

MORPHOLOGICAL ASPECTS OF THE COMMON CAROTID ARTERY IN TURCANĂ LAMBS

ASPECTE MORFOLOGICE ALE ARTEREI CAROTIDE COMUNE LA MIEII DE RASĂ ȚURCANĂ

C. MARTONOS¹⁾, A. GUDEA^{1),*},
V. RUS¹⁾, V. MICLĂUȘ¹⁾, A. DAMIAN¹⁾,
D. PIVARIU¹⁾, A.F. GAL¹⁾, F. STAN¹⁾

ABSTRACT | REZUMAT

The present paper deals with the description of peculiar structures found within the wall of the common carotid artery in the Turcana breed of sheep. The aims to approach a detailed histological investigation by means of regular histologic staining methods, assessing the components of the arterial wall in the area of the common carotid artery in this primitive sheep breed. Based on morphological evaluations, the common carotid artery in Turcana lambs can be classified as an elastic type of vessel, with a series of particular features such as an evident internal limiting membrane for the internal layer, the existence of a slightly higher number of elastic laminae, interlaminar anchoring bridges for the media and a thicker adventice with a significant elastic tissue content.

Keywords: arterial wall, histology, media, Turcana sheep, vascular morphology

Lucrarea de față abordează particularități ale structurilor găsite în peretele arterei carotide comune la oile din rasa Țurcană. Scopul este de a aborda o investigație histologică detaliată prin intermediul metodelor de colorare histologică obișnuită, apreciind componentele peretelui arterial din zona arterei carotide comune la această rasă primitivă de oaie. Pe baza evaluărilor morfologice, la mieii rasei Țurcane artera carotidă comună poate fi clasificată ca un tip de vas elastic, cu o serie de caracteristici particulare precum o membrană limitativă internă evidentă pentru stratul intern, existența unui număr ceva mai mare de lamine elastice, punți de ancorare interlaminare pentru mede și o adventice mai grosă cu un conținut semnificativ de țesut elastic.

Cuvinte cheie: perete arterial, histologie, medie, oaie Turcana, morfologie vasculară

Turcana is an ancient sheep breed that is one of the principal breeds in Romania used for milk, meat, and coarse wool. The breed has evolved since prehistoric times, principally in the Carpathian Mountains.

The breed (with a high heterogeneity within the breed) belongs to the east European zackel group and accounts for up to 70% of the national flock (9). In terms of productivity, nowadays a series of cross-breeds between the local stock and improved breeds are constantly tested, combining the genetic ancient features of the local breed and improved morphology and productivity in order to obtain hybrids with balanced morphological and productive features (50).

Apart from being farm animals, sheep have been proven as a good experimental animals. Important fields of studies are biomaterials, immunology (1, 34), neurology and behavioural studies (22, 49), genetics and evolution (2, 31, 42), or systematic pathology (11, 30). A significant share of studies is dedicated to vascular system-related investigations (3, 8, 10, 13, 15, 16, 24-26, 35, 36), as animal models may serve as

a good starting point for other comparative studies in the field of micro and macro morphology.

The common carotid arteries (*Arteria carotis communis*) originate from the cardiac mediastinum, ascend the ventral cervical area into the area of the jugular groove to terminate in the cephalic area by branches that nourish regional cephalic and cervical structures (36). In small ruminants, the common carotid arteries originate from the brachiocephalic trunk in an acute angle. In other species, the detachment of the left common carotid artery (*Arteria carotis communis sinister*) is cited, followed after a distance by the right common carotid artery (*Arteria carotis communis dexter*) (6). Deep into the parotid region, ventrally to the atlantal wing in a retromandibular position, the common carotid arteries in sheep terminate by the external carotid artery (*Arteria carotis externa*) and the occipital artery (*Arteria occipitalis*) (6), where the carotid body and carotid sinus are described as present (44). Histological investigations on the structure of the arterial wall of the common carotid arteries were previously reported in the literature for dog, sheep, goat, rats, mice, and humans (7, 17, 20, 21, 23, 28, 36, 39, 47). Structurally, the arterial wall is composed of three overlapping tunics: the internal tunic (intima), the mi-

1) University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj-Napoca, Romania

*) Corresponding author: alexandru.gudea@usamvcluj.ro

middle tunic (media), and the external tunic (adventice) (19). The internal tunic (intima) is the innermost layer that comprises three distinctive components: the endothelium, the subendothelial tissue, and the internal limiting membrane (38). The middle tunic consists of smooth muscle cells, elastic fibers, and collagenous bundles. This layer is separated by the internal tunic from the internal limiting membrane and the adventice by an external limiting membrane. According to literature sources (45), the internal limiting membrane may serve as a barrier between the vascular endothelial cells and the smooth muscle cells with significant input in atherogenesis. Depending on the prevalence of the elastic tissue of each tunic, arteries can be categorized into elastic or muscular types (19).

The media plays the most important hemodynamic role (windkessel function), related to the influence of the systolic pressure and turbulent blood flow during ventricular systole (18, 19, 32, 33). Series of studies demonstrated that an increase in the width of intima and media of the common carotid arteries may be a predictor of cardiovascular risk in humans (37, 39). More than that, a "muscularity index" was devised by some authors to compare the relative muscularity of arteries within the wall of different branches (12).

The external tunic (adventice) is formed by dense connective tissue that embeds fibroblasts, fibrocytes, and collagenic fibers. Due to its components, adventice plays an essential function in stabilizing and strengthening the arterial wall (19).

The present study aims to approach a detailed histological investigation, assessing the components of the arterial wall in the area of the common carotid artery in this primitive sheep breed, highlighting some peculiar features of these components as opposed to the standard, well-known description of the arterial wall in classic histology sources. The main purpose of this study was to investigate the particularities of the arterial wall, targeting the minor differential and peculiar aspects of the core structure that was not mentioned up until now in the speciality literature.

MATERIALS AND METHODS

The biological material originates from five 3-6 months old Tsurcana lambs slaughtered for commercial reasons in an approved CE slaughterhouse. The procedure is in line with the recommendations of the permanent OIE (World Organisation for Animal Health) Animal Welfare Working Group for Humane slaughter of animals and AVMA Guidelines for the Humane Slaughter of Animals. The area of the neck was approached immediately for the collection of the common carotid samples into special containers with preserving fluid. Fragments were immersed in 10% formaldehyde solution for a period of three days. A de-

hydration process followed in successive baths of increasing concentration of ethyl alcohol (70%, 96%, and absolute), then clarification on N-butanol successive baths (1 hour), followed by paraffin inclusion.

Slices of 5 µm were stained with Tricromic Goldner (for general aspects) and Verhoeff (for elastic component) stains. An Olympus BX41 phase contrast and darkfield microscope with a digital Olympus E330 camera were used for the assessment of the histological slides. Further digital processing was performed with Adobe Photoshop and Image-J software packages.

RESULTS AND DISCUSSIONS

Both Verhoeff (Fig. 1) and Tricromic-Goldner (Fig. 2) staining methods allowed a good identification of the three tunics of the arterial wall.

The internal tunic (Fig. 3 and Fig. 4) comprises the endothelial cells, the subendothelial connective tissue, and the internal elastic lamina. The endothelial cells (Fig. 3) appear flattened due to direct contact with the blood flow. They seem to be placed in an organized and ordered placement onto the entire surface of the subendothelial connective tissue. Structurally, the endothelial layer includes fine meshes of elastic and collagenic fibers. Externally, under the subendothelial layer, a continuous elastic stripe makes the internal elastic lamina (Fig 3 and Fig. 4). This well-defined layer seems wavy with a pronounced ripple appearance and a clear bounding between the internal and middle tunic. The middle layer (media) (Fig 5 and Fig 6) was easily assessed on both staining methods. The elastic fibers, smooth muscle cells, and connective tissue were clearly made visible. The elastic laminae (11-13 in number, depending on the analyzed sample) had a circumferential placement. In the internal third, their number seemed higher, with a smaller width. The wavy appearance (folding degree) seems slightly reduced and less evident when compared to the situation of the adventitial third of the studied sector. The elastic laminae also showed a certain degree of fragmentation. In the external third of the media (adventitial) the number of the elastic laminae decreases significantly in favour of an accentuated folding degree with less fragmentation.

On Verhoeff stained samples, small elastine anchoring fibers sent from the elastic laminae towards the interlamellar spaces were made visible. These anchoring fibers seemed sometimes interconnected, creating bridges between neighbouring laminae. Such a situation was noticed both in the intimal and adventitial thirds of the media. Another note should be made on the interlamellar spaces- in the intimal third, these spaces are smaller and less identifiable as opposed to those in the adventitial third, that are slightly larger and more noticeable.

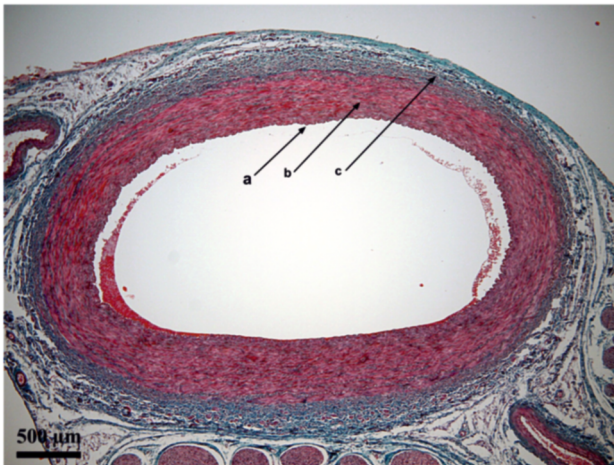


Fig. 1. Structure of the arterial wall, Tricrom-Goldner staining, a. internal tunic, b. middle tunic, c. external tunic

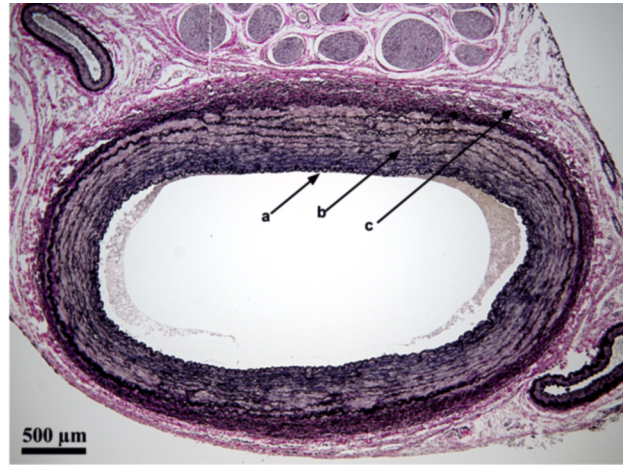


Fig. 2. Structure of the arterial wall, Verhoeff staining, a. internal tunic, b. middle tunic, c. external tunic

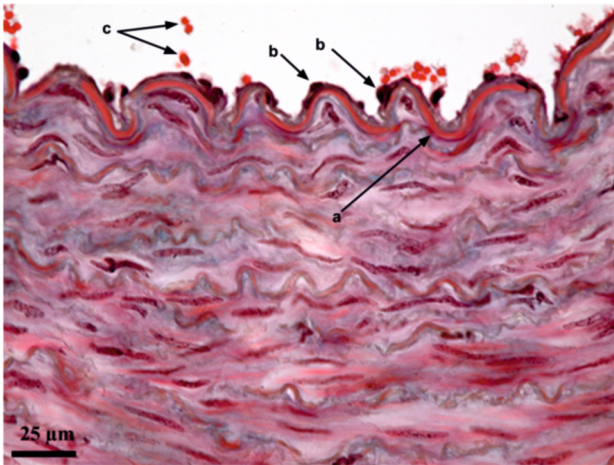


Fig. 3. Morphological aspects of the internal tunic, Tricrom-Goldner staining, a. internal limiting membrane, b. endothelial cells, c. red blood cells

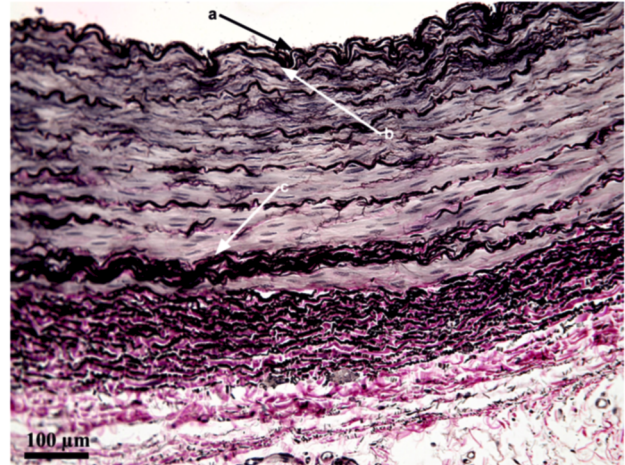


Fig. 4. Internal tunic, Verhoeff staining, a. intima, b. internal limiting membrane, c. external limiting membrane-fenestrated

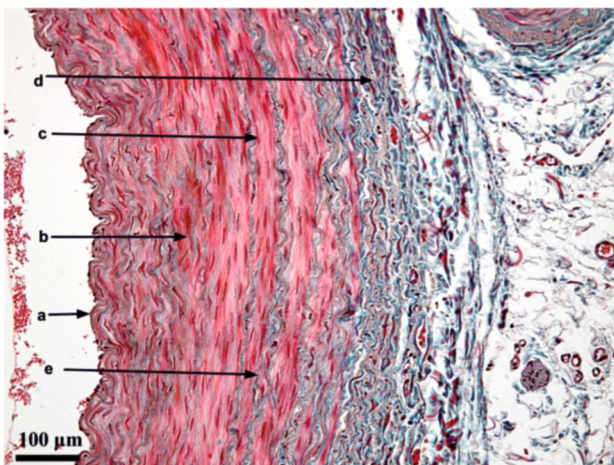


Fig. 5. Middle tunic, Tricrom-Goldner staining, a. internal tunic, b. middle tunic, c. smooth muscle cells, d. external tunic, e. connective tissue

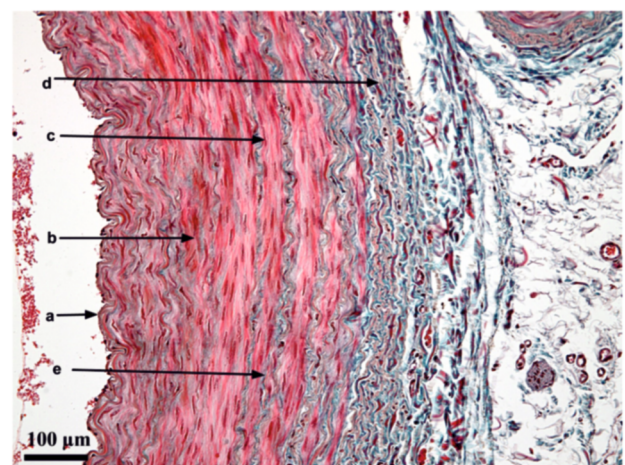


Fig. 6. The middle tunic, Verhoeff staining, a. elastic laminae, b. external limiting membrane (double), c. anchoring fibers (elastin fibers), d. external tunic, e. internal limiting membrane (elastic)

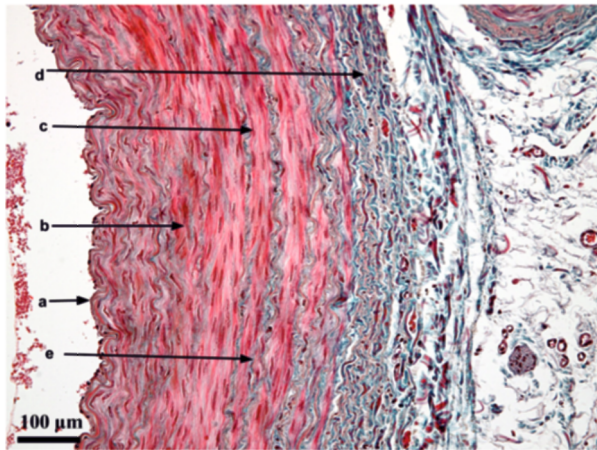


Fig. 7. The external tunic, Tricrom-Goldner staining, a. connective tissue, b. smooth muscle fibers circumpherentially disposed, c. external limiting membrane, d. smooth muscle cells, obliquely or longitudinally placed

The interlamellar space is filled with smooth muscle cells and scant connective tissue. The circumferential placement of the smooth muscle cells is variable. At the level of the internal half of the media, the number of the overlapping lining layers was at a maximum of 3. As we noticed, this number increased to 5 in the external (adventiceal) half.

Marginally, the middle tunic is continued with the external elastic lamina that is significantly thicker than the internal one. More than that, a doubling or even a tripling of this layer might be noticed in some segments of the analyzed arteries (Fig. 6) with a certain degree of fenestration (Fig. 4).

The external tunic (Fig. 7 and Fig. 8) is smaller in size when compared to the middle tunic. Its main components are represented by the connective fibers intermingled with reduced small-caliber blood vessels, nervous fine bundles, elastic tissue, and smooth muscle fibers. A significant amount of elastic fibers, mainly placed in circumferential distribution, can be observed, with a reduced amount of fibers that are either obliquely or longitudinally placed. The elastic fibers are denser in the internal half part of the tunic but thinner when compared to the outer ones. Corresponding to the fenestrated area of the internal limiting membrane, smooth muscular tissue organized as bundles reach the core of the adventice. The muscular bundles are mainly circumferential, but longitudinally-oriented fibers are noticeable, too.

On the basis of the observed structural details, one can state the fact that the common carotid artery in Turcana lambs can be framed as an elastic artery. Similar data were presented for the common carotid artery in goat and sheep (39), brachiocephalic trunk in Japanese quail (46), and chicken (41), the aorta of

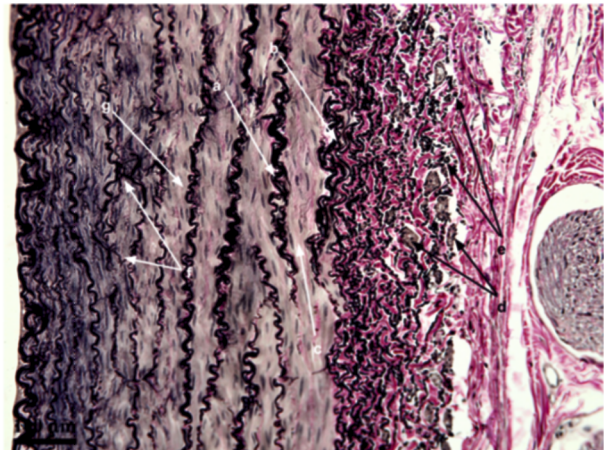


Fig. 8. The external tunic, Verhoeff stainig, a. External limiting membrane-fenestrated, b. circumferential elastic fibers, c. circumferential smooth muscle fibers, d. longitudinal muscle fibers, e. Longitudinal elastic fibers, f. anchoring fibers, g. Elastic laminae of the middle tunic

chinchilla (29), and guinea-pig (4). The same stands for the subclavian artery in mini pig (48), and ascending aorta for dogs (40). Even if the inclusion in the category of elastic arteries is quite obvious, it should be noted that for this species, there are a number of structural formations which make the common carotid arteries in the Turcana breed lambs a particular type of elastic arteries (17).

The structural details of the internal tunic observed by us were similar to the aspects mentioned in the case of elastic arteries in sheep (39), goat (38), and rat (5). In Turcana specimens, the internal elastic limiting membrane is described as wavy, evident, and easily identifiable with respect to the rest of the media elastic laminae. In the case of the goat aorta (38) this segment seems hard to identify (51). In the case of large-caliber arteries, in humans, the membrane is hard to identify or even missing (27). A similar situation was also reported in the case of birds (27, 46).

For the internal iliac arteries in the lamb, the literature also reports cases where the internal elastic limiting membrane is discontinuous or even absent (43). A low degree of folding (or even the total absence of folding) is reported in the literature as an early change linked to aneurysms in major cerebral arteries in rats (51).

The middle tunic (the largest of three) comprises the elastic laminae, smooth muscle fibers, and connective tissue. The same components were noted for goat and sheep (common carotid artery) (39), rat (aorta) (5), chinchilla, and Guinea pig (brachiocephalic trunk, aorta) (4, 29). The number of elastic laminae observed on our samples was slightly higher than the reports found in the literature. It seems that this number is gradually depending on the caliber of the vessel

and the anatomico-topographical distance from the hearth (4). The existence of the interlamellar anchoring bridges has been earlier reported in the aortic segments in humans and rodents (14). In the light of these findings, one can assume the fact that these connections play a significant role in maintaining the characteristics of the interlamellar spaces, given the blood flow pressures within the vascular bed.

The external tunic follows generally the morphological and morphometrical features of the classically-described adventice for the arteries of the elastic type (4, 14, 29). A peculiar aspect has to be mentioned for the Turcana examined samples that address the overall dimensions: significantly higher than in the typical elastic arteries with a higher concentration of elastic tissue. Also, a certain amount of muscular tissue with somehow zonal/regional disposition should be mentioned, as described in the previous section.

CONCLUSIONS

On the basis of the morphological characteristics earlier described in the case of the common carotid samples in Turcana lambs, we can state the framing of the arteries into the elastic type, with some particular aspects. The interesting feature is represented by the existence of the evident internal limiting membrane for the internal layer, the existence of a slightly higher number of elastic laminae, and the interlaminar anchoring bridges for the media and a thicker adventice with a significant elastic tissue content. The identification of these characteristics of the carotid arteries in this primitive race may be proof of some of the morphological features that may have been lost as a result of changes brought by the improvement and selection of the sheep in order to obtain highly-productive commercially exploited breeds.

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Author contributions

CM was the major contributor to the experimental design, dissected and harvested the samples, analyzed data, assisted in all areas of producing the manuscript, and conducted the overall study, GA drafted and wrote the manuscript, partly offered advice in the experimental design and analyzed data. R.V, M.V., and

G.A.F. analyzed data and provided advice and assistance on laboratory work. D.A., S.F., and P.D. provided advice on the experimental design. All authors have read and approved the final version of this manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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