# EKSPERIMENTINIAI TYRIMAI

## Collagen network changes in basilar artery in aging

**Devika Gudienė, Angelija Valančiūtė, Juozas Vėlavičius[1]**
*Department of Histology and Embryology, [1]Department of Pathological Anatomy,*
*Kaunas University of Medicine, Lithuania*

***Summary.*** *Objective. The aim of this study was to examine and evaluate morphometrically age-related changes in collagen network in the tunica media of human basilar artery.*

*Materials and methods. Histological analysis of the basilar arteries obtained from 89 individuals, aged 20 to 85 years, was performed. The study has been carried out by standard histological technique; histological samples were stained with picrosirius red. Detailed quantitative analysis of collagen bundle network in the tunica media of basilar artery was performed.*

*Results. We analyzed collagen network area, perimeter and number of collagen bundles in the tunica media of the basilar artery. After investigation of age-related changes in collagen network area in men and women, we determined that in both genders collagen network area in the tunica media of the basilar artery increased with age. Analyzing the perimeter and number of collagen bundles in both genders by different age groups, it was found that they decreased with age. We found a statistically significant correlation between all the measured parameters and the age.*

*Conclusions. The area of collagen bundles increased, the number and perimeter of collagen bundles decreased with age in both genders in the tunica media of human basilar artery. While aging, the structure of collagen bundle network became less branchy, collagen fibers merged, their cross section area enlarged.*

### Introduction

One of the main hallmarks of arterial aging is arterial wall remodeling, which is the outcome of degenerative processes, but also involves adaptive and compensatory processes (1). With advancing age, a series of structural, architectural, and compositional modifications take place in the vasculature (2). Vascular aging is associated with changes in the mechanical and structural properties of the vascular wall, which leads to the loss of arterial elasticity and reduced arterial compliance (3, 4). In aging, the structural properties of the arterial network are significantly altered. These processes are unrelated to hypertensive or vascular diseases. The collagen, elastin, and smooth muscle contents of the tunica media and the geometrical arrangement of arterial wall components are constantly modified according to the subject's age. These morphometric changes are responsible for changes in the mechanical properties of the arterial walls leading to rigidity (5, 6). The diameter of the vessels tends to

increase. It partially compensates for the effects of arterial wall rigidity on vascular compliance, thereby limiting the hemodynamic and functional changes that occur in blood circulation (6, 7).

Thickening of tunica intima and tunica media is often observed. In the subendothelial space, blood-derived leukocytes and an increased amount of "activated" smooth muscle cells are present. Extracellular matrix accumulates and becomes particularly rich in glycosaminoglycans. Collagen content increases, while elastic fibers appear progressively disorganized, thinner, and frequently fragmented. These changes in the normal architecture of the vessel wall are likely to be the consequence of adaptive mechanisms to maintain normal conditions of flow, mechanical stress, and/or wall tension (2).

Recently more scientific studies have been performed in researching remodeling of collagen. Collagen is one of the most important structures of the connective tissue. It represents a series of proteins that are

broadly related in terms of chemical features, structure, and function (8). Collagens are centrally involved in the formation of fibrillar and microfibrillar networks of the extracellular matrix, basement membranes, as well as other structures of the extracellular matrix (9).

There are few extensive morphometric studies, which evaluated changes of collagen network in human cerebral arteries. A lot of studies were performed, which investigated structural and biochemical changes in other cerebral arteries in pathological cases, but not in relation with aging (10, 11). Evaluation of age-related changes in collagen network in human basilar artery wall might be important for understanding general processes of cerebrovascular aging and vascular pathology.
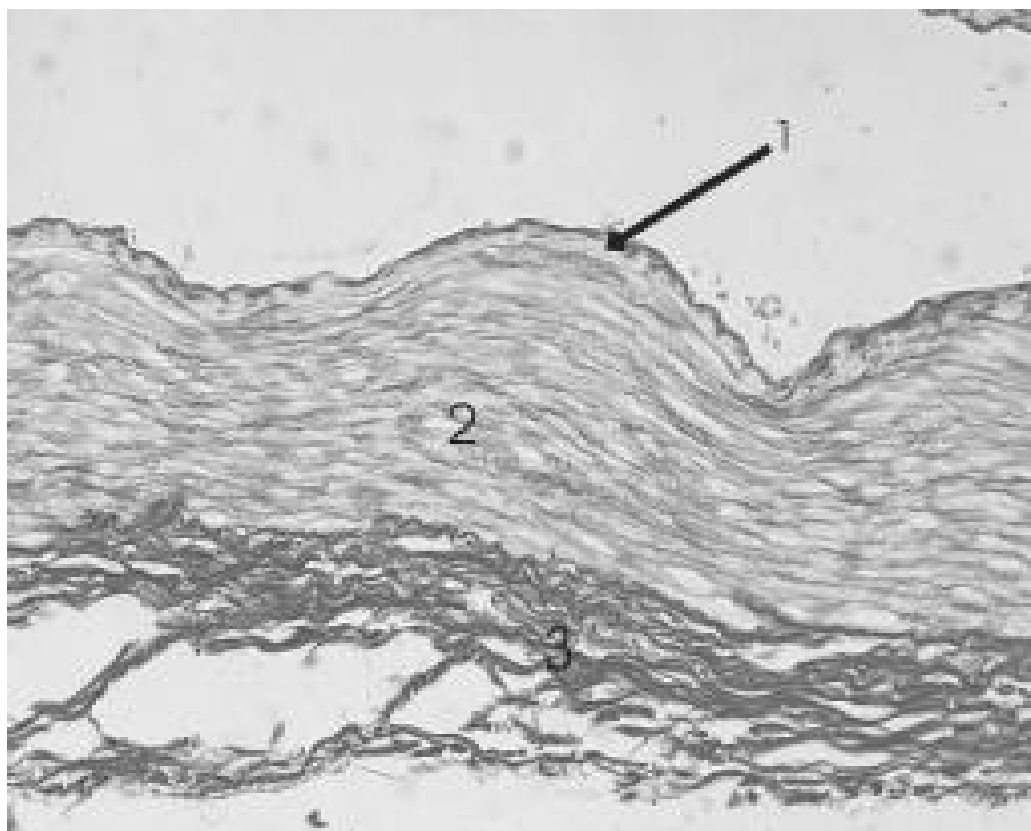
The objective of this study was to examine and evaluate morphometrically age-related changes in collagen network in the tunica media of basilar artery of persons who died from violent death and who were not diagnosed with cerebrovascular diseases or severe atherosclerosis.

**Material and methods**

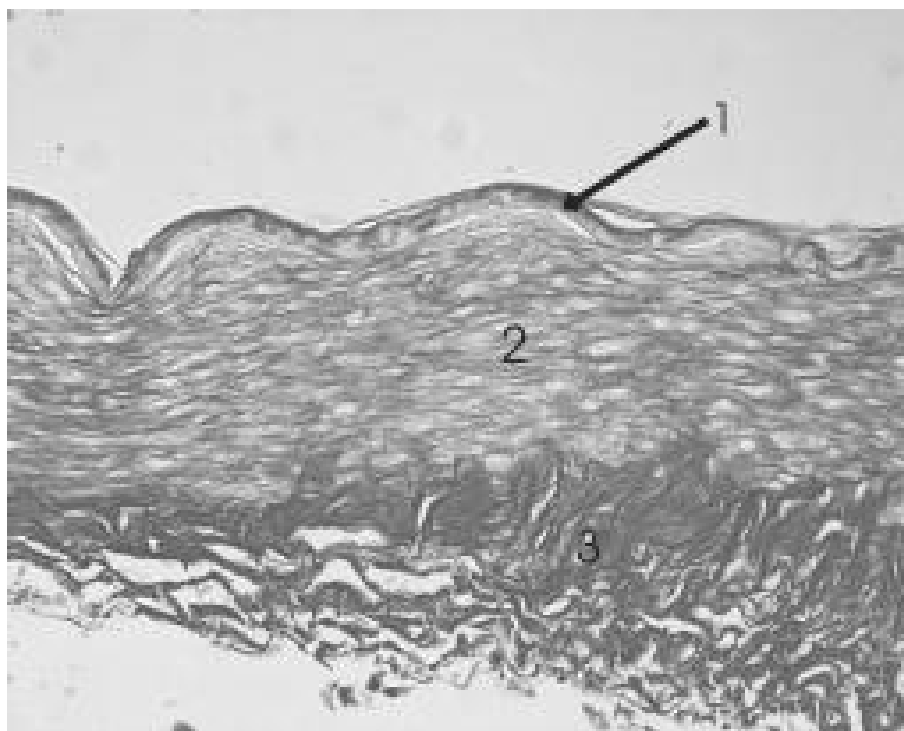Study material consisted of 89 basilar arteries ob-

tained from persons aged 20 to 85 years. We investigated basilar arteries of 44 men and 45 women. Cases were divided in age groups by decades. The young age group consisted of 18 men and 6 women aged 20 to 40 years (mean ages were 28±6.75 years and 36.8± 2.31 years, respectively); the middle-aged group included of 16 men and 20 women aged 40 to 59 years (mean ages were 48±5.10 years and 50.9±3.73 years, respectively). The third group was composed of 11 men and 19 women aged 60 to 85 years (mean age 67±6.65 years and 72±7.46 years, respectively). The formation of human blood vessel wall ends after age of about twenty, when the main parameters of arteries are stabilized. That is why our study material included samples of basilar arteries obtained from victims not younger than 20 years. Cases with specific cerebrovascular diseases or severe atherosclerosis were excluded.

Study material was taken from the middle part of basilar arteries. Samples were fixed in 10% neutral formalin and embedded into paraffin by the standard technique. Paraffin blocks were crosswise sectioned into slices of 5 μm in thickness using a microtome. Histological sections were stained with picrosirius red (Fig. 1 and 2). Preparations were analyzed using optic



**Fig. 1.** **Transverse section of basilar artery of a 20-year-old man, stained with picrosirius red (magnification ×20)**
1 – internal elastic membrane; 2 – tunica media of *a. basilaris*; 3 – adventitia.

**Fig. 2.** **Histological sample of transverse section of basilar artery obtained from a 69-year-old man, stained with picrosirius red (magnification ×20)**
1 – internal elastic membrane; 2 – tunica media of *a. basilaris*; 3 – adventitia.

microscope, magnifying ×20. Ten images of each slice were registered using microscope supplied with photo camera for examination of collagen network in media of basilar artery. Images were selected visually based on optimal clearness of examined structure. Totally 890 basilar artery fields were examined using semi-automated image analysis system. Images of histological samples were analyzed using special image processing program Image-Pro Plus v. 6. Detailed quantitative analysis of collagen bundle network in the tunica media of basilar artery was performed: area, perimeter, and number of collagen bundles were calculated. Data were processed and statistically analyzed using Statistica 6.0 program.

### Results

Morphometric analysis revealed an increase in the area of collagen network with age. The area of collagen bundles was 3.4% higher in middle-aged male group than in young group ($P>0.05$). Difference in the area of collagen bundles in the tunica media between old and middle-aged male groups was 8.5% ($P<0.05$). There was an 11.9% increase in the area of collagen network comparing old and young men ($P<0.05$) (Fig. 1). It was found that there was no sta-
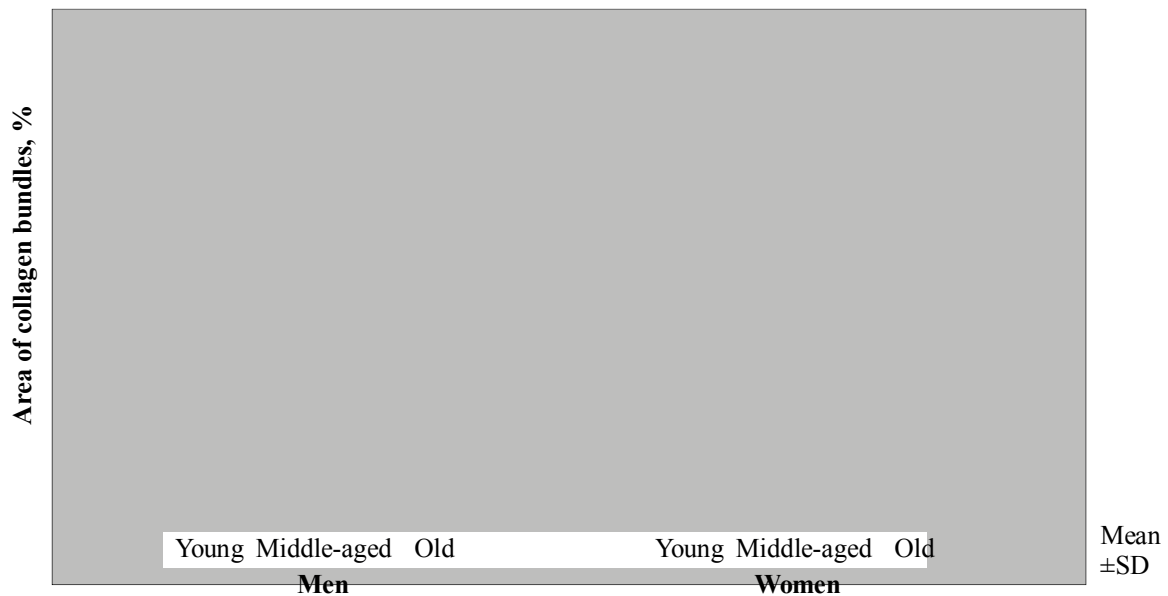
tistically significant difference in the area of collagen bundles only between young and middle-aged group.

Analysis of female groups showed that there was a significant difference in collagen network area in the tunica media of the basilar artery between all age groups ($P<0.05$). The area of collagen bundles was 7.8% higher in middle-aged female group than in young group and 5.6% lower than in old age group. A 13.4% increase in the area of collagen bundles was found in old female group as compared to youngest one (Fig. 3).

Analyzing collagen network area between male and female groups of the same age, it was found that collagen network area was higher in female groups. The difference was statistically significant comparing men and women aged 40–59 years ($P<0.05$).

Correlation between the area of collagen bundles (%) and age was determined using a regressive analysis. Correlation between collagen network area and age was strong in the groups of both genders ($r=0.56$ in men and $r=0.6$ in women; both $P<0.05$).

Analyzing the perimeter of collagen bundles in both genders by different age groups, we found out that it was decreasing with age (Table 1, 2). Morphometrical analysis showed a 21.2% decrease in the pe-

***Fig. 3.*** **The area of collagen bundles in the tunica media of basilar artery
in men and women by all age groups**

***Table 1.*** **Number and perimeter of collagen bundles in men by different age groups**

| Age group | Number of cases | Perimeter of collagen bundles mm/mm$^2$ | Standard deviation | Number of bundles in mm$^2$ | Standard deviation |
|---|---|---|---|---|---|
| <40 | 18 | 135.49 | ±0.073 | 4173.84 | ±2195 |
| 40–59 | 16 | 107.64 | ±0.053 | 3541.46 | ±2161 |
| 60> | 11 | 66.65 | ±0.046 | 2101.88 | ±1547 |

***Table 2.*** **Number and perimeter of collagen bundles in women by different age groups**

| Age group | Number of cases | Perimeter of collagen bundles mm/mm$^2$ | Standard deviation | Number of bundles in mm$^2$ | Standard deviation |
|---|---|---|---|---|---|
| <40 | 6 | 126.32 | ±0.083 | 3798.13 | ±2760 |
| 40–59 | 20 | 82.58 | ±0.051 | 2394.24 | ±1866 |
| 60> | 19 | 55.75 | ±0.033 | 1515.58 | ±1258 |

rimeter of collagen bundles in middle-aged male group as compared to young group (P>0.05). The difference in the perimeter of collagen bundles between old and middle-aged male groups was 37.6% (P>0.05). The decrease in the perimeter was 51% comparing young and old men (P<0.05) (Table 1).

Morphometric study revealed that in women, the perimeter of collagen bundles was by 34.7% lower in middle-aged group than in young group (P>0.05) and by 32.6% higher than in old group (P>0.05). A 56%

decrease in the perimeter of collagen bundles was found when comparing old and young women (P<0.05) (Table 2).

There was a 16.24% decrease in the number of collagen bundles in middle-aged male group as compared with young male group (P>0.05), whereas a 39.9% decrease was not statistically significant comparing old and middle-aged groups (P>0.05). The decrease in the number of collagen bundles comparing young and old men was 49.7% (P<0.05) (Table 1).

Morphometric study showed that in women, the number of collagen bundles was by 37% lower in middle-aged group than in young group (P>0.05) and by 36.7% higher in old group (P>0.05). A 60.1% decrease in the number of collagen bundles was noted comparing old and young women (P<0.05) (Table 2).

Analysis of age-related changes in perimeter and number of collagen bundles showed that there was no statistically significantly difference between female and male groups of the same age (P>0.05). It was found that perimeter and number of collagen bundles in the tunica of media basilar artery in men and women decreased with age.

Correlation between the perimeter of collagen bundles in the basilar artery and age in men and women was statistically significant in both groups (r=–0.436 in men and r=–0.478 in women; both P<0.05). Correlation between the number of collagen bundles and age was also statistically significant in men (r=–0.41; P<0.05) and women (r=–0.45; P<0.05).
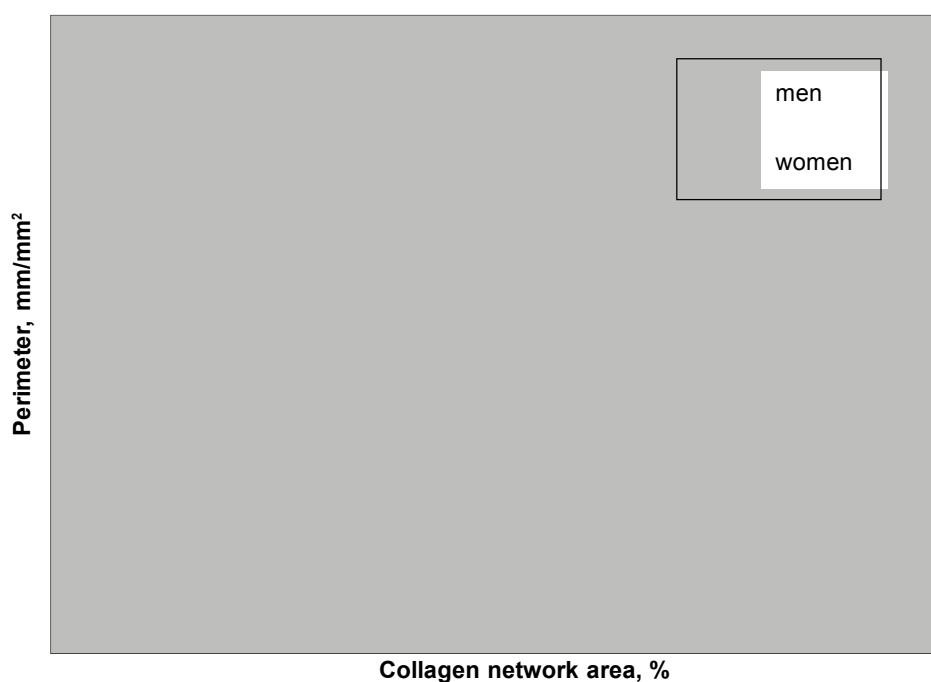
Fig. 4 shows the correlation between the perimeter and area of collagen bundles in both genders.

Regressive analysis showed a statistically significant correlation between area and perimeter of collagen bundles in the tunica media of basilar artery (r=–0.86 in men and r=–0.88 in women, respectively). A strong correlation between the number of collagen bundles in 1 mm$^2$ and area of collagen bundles was found (r=–0.9 in men and r=–0.87 in women) (Fig. 5).

## Discussion

Physicochemical characteristics of the collagen change with age. Changes and regeneration of collagen network are going on constantly. Even in old age, collagen fibers are fragmenting and forming again (7). The area of collagen network increases, but perimeter and number of bundles decrease with age. Similar age-related changes were noticed in studies analyzing changes in the structure of collagen network in aging in other blood vessels and heart (10, 12, 13). We determined that in both gender groups with age, percentage of collagen network area in human basilar artery increased, and perimeter of collagen bundles and their number decreased.

There was a 1.2% increase in the area of collagen bundles in the tunica media in women aged less than 40 years, a 7.2% increase in middle-aged women, and a 2.9% increase in women older than 60 years as compared to men of the same age groups. Age-related changes in collagen area in the basilar artery seem to be more obvious in women. The area of collagen bundles was higher in middle-aged women than in young ones (by 34.7%). At the same time, difference between young and middle-aged men was 21%, while the perimeter of collagen bundles and number of bundles in 1 mm$^2$ were higher in men than in women in all age groups. The correlation between the area, perimeter, and number of collagen bundles and age was slightly stronger in women than in men.



*Fig. 4.* **Correlation between area and perimeter of collagen bundles in men and women**

**Fig. 5.** Correlation between collagen area and collagen bundles number
in mm² in men and women

Analyzing the groups of different age of both genders together, it was observed that collagen area was by 8.6% higher in the middle-aged group than in the young group and by 9.6% lower than in the old group. Differences appearing in collagen area every twenty years are about 10%.

In addition that collagen network area was determined, perimeter of its bundles and number of bundles were also calculated. This widens possibilities for quantitative assessment and provides objective information on qualitative characteristics of collagen network. An increase in the area and decrease in the perimeter and number of collagen bundles indicate the presence of larger collagen fiber bundles in aging tunica media of the basilar artery. Collagen network area increasing with age, judging by in parallel decreasing total perimeter and number of bundles, becomes less branchy. While aging, collagen looses its toughness, and the fibers start to stiffen and thicken (7). With an increase in the amount of collagen, elasticity of blood vessels decreases. The structural changes of arteries with the age, with predominance of collagen over elastin and reticulin, could be reason of their fragility (10).

There are few studies on age-related changes in collagen network in the tunica media of the basilar artery. Most of studies (14–16) analyzed structural and more frequent functional changes in cases of various pathologies: aneurysms, strokes, posttraumatic infarc-tions, thrombosis, *etc*. Our data obtained using quantitative analysis of collagen network changes in the tunica media of the basilar artery will enrich knowledge about processes of aging in vascular wall.

Dissecting aneurysms or thrombosis appear to be the cause of vertebro-basilar insufficiency after manipulations in the neck, and the clinical outcome is often poor, with severe long-term neurological disability and mortality. Detailed quantitative analysis of important age-related changes in the basilar artery wall might be important in morphological evaluation of vascular pathology in fatal artery injuries.

**Conclusions**

Quantitative parameters of collagen network – area, number, and perimeter of collagen bundles – provided additional information about structural changes, which occur in the tunica media of human basilar artery in aging.

The area of collagen bundles increased, number and perimeter of collagen bundles decreased with age in both genders in the tunica media of human basilar artery.

The area of collagen bundles increased with age. Its network structure, judging by in parallel decreasing total perimeter and number of bundles, became less branchy. It is feasible that separate collagen fibers merge and their cross section area enlarges.

# Pamatinės arterijos kolageno tinklo amžiniai pokyčiai

**Devika Gudienė, Angelija Valančiūtė, Juozas Vėlavičius[1]**
*Kauno medicinos universiteto Histologijos ir embriologijos katedra, [1]Patologinės anatomijos katedra*

**Santrauka.** *Tikslas*. Išnagrinėti ir įvertinti amžinius morfometrinius žmogaus pamatinės arterijos sienelės medijos kolageno tinklo pokyčius.

*Medžiaga ir metodai*. Histologiškai ištirti 89 žmonių, kurių amžius buvo nuo 20 iki 85 metų, pamatinių arterijų histologiniai preparatai. Preparatai paruošti įprastine histologine metodika, dažyti Pikro-sirijaus raudonuoju. Atlikta nuodugni kiekybinė pamatinės arterijos medijos kolageno tinklo analizė.

*Rezultatai*. Ištirtas kolageno tinklo plotas, skaidulų pluoštų perimetras ir pluoštų kiekis pamatinės arterijos medijoje. Išnagrinėję amžinius kolageno tinklo pokyčius (vyrų ir moterų), nustatėme, kad abiejų lyčių pamatinės arterijos medijoje kolageno plotas didėjo. Tiriant kolageno pluoštų perimetrą ir kiekį, abiejų lyčių, priklausomai nuo amžiaus, nustatėme, kad jie su amžiumi mažėjo. Mes nustatėme statistiškai reikšmingą ryšį tarp visų išmatuotų parametrų ir amžiaus.

*Išvados*. Kolageno pluoštų plotas žmogaus pamatinėje arterijoje su amžiumi didėja, o kolageno pluoštų kiekis ir perimetras mažėja abiejų lyčių. Kolageno tinklo struktūra su amžiumi tampa mažiau šakota, kolageno skaidulos persipina, jos užima didesnį plotą.

Adresas susirašinėti: D. Gudienė, KMU Histologijos ir embriologijos katedra, A. Mickevičiaus 9, 44307 Kaunas
El. paštas: devikagud@yahoo.com

## References

1. Nagai Y, Metter EJ, Earley CJ, Kemper MK. Artery changes with aging: degeneration or adaptation? Dialogues in Cardiovascular Medicine 2001;6:104-11.
2. Bilato C, Crow MT. Atherosclerosis and the vascular biology of aging. Aging (Milano) 1996;8(4):221-34.
3. Jani B, Rajkumar C. Ageing and vascular ageing. Postgrad Med J 2006;82(968):357-62.
4. Dekoninck WJ. Ageing and cerebral vascular sclerosis: myth or reality? Pathol Biol (Paris) 1982;30(5):303-9.
5. Levy BI. Aging of the arterial system. Presse Med 1992; 21(26):1200-3.
6. Ooyama T, Sakamoto H. Arterial ageing of aorta and atherosclerosis – with special reference to elastin. Nippon Ronen Igakkai Zasshi 1995;32(5):326-31.
7. Hegedus K, Molnar P. Age-related changes in reticulin fibers and other connective tissues elements in the intima of the major intracranial arteries. Clin Neuropathol 1989;8(2):92-7.
8. Gay S, Miller EJ. What is collagen, what is not. Ultrastruct Pathol 1983;4(4):365-77.
9. Gelse K, Pöschl E, Aigner T. Collagens – structure, function, and biosynthesis. Adv Drug Deliv Rev 2003;55:1531-46.
10. Johnson CP, Baugh R, Wilson CA, Burns J. Age related changes in the tunica media of the vertebral artery: implications for the assessment of vessels injured by trauma. J Clin Pathol 2001;54(2):139-45.
11. Debessa CRG, Maifrino LB. Age related changes of the collagen network of the human heart. Mech Ageing Dev 2001;122(10):1049-58.
12. Burkauskienė A. Age-related changes in the structure of myocardial collagen network of auricle of the right atrium in healthy persons and ischemic heart disease patients. Medicina (Kaunas) 2005;41(2):145-54.
13. Mirvis SE, Wolf AL, Numaguchi Y, Corradino G, Joslyn JN. Posttraumatic cerebral infarction diagnosed by CT: prevalence, origin, and outcome. AJNR Am J Neuroradiol 1990;11(2): 355-60.
14. Marino R, Gasparotti R, Pinelli L, Manzoni D, Gritti P, Mardighian D, et al. Posttraumatic cerebral infarction in patients with moderate or severe head trauma. Neurology 2006;67(7): 1165-71.
15. Hu JJ, Baek S, Humphrey JD. Stress-strain behavior of the passive basilar artery in normotension and hypertension. J Biomech 2007;40(11):2559-63.
16. Tanaka H, Fujita N, Enoki T, Matsumoto K, Watanabe Y, Murase K, et al. Relationship between variations in the circle of Willis and flow rates in internal carotid and basilar arteries determined by means of magnetic resonance imaging with semiautomated lumen segmentation: reference data from 125 healthy volunteers. AJNR Am J Neuroradiol 2006;27(8): 1770-5.