μm.
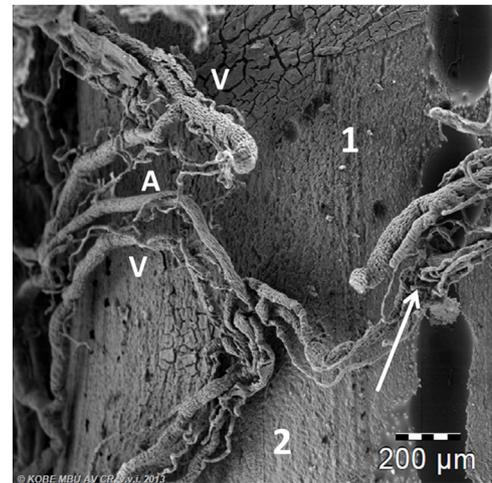
the wall of the AIB and its immediate surroundings. In the majority of the samples the arteries feeding the VV system arose from branches of the AIB and branched either in the subepicardial adipose tissue or, on the side facing the heart, within the myocardium (Fig. 6).

The feeding vessels consisted of an arteriole and venule running usually together as a bundle in the most superficial layer of the adventitia. The largest diameters of the lumen of the arteriole and venule were 40 μm and 50 μm, respectively (Fig. 7). We found no relation between the directions of the trunks of these vessels and the longitudinal axis of the AIB. The feeding vessels branched into an irregular capillary network spreading over the entire circumference of the AIB (Fig. 8).
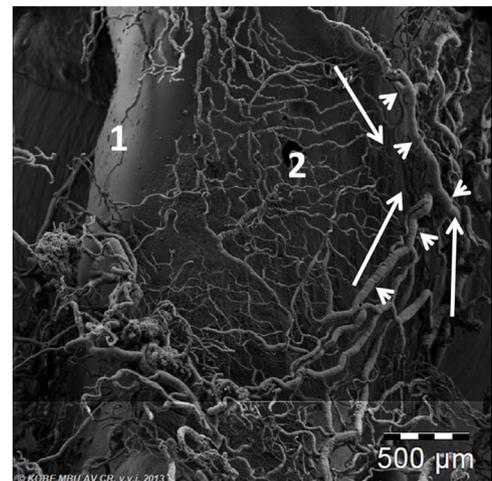
The postcapillary venules on the periphery of the capillary network gradually joined together to form the adventitial venules, which had an average diameter of 50–60 μm and ran in a direction away from the arteries to drain into larger veins, ultimately draining the entire periarterial space.

3.5. Arteries resembling the VVI

Despite being the main focus of this study, arteries originating in the lumen of the AIB and resembling the VVI were detected in only two (4.8%) out of the 42 samples examined (Figs. 9 and 10). The origin and course of these two vessels very strongly matched that of the VVI described in the literature. Our analysis of these vessels focused on their diameter at their point of origin on the AIB, their length and the character of their branching pattern.

The first such artery originated from the middle segment of the AIB with a lumen diameter of 170 μm (Fig. 9). It followed the course of the AIB at a distance of 400 μm and gave off branches in the subepicardial space. Its first branch was located at a distance 1100 μm from its origin, where it divided into two branches with diameters of 100 and 90 μm. On the left hand side of the image the left branch can be seen entering a rich capillary network, which deepest layer may already be part of the capillary network of the VV in the most superficial layer of the adventitia of the AIB.

In the second sample (Fig. 1) we found a small artery, leaving from the subepicardial surface of the upper segment of the AIB, with an initial diameter of 180 μm. At a distance of 400 μm from its origin, it angled away from the AIB. Later, at a distance of 660 μm from its origin, it changed its course to travel parallel to the AIB. All
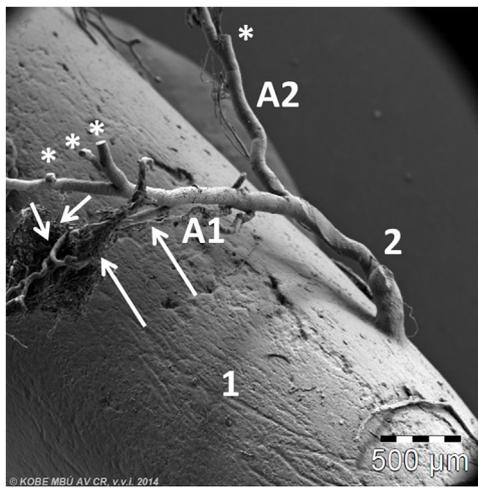
Fig. 9. Mercox corrosion cast of the middle segment of the AIB (1) giving off a small artery (2) with an initial lumen of 170 μm. It takes arched shaped course as it follows the curvature of the AIB at a distance of 400 μm above the lumen. At a distance of 1 mm from its origin the artery divides into two arterioles: A1 with a diameter of 100 μm and A2 with a diameter of 90 μm. Both these vessels give rise to multiple generations of branches (*) within the subepicardial space, and only their several thinnest branches run along the external surface of adventitia (arrows). Bar = 500 μm.
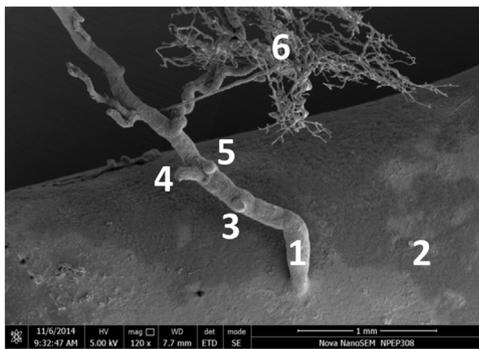
Fig. 10. Mercox corrosion cast of the upper segment of the AIB. A small artery (1) with a lumen of 180 μm is seen arising directly from the trunk of the AIB (2). It travels at a distance of 600 μm above the lumen of the AIB. At a distance of 750 μm from its origin it gives of its first three branches (3–5) into the subepicardial microcirculatory bed (6), which is located approximately 1500 μm above the lumen of the AIB. Bar = 1 μm.

of its initial branches left the vessel at an average of 750 μm from its origin, had diameters between 90 and 100 μm and transmitted their peripheral branches far above the outermost layers of the adventitia, predominantly into the subepicardial vascular network.

3.6. Analysis of the results of Gössl et al. (2003a)

We compared our results with the measurements of porcine AIB (LADA) presented by Gössl et al. (2003a,b) (Table 2). In summary we can state that their values of the diameter of the lumen and the thickness of the media of the AIB as well as the amount of trunks forming the VVE and VVI and their length and diameter are almost identical to the parameters we found in our present study.

4. Discussion

From 42 SEM samples from the upper, middle and lower segments of porcine AIB we were able to identify two cases of a small artery, originating from the lumen of the AIB and branching within the wider perivascular space. No such vessels were found in any of

Table 2
Comparison of our results (P) with results of Gössl et al. (2003a) (G). All values are given in micrometers (μm). Staining in Group/Column P: Weigert's elastic stain with van Gieson counterstain. Group/Column G: No stain was used, all values were obtained from micro-CT measurements.

Object	P	G
Size of LADA – lumen		
Upper segment	1860	1855
Middle segment	1217	–
Lower segment	714	1080
Thickness of media		
Upper segment	246	183
Middle segment	192	–
Lower segment	156	–
# of detected VVI	2	6
Diameter of VVI origin	172, 180	200
Diameter of VVE origin	220, 160	143, 200
Distance: Stem of VVI ↔ surface of LADA lumen	400, 750	570
Distance: Stem of VVE ↔ surface of LADA lumen	625, 780	790
Distance: Origin of VVI ↔ 1st branching	1130, 750	570
Distance: Origin of VVE ↔ 1st branching	1750, 970	714, 800

the histological samples. Although it may at first appear that our results confirm the findings by Gössl et al. (2003a), in reality they bring several points against them.

The first problem arises in the scheme Gössl et al. (2003a) used to present the classification of the VV into VVI, VVE and VVV, a scheme based on the results by Schönenberger and Müller (1960). The scheme published by these authors showed two different types of VV participating in the blood supply of the media of a bovine aorta; the VVI were shown only on two micro X-rays of poor quality. The richly vascularized media of the aorta has been described in various species by a number of authors (Woodruff, 1926; Smetana, 1929; Robertson, 1929; Igarashi, 1960; Wolinsky and Glagow, 1967). In 1960 Igarashi emphasised the distinctive difference between the composition of the media of the aorta and the coronary vessels, a finding confirmed by our results. Coronary arteries are muscular arteries and thus, under normal circumstances have an avascular media. This is evidenced also by the histological description of the AIB by Gössl et al. (2003b), which showed that all parts of the VV are located above the surface of the media, that is, exclusively in the adventitia. Thus, it is clear that as the composition of the wall of two different types of arteries – in our case that of the aorta and of the coronary artery – is different, so to must be their vascularization.

The second problem is that the schemes by Gössl et al. (2003a) do not differentiate the layers of the vascular wall and therefore do not show the extent of the distribution of the VVI and VVE into the media and adventitia of the coronary arteries. Equally ambiguous is that they repeatedly mention that the VV "supply the vessel wall", without any distinction between its two constituents, which is anatomically very misleading.

Gössl et al. (2003b) calculated the average thickness of the media in the upper segment of the AIB to be 183 μm. In our analysis, however, we found the average thickness to be 246 μm in the upper segment, 192 μm in the middle segment and 156 μm in the lower segment. Knowledge of the thickness of the media is extremely important when analyzing the spatial distribution of the VV because while both corrosion casting and 3D micro-CT allow visualization of the casted VV lumina, they do not depict the tissues of the vascular wall as well as the soft tissue of the surrounding space. Thus, only by combining corrosion casts with histological

findings can the distance of the VV to the individual layers of the parent vessel be ascertained.

Here we come to the third and very fundamental problem. While our values concerning the diameters of the feeding arteries of the VVE and VVI, the distance to their first branchings and their lengths are similar to the values reported by Gössl et al. (2003a), when reading Gössl's results it is not possible to establish anything more than the feeding arteries of the VV originate directly from the lumen of the AIB and give off their first branches far above the most superficial layer of the adventitia. In addition, as shown by the results of all previous authors and our own, the number of these small arteries, or rather arterioles, arising from the lumen of the parent vessel is generally considered to be very small (Gössl et al., 2003a, less than 5% in our study). As a result, their actual contribution to the construction of the VV network is minimal and absolutely insignificant in the nutrition of the parent artery's wall.

The fourth problem concerns the much lower resolving power of 3D micro-CT ($20\,\mu m$) compared to scanning electron microscopy. Kwon et al. (1998), found, in the same material and by using the same method (3D micro-CT), VVE vessels of only first and second order, which contrasts with the results of Gössl et al. (2003a). In addition, Gössl et al. (2003b) found a significantly higher density of VV in both subepicardial quadrants of the AIB than on the opposite side. However, they included a relatively large area, which contained not only the VV themselves, but also other blood vessels that clearly belong to the subepicardial vascular bed, a different topographical area, which is significantly more vascularized. Therefore, the question is, when comparing the low resolution of 3D micro-CT with the significantly higher imaging quality of the SEM, how high the predictive value can be for the subsequent sophisticated mathematical analysis (Gössl et al., 2007). Additionally, novel methods for visualising feeding vessels and the determining the size of the VV in man and experimental animals including pigs under normal and pathological conditions work exclusively with only the adventitial VV layer (Nishimiya et al., 2015a,b; Aoki et al., 2015; Park et al., 2016).

After considering all of the above mentioned points, it can be clearly concluded that the occasional occurrence of very small arteries originating directly from the lumen of the paternal artery, in our case that of the AIB, cannot be denied. But, on the other hand, with regard to their low number and to the fact that they predominately branch in the perivascular space, giving only a very few branches to the adventitial VV we do not consider it appropriate to continue to designate vessels of this kind as "vasa vasorum interna", especially in the region of the arteries of the muscular type.

5. Conclusion

We failed to prove the absence of small arteries originating directly from the lumen of the AIB. In more than 40 samples, from all three major segments of the AIB, we found them in two cases only. However, neither of them could be classified as belonging to the VVI because they travelled through the entire thickness of the wall of the AIB without branching. Their first branches were given off above the adventitia and they predominantly supplied the periarterial and subepicardial spaces. Because of their very small number and minimal contribution to the blood supply to the AIB wall we do not recommended considering these arteries as being a special type of vasa vasorum. Instead we consider them as being the smallest branches of AIB supplying its wide perivascular space and part of the subepicardial space.

Ethics approval

The project was approved by the Ethics Committee of the Third Faculty of Medicine, Charles University, Prague, Czech Republic – Ethics Committee Project Nr. 47/2013.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aanat.2019.02.006.

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