Arch. Biol. Sci., Belgrade, 65 (4), 1271-1278, 2013 DOI:10.2298/ABS1304271T

ULTRASTRUCTURAL CHARACTERISTICS OF THE VASCULAR WALL COMPONENTS OF RUPTURED ATHEROSCLEROTIC ABDOMINAL AORTIC ANEURYSM

IRENA TANASKOVIĆ[1], VESNA LAČKOVIĆ[2], D. RADAK[3], SLAVICA KNEŽEVIĆ USAJ[4], MILENA LAČKOVIĆ[5], VESNA STANKOVIĆ[6], MAJA VULOVIĆ[7], LIDIJA POČEK[8] and V. KANJUH[9]

[1] *Department of Histology and Embryology, Faculty of Medical Sciences, University of Kragujevac,* 34000 Kragujevac, Serbia
[2] *Institute of Histology and Embryology "Aleksandar Dj. Kostic", Faculty of Medicine, University of Belgrade,* 11000 Belgrade, Serbia
[3] *Department of Surgery, Faculty of Medicine, University of Belgrade,* 11000 Belgrade, Serbia *and "Dedinje" Institute for Cardiovascular Diseases,* 11040 Belgrade, Serbia
[4] *Department of Pathology, Medical Faculty University of Novi Sad,* 21000 Novi Sad, Serbia
[5] *Zemun Clinical Center,* 11080 Belgrade, Serbia
[6] *Department of Pathology, Faculty of Medical Sciences, University of Kragujevac,* 34000 Kragujevac, Serbia
[7] *Department of Anatomy, Faculty of Medical Sciences, University of Kragujevac,* 34000 Kragujevac, Serbia
[8] *Department of Histology and Embryology, Faculty of Medicine University of Montenegro,* 81000 Podgorica, Montenegro
[9] *Serbian Academy of Sciences and Arts,* 11001 Belgrade, Serbia

Abstract - The aim of this study was to determine the ultrastructural characteristics of cell populations and extracellular matrix components in the wall of ruptured atherosclerotic abdominal aortic aneurysm (AAA). We analyzed 20 samples of ruptured AAA. For orientation to the light microscopy, we used routine histochemical techniques by standard procedures. For ultrastructural analysis, we applied transmission electron microscopy (TEM). Our results have shown that ruptured AAA is characterized by the remains of an advanced atherosclerotic lesion in the intima followed by a complete absence of endothelial cells, the disruption of basal membrane and disruption of internal elastic lamina. On plaque margins as well as in the inner media we observed smooth muscle cells (SMCs) that posses a euchromatic nucleus, a well-developed granulated endoplasmic reticulum around the nucleus and reduced myofilaments. The remains of the ruptured lipid core were acellular in all samples; however, on the lateral sides of ruptured plaque we observed a presence of two types of foam cells (FCs), spindle- and star-shaped. Fusiform FCs possess a well-differentiated basal lamina, caveolae and electron dense bodies, followed by a small number of lipid droplets in the cytoplasm. Star-shaped FCs contain a large number of lipid droplets and do not possess basal lamina. On the inner margins of the plaque, we observed a large number of cells undergoing apoptosis and necrosis, extracellular lipid droplets as well as a large number of lymphocytes. The media was thinned out with disorganized elastic lamellas, while the adventitia exhibited leukocyte infiltration. The presented results suggest that atherosclerotic plaque in ruptured AAA contains vascular SMC synthetic phenotype and two different types of FCs: some were derived from monocyte/macrophage lineage, while others were derived from SMCs of synthetic phenotype. The striking plaque hypocellularity was the result of apoptosis and necrosis of different cell populations.

Key words: Ruptured aneurysm wall, foam cells, plaque, smooth muscle cells

INTRODUCTION

An aneurysm is a local dilatation of the artery that arises due to congenital or acquired damage to the wall, especially the tunica media, causing a consequent weakness of the wall. The aortic aneurysm is

usually localized in the abdominal area (abdominal aortic aneurysm - AAA), mainly from the distal opening of the renal arteries. It rarely occurs in the thoracic aorta or in the upper abdominal aorta. Sometimes multiple lesions can be observed along the aorta. The most common pathological processes that cause aneurysm development are hereditary metabolic disorders of connective tissue, primarily a congenital defect in the synthesis of collagen III, but also dystrophic changes, atherosclerosis, inflammation and trauma (Bengtsson et al., 1996).

Atherosclerotic aortic aneurysms occur because of the pathogenesis of atherosclerotic changes that cause a weakening of the aortic wall. Atherosclerosis primarily damages the intima, but due to the pressure of atherosclerotic plaques, the media also undergoes atrophy followed by fibrosis and the loss of elastic lamellas. Ulceration of the plaque conjoined with parietal thrombus formation, which is a common feature of advanced atherosclerotic lesion, could further promote the development of aneurysms (Zhao et al., 2004; Stary et al., 1995).

The rupture of AAA is the most significant complication of aneurysm (Di Martino et al., 1998). However, the impact of all the cellular and matrix components that could lead to the development of an aneurysm and their role in the formation and rupture of the AAA are not completely understood (Lackovic et al., 2011; Pearce and Shively, 2006).

The purpose of this study was to determine the ultrastructural characteristics of the cells and extracellular matrix components in the aortic wall in ruptured atherosclerotic AAA.

MATERIALS AND METHODS

Twenty samples of ruptured atherosclerotic AAA were analyzed, all of them obtained during autopsies of both sexes performed at the Department of Pathology, Medical Faculty in Kragujevac. Routine histochemical techniques were used (Heidenhain's Azan and Periodic acid Schiff – PAS stains) and standard procedures (Bancroft and Gamble, 2002).

For ultrastructural analysis, we used transmission electron microscopy (TEM). Samples were primarily fixed in a solution of 2.5% glutaraldehyde in 0.1 M sodium cacodylate-HCl buffer (pH 7.4) for 24 h at 4° C The specimens were post fixed for 1 h at 4°C in 1% osmium tetraoxide in 0.1 M cacodylate buffer and 4.8% uranyl acetate for 24 h at 4°C. The samples were dehydrated in increasing ethanol series (70-100%) and embedded in Epon 812. The samples were cut with a diamond knife on an LKB Ultrotome. Ultra-thin sections were stained with 2% uranyl acetate and alkaline lead citrate (Bancroft and Gamble, 2002; Vukovic et al., 2006).

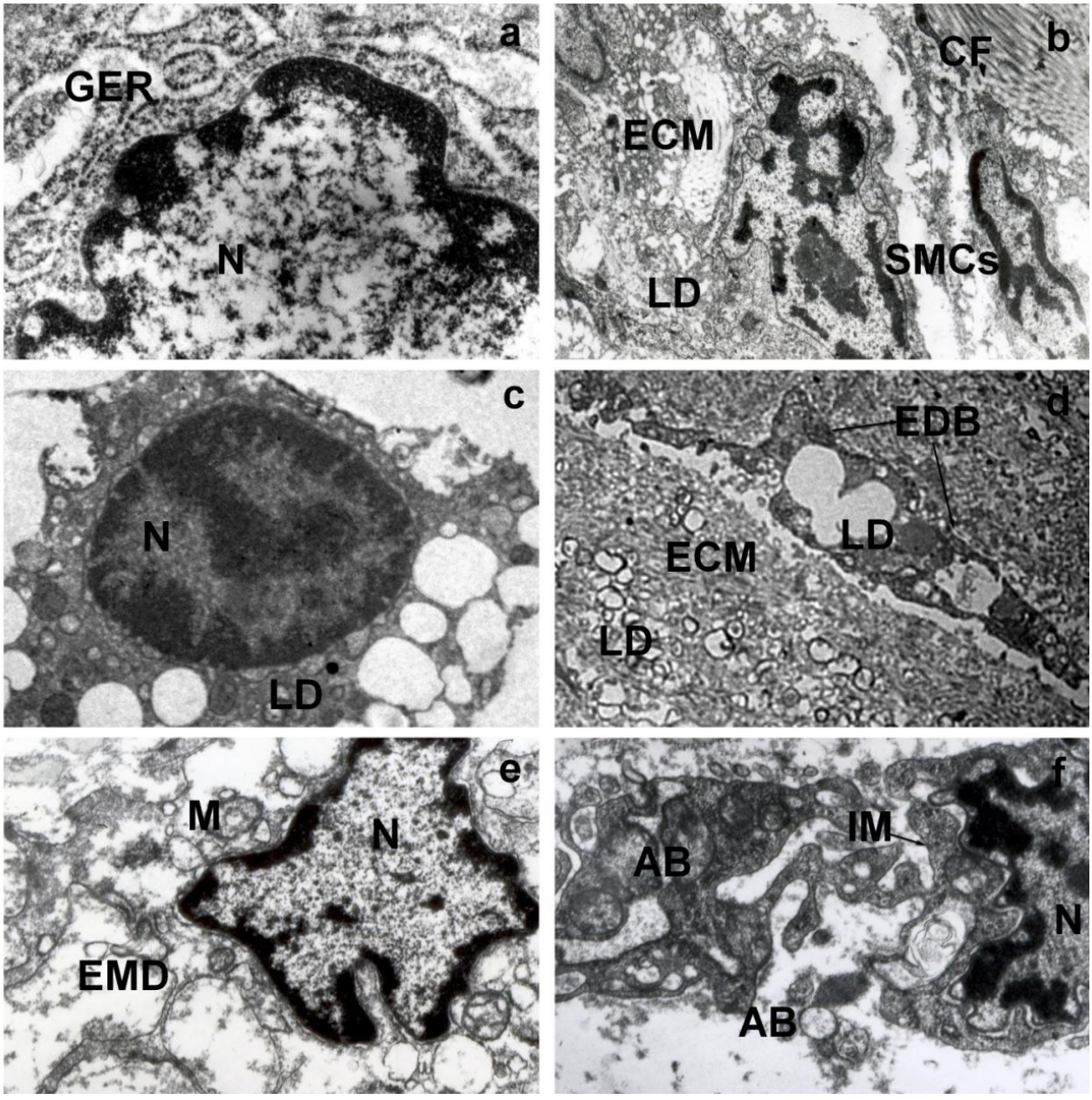
RESULTS

The results obtained by light microscopy showed that in all the samples atherosclerotic changes had occurred in all layers of the aortic wall. In all analyzed samples, we observed the remains of advanced atherosclerotic lesions in the intima, thinned-out and atrophic media, as well as adventitia with a chronic inflammatory infiltrate, followed by damage to the vasa vasorum and signs of hemorrhage. Further results obtained by TEM confirmed those obtained by light microscopy.

The results of TEM analysis showed that the intima of all analyzed samples was characterized by a complete absence of endothelial cells, the disruption of the basal membrane, as well as a presence of the remains of advanced atherosclerotic plaques and consequent signs of hemorrhage due to the rupture of vasa vasorum.

The ruptured lipid core in all samples was acellular and filled with cholesterol crystals. On the margins of the ruptured plaques, there was a heterogeneous cell population composed of foam cells (FCs) and smooth muscle cells (SMCs) of different shapes and characteristics, as well as cell detritus.

Analysis of the ultrastructural characteristics of the SMCs at the margins of the plaques and in the inner media showed that these cells had a euhromatic nucleus, well-developed granulated endoplasmic re-



Figs. 1. Ultrastructural analysis of cell populations of ruptured atherosclerotic AAA. **A** – smooth muscle cell of synthetic phenotype with an euchromatic nucleus (N) and a well-developed granulated endoplasmic reticulum (GER) around the nucleus (TEM, x 36875); **b** – smooth muscle cells (SMCs) on the plaque margin between collagen fibers (CF). Extracellular lipid droplets (LD) bound to extracellular matrix components (ECM) (TEM, x 8250) can be observed. C – star-shaped foam cell without basal lamina which points to their monocyte/macrophage origin; N - nucleus; LD - lipid droplets (TEM, x 11250); **d** – spindle-like foam with a well-differentiated basal lamina and electron dense bodies (EDB) which suggests their smooth muscle cell origin. Lipid droplets (LD) are present in foam cell as well as in extracellular matrix (ECM). (TEM, x 8250). **E** – necrotic cell. Extensive membrane damage (EMD), as well as damages to the cytoplasm and mitochondria (M) (TEM, x 11250) can be observed. **F** – apoptotic cell. We can notice intact membrane (IM) and blebbing on the surface of the cell. Parts of the plasma membrane, i.e. blebs, separate from the cell, taking a portion of cytoplasm with them, to become apoptotic bodies (AB). In the nucleus (N), we can observe nuclear fragmentation and chromatin condensation (TEM, x 16875).

ticulum around the nucleus and a reduced number of microfilamentes (Figs. 1a, b).

TEM analysis of the cell populations at the margins of the plaques revealed the presence of two types of FCs: star-shaped and spindle-shaped. The star-shaped FCs contained a large number of lipid droplets and did not possess basal lamina (Fig. 1c). The spindle-shaped foam cells contained a small number of lipid droplets in the cytoplasm and had a clearly differentiated basal lamina, caveolae and dense bodies (Fig. 1d). These cells were localized predominantly in the deeper areas of the intima and on its border with the media.

TEM analysis of the cell detritus at the margins of the plaque uncovered at least two types of cell debris. In one type we observed groups of cells that were characterized by edema of the cell and organelles (especially mitochondria), lysis of the organelles, extensive membrane damage followed by the release of cytoplasm in the extracellular matrix, and inflammation and damage to surrounding tissue (Fig. 1e). The second type of cells were characterized by an intact membrane, nuclear fragmentation and chromatin condensation along the inner nuclear membrane as well as apoptotic bodies (Fig. 1f).

The tunica media was characterized by thinning, fragility and a prominent loss and disruption of elastic lamellae. In all analyzed samples, blood dissection spread from the intimal rupture to the media, partly through the newly formed medial blood vessels.

The adventitia contained connective tissue with collagen fibers, fibroblasts, extensive leukocyte infiltration and adventitial blood vessels (vasa vasorum) with thickened walls.

DISCUSSION

The results of our study confirmed that ruptured atherosclerotic AAA is characterized by the presence of atherosclerotic plaque remains with hemorrhage and developed thrombotic masses. Within the plaque, there are the remains of smaller, secondary plaques mutually separated by newly formed lumens. These lumens represent the points through which the wall dissection expands. In all the samples analyzed, we observed an acellular lipid core surrounded by collagen fibers. At the plaque margins we noticed heterogeneous cell populations. The results are in accordance with our previous results, as well as the studies of other authors (Tanasković et al., 2010; Lackovic et al., 2011; Richardson et al., 1989; LeMaire et al., 2005; van der Wal et al., 1994).

According to the current classification of atherosclerotic lesions, the described changes in our samples correspond to the advanced atherosclerotic lesion type VI (subtype VIa, b, c). The main characteristic of advanced lesion type VI is the presence of complicated plaque, derived from atheroma (type IV lesions), and subsequently complicated by disruption, hemorrhage and thrombosis (Stary et al., 1995).

According to literature data, structural weakness in the wall is caused by the composition of the lesions, which suggests that unstable plaques are more susceptible to rupture, which could lead to the rupture of the entire aortic wall (Richardson et al., 1989). Beside the stage of the plaque, other factors of plaques stability could be the presence of macrophages and foam cells derived from macrophages, their proteolytic enzymes in the plaque (LeMaire et al., 2005), as well as the presence of inflammatory cells in the lesion (van der Wal et al., 1994). The exposure to increased hemodynamic wall stress could also contribute to the rupture of AAA (Lacković and Vuković, 2006. These data clearly indicate that the morphological composition of the lesion, as well as the ultrastructural and morphofunctional characteristics of the cells within it, are of crucial importance in the pathogenesis of AAA. By comparing the results of our study with the previously presented factors, we can assume that one of the most important causes of the rupture of AAA could be the composition of the atherosclerotic lesion or structural weakness of the wall caused by the presence of unstable atheroma. Other authors have previously shown that lesions with a large lipid core, as is the case with atheroma (type IV lesion), are more susceptible to rupture when compared to more

stable fibroatheroma (type V lesion) (Falk, 1992). It is considered that the existence of a thick fibrous cap, as well as the presence of functionally preserved SMCs on the margins of the plaque, contribute to the stability of fibroatheroma (Chan et al., 2005; Thyberg et al., 1995).

Another factor that contributes to the rupture is the composition of the cell population in the plaque, as well as the morphofunctional preservation of cells. Our results obtained by TEM have shown the presence of SMCs, characterized by a euchromatic nucleus with prominent nucleolus, surrounded by a well-developed rough endoplasmic reticulum at the plaque margins in all samples, which suggests the synthetic phenotypes of these cells. This result is in accordance with our previous immunohistochemical results that have shown the presence of SMCs expressing α-SMA and vimentin on the plaque margins, followed by an absence of desmin, which suggests that these SMCs succumb to modulation from a contractile to a synthetic phenotype (Tanasković et al., 2010; Lackovic et al., 2011; Vukovic et al., 2010). According to the literature, SMCs of synthetic phenotype in the plaque of AAA originate from the main population of intimal SMCs, as well as from the cells that migrate from the media to the intima (Gabbiani et al., 1981; Vukovic et al., 2010; Tanaskovic et al., 2011). The migration of cells from the media takes place under the influence of PDGF that is synthesized by activated macrophages in the lesion (Libby and Clinton, 1993; Xu et al., 2001). In response to the effects of PDGF, SMCs migrate into the intima, proliferate and synthesize components of the extracellular matrix, predominantly proteoglycans, which contribute to plaque formation in the early stages of atherosclerosis (Tanaskovic et al., 2011; Guyton JR, Klemp, 1994).

Besides SMCs of synthetic phenotype, we observed two types of foam cells on the plaque margins: spindle-shaped and star-shaped cells. The spindle-shaped SMCs have a well-differentiated basal lamina, caveolae, dense bodies and lipid drops in the cytoplasm, which give the impression of foam cells. These foam cells are derived from smooth muscle cells of synthetic phenotype. Star-shaped foam cells have cytoplasmic processes, contain a large number of lipid droplets in the cytoplasm and do not have a basal lamina, which suggests that they originate from macrophages. These results are consistent with the results of our previous studies, as well as studies by other authors (Tanasković et al., 2010, 2011; Lackovic et al., 2011; Vukovic et al., 2010; Guyton and Klemp, 1994).

According to literature data, in the early stages of the lesion, in the phase of endothelial activation and the fatty streak stage, monocytes or macrophages are the main precursors of foam cells, whereas in the later stages most of the foam cells are formed by SMCs (Tanasković et al., 2010, 2011; Lackovic et al., 2011; Vukovic et al., 2010; Guyton and Klemp, 1994) It was also found, as previously noted, that the presence of macrophages in the lesion is an important predisposing factor for aneurysm rupture. Macrophages influence the pathogenesis of AAA in two ways. It is well known that foam cells in the early stages of atherosclerosis accumulate lipids intracellularly. The development of the lesion volume exceeds the capacity of lipid foam cells; they undergo necrosis and release their contents in the extracellular space, which causes a large mass of lipid in the plaque. Thus, macrophages directly influence the creation of unstable atheroma in the intima and contribute to the development of rupture (Vukovic et al., 2006). On the other hand, macrophages synthesize matrix metalloproteinases (MMPs) that degrade components of extracellular matrix intima and media, and also influence cell proliferation, migration, differentiation, angiogenesis, apoptosis and activation of chemokines, which indirectly and directly contribute to the weakening of the vascular wall and promote rupture of the aneurysm (LeMaire et al., 2005).

Bearing in mind the impact of cell populations on the rupture of atherosclerotic AAA, in the light of the morphofunctional characteristics of the cells analysis of the cell remains in the plaque, and consequently the presence of leukocyte infiltration is indispensable. TEM analysis of the samples in our study showed that the lipid cores are acellular, while on the

plaque margins there is a presence of extensive leukocyte infiltration, as well as two types of cell debris. A number of recorded cell fragments are characterized by edema of cells and organelles, especially mitochondria, lysis of organelles, extensive damage to the basal membrane followed by the release of cytoplasm contents, which is accompanied by inflammation and damage to surrounding tissue. According to the results of other authors, the described changes correspond to cell necrosis (Wang et al., 2000). The second type of cell residues are characterized by intact membrane, nuclear fragmentation and chromatin condensation to the inner nuclear membrane as well as parts of plasma membrane that separate from the cell, taking a portion of cytoplasm with them to become apoptotic bodies which, according to literature data, is characteristic of cells in apoptosis (Wang et al., 2000). The results suggest that extreme plaque hypocellularity is formed because of apoptosis and cell necrosis, which is followed by an intensive inflammatory reaction. Hypocellularity also contributes to plaque instability and rupture of the lesion. According to the literature, the presence of SMCs on the plaque margins and their morphofunctional preservation promote plaque stability, while the loss of contractile characteristics of SMCs causes atrophy of the wall and contributes to its rupture (Tanasković et al., 2010; Chan et al., 2005).

Beside smooth muscle cells and foam cells of different origin, in all the analyzed samples we observed leukocyte infiltration in all parts of the wall. The largest number of leukocytes was present at the margins of the plaque, outer media and adventitia, while at the rupture site the number of leukocytes was significantly lower. This result is in accordance with recent studies on the impact of leukocyte infiltration in the rupture of aneurysms, which have shown that inflammation of the tissue has little influence on the process of aneurysm rupture (Chan et al., 2005).

Finally, all analyzed changes can be viewed comprehensively with aspects of vascular remodeling. According to the modern concept of pathogenesis, atherosclerosis is a complex disease that can be defined as a remodeling of the arterial wall under conditions of hypertension associated with hypercholesterolemia and consequent inflammation. According to the available literature data in terms of hypertension, in the artery wall changes develop that are characterized as vascular remodeling (Lacković et al., 2006). These changes include the primary compensatory dilatation, proliferation of SMCs and a consequent thickening of the wall, and in later the stages the activation of apoptosis (Falk, 1992). Synthetic SMCs synthesize components of connective tissue, predominantly proteoglycans, during the early response of the vascular wall to hypertension (Tanaskovic et al., 2011). When hypertension is associated with hypercholesterolemia, the accumulation of lipid droplets in the subendothelium of the intima is observed, as well as their binding to proteoglycans (a product of the synthesis of SMCs), their oxidation or other types of chemical modifications. Oxidized LDL (ox-LDL) activates endothelial cells to express adhesion proteins from the family of immunoglobulin, selectins and specific cytokines/chemokines (Libby and Clinton, 1993). In the initial stage of atherosclerosis, endothelial cells express VCAM-1, which leads to the adhesion of monocytes and T lymphocytes to the endothelial surface (Tanaskovic et al., 2011; Libby and Clinton, 1993). Adhered monocytes and T lymphocytes under the influence of selectin "rolling" on the endothelium then migrate under the influence of chemokines in the subendothelium. Monocytes are transformed to macrophages, take ox-LDL through scavenger receptors and transform into foam cells (Guyton and Klemp, 1994). In addition, ox-LDL stimulates MMP synthesis in macrophages and initiates a cycle of degradation of extracellular matrix components (Choke et al., 2006).

According to literature data, all atherosclerotic AAA samples analyzed in this study are in the late stages of vascular remodeling that is accompanied by extensive development of atherosclerotic plaque, media fibrosis and loss of contractile properties of the SMCs, as well as a reduction in the number of cells due to the activation of apoptosis and necrosis. The described degenerative changes in atherosclerotic AAA lead to the reduced elasticity and weak-

ened resistance of the aortic wall, which can cause its rupture.

CONCLUSION

Atherosclerotic aortic aneurysm is the result of complex changes in the aortic wall. In the ruptured intima of atherosclerotic AAA are the remains of complicated atherosclerotic lesions classified as type VI. On the margins of ruptured plaques there are SMCs of the synthetic phenotype, as well as foam cells; one number is derived from monocyte/macrophage lineage, while others originate from SMCs of synthetic phenotype. The marked hypocellularity of the plaque is a result of the apoptosis and necrosis of SMCs. Necrosis is accompanied by leukocyte infiltration, suggesting an inflammatory process.

The cytohistological and ultrastructural characteristics of AAA wall components point to several processes that can promote rupture: the presence of unstable, complicated atherosclerotic plaque in the intima, the presence of macrophages, hypocellularity and loss of elastic properties of the media, as well as the loss of contractile properties of SMCs. Due to the described degenerative changes, the wall of atherosclerotic AAA loses its ability to adapt to compensatory dilatation and in conditions of elevated pressure is subject to rupture. Based on these results, it can be concluded that the changes observed in atherosclerotic AAA correspond to the late stage of remodeling of the vascular wall. Hypocellularity of the plaque is a consequence of apoptosis and necrosis.

Acknowledgments - This research is supported by the Ministry of Education, Science and Technological Development Government of the Republic of Serbia Grant III41007 and Grant 41002, and the Ministry of Science Government of Montenegro Grant 01-1372.

REFERENCES

Bancroft, J.D. and *M. Gamble* (2002) Theory and practice of histological techniques. 5th edition. Churchill Livingstone, Edinburgh, London, New York, Oxford.

Bengtsson, H., Svensson, B. and *D. Bergqvist* (1996) Incidence and prevalence of abdominal aortic aneurysms, estimated by necropsy studies and population screening by ultrasound. In: The abdominal aortic aneurysm - genetics, pathophysiology and molecular biology, David Tilson and Charles Boyd (eds.), *Annals of the New York Academy of Sciences* (volume 800), New York, pp 44-56.

Chan, W.L., Pejnovic, N., Hamilton, H., Liew, T.V., Popadic, D., Poggi, A. and *SM. Khan* (2005) Atherosclerotic abdominal aortic aneurysm and the interaction between autologous human plaque-derived vascular smooth muscle cells, type 1 NKT and helper T cells. *Circ Res*; **96**(6), 675-83.

Choke, E., Thompson, M.M., Dawson, J., Wilson, W.R., Sayed, S., Loftus, I.M. and *G.W. Cockerill* (2006) Abdominal aortic aneurysm rupture is associated with increased medial neovascularization and overexpression of proangiogenic cytokines. *Arterioscler Thromb Vasc Biol*; **26**(9), 2077-82.

Di Martino, E., Mantero, S., Inzoli, F., Melissano, G., Astore, D., Chisea, R. and *R. Fumero* (1998) Biomechanics of abdominal aortic aneurysm in the presence of endoluminal thrombus. Experimental characterisation and structure static computational analysis. *Europ J Vasc Endovasc Surg*; **15**, 290-9.

Falk, E. (1992) Why do plaques rupture? *Circulation*; **86** (3), 30-42.

Gabbiani, G., Schmid, E. and *S. Winter* (1981) Vascular smooth muscle cells differ from other smooth muscle cells: predominance of vimentin filaments and a specific type actin. *Proc Natl Acad Sci USA*; **103**, 2787-2796.

Guyton, J.R. and *K.F. Klemp* (1994) Development of the atherosclerotic core region. Chemical and ultrastructural analysis of microdissected atherosclerotic lesions from human aorta. *Arterioscler Thromb*; **14**(8),1305-14.

Lacković, V. and *I. Vuković* (2006) Cytohistological and immunohistochemical characteristics of vascular remodeling in diseases of the blood vessels. *Srp Arh Celok Lek*;**134**(1), 9-16.

Lackovic, V., Tanaskovic, I., Radak, Dj., Nesic, V., Gluvic, Z., Lackovic, M., Asanin, B., Radovic, S., Stankovic, V., Djuric, J., Nesic, J. and *V. Kanjuh* (2011) Morphofunctional characteristics of endothelial cells in coronary atherosclerosis. *Arch Biol Sci*; **63**(4), 921-932.

LeMaire, S.A., Wang, X., Wilks, J.A., Carter, S.A., Wen, S., Won, T., Leonardelli, D., Anand, G., Conklin, L.D., Wang, X.L., Thompson, R.W. and *J.S. Coselli* (2005) Matrix metalloproteinases in ascending aortic aneurysms: bicuspid versus trileaflet aortic valves. *J Surg Res*; **123**(1), 40-8.

Libby, P. and *S.K. Clinton* (1993) The role of macrophages in atherogenesis. *Curr Opin Lipidol*; **4**, 355-363

Pearce, W.H. and *V.P. Shively* (2006) Abdominal aortic aneurysm as a complex multifactorial disease: interactions of poly-

morphisms of inflammatory genes, features of autoimmunity and current status of MMPs. *Ann N Y Acad Sci*; **1085**, 117-32.

Richardson, P.D., Davies, M.J. and *G.V. Born* (1989) Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaques. *Lancet*; **2**, 941-944.

Stary, H.C., Chandler, A.B., Dinsmore, R.E., Fuster, V., Glagov, S., Insull, W. Jr, Rosenfeld, M.E., Schwartz, C.J., Wagner, W.D. and *R.W. Wissler* (1995) A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the committee on vascular lesions of the council on arteriosclerosis, *American Heart Association*; **92**, 1355-1374.

Tanaskovic, I., Lackovic, V., Gluvic, Z., Lackovic, M., Nesic, V., Stankovic, V., Labudovic Borovic, M., Radovic, S. and *B. Asanin* (2011) The influence of extracellular matrix composition on the pathogenesis of coronary atherosclerosis. *Arch Biol Sci*; **63**(2), 333-343.

Tanasković, I., Mladenović-Mihailović, A., Ušaj-Knežević, S., Stanković, V., Aleksić, A., Kastratović, T. et al. (2010) Histohemijska i imunohistohemijska analiza zida aterosklerotične abdominalne aneurizme aorte sa rupturom zida. *Vojnosanit Pregl*; **67**(12), 959-964.

Thyberg, J., Blomgren, K., Hedin, U. and *M. Dryjski* (1995) Phenotypic modulation of smooth muscle cells during the formation of neointimal thickenings in the rat carotid after balloon injury: an electron-microscopic and stereological study. *Cell Tiss Res*; **281**, 421-433.

van der Wal, A.C., Becker, A.E., van der Loos, C.M. and *P.K. Das* (1994) Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation*; **89**, 36-44.

Vukovic, I., Arsenijevic, N., Lackovic, V. and *V. Todorovic* (2006) The origin and differentiation potential of smooth muscle cells in coronary atherosclerosis. *Exp Clin Cardiol*; **11**(2), 123-128.

Vukovic, I., Lackovic, V., Raicevic, R., Lazic, Z., Milosavljevic, Z., Kastratovic, T., Mladenovic Mihailovic, A., Stankovic, V. and *M. Lackovic* (2010) Recent views on cytohistological characteristics and pathogenic mechanisms of the atherosclerotic lesions types I, II and III. *Vojnosanit Pregl*; **67**(12), 1007-1014.

Vukovic, I., Lackovic, V., Todorovic, V., Kanjuh, V. and *S. Ilic* (2004) Cytohistologic and immunohistochemical characteristics of the aortic intima and media in coarctation of the aorta of the adult type. *Srp Arh Celok Lek*; **132**(1), 66-71.

Wang, A.Y., Bobryshev, Y.V., Liang, H., Cherian, S.M., Inder, S.J., Ashwell, K.W., Farnsworth, A.E and *R.S. Lord* (2000) Electron-microscopic detection of apoptotic and necrotic cell death in non -atherosclerotic areas of stenotic aorto-coronary saphenous vein bypass grafts. *J Submicrosc Cytol Pathol*, **32**(2), 209-19.

Xu, K., Yen, T. and *C. Geczy* (2001) IL-1 up-regulates macrophage expression of the S-100 protein S-100A8. *J Immunnol*, **166**, 6358-6366.

Zhao, L., Moos, M., Grabner, R., Pedrono, F., Fan, J., Kaiser, B., John, N., Schmidt, S., Spanbroek, R., Lotzer, K., Huang, L., Cui, L., Rader, D., Evans, J., Habenicht, J. and *C. Funk* (2004) The 5 lipoxygenase pathway promotes pathogenesis of hyperlipidemia-dependent aortic aneurysm. *Nat Med*; **10**, 966-973.