Int. J. Morphol.,
40(5):1236-1241, 2022.

Are Statins a Risk Factor in Patients with Atherosclerosis?

¿Son las Estatinas un Factor de Riesgo en Pacientes con Aterosclerosis?

Andrejic Visnjic Bojana[1]; Samardzija Golub[2,3]; Bosanac Milana[1]; Pantic Teodora[3]; Kolarov-Bjelobrk Ivana[4,5]; Radic Jelena[4,5]; Jelena Ilic Sabo[1,6]; Jelena Amidzic[1,6] & Gvozdenovic Nemanja[7,8]

ANDREJIC VISNJIC B.; SAMARDZIJA, G.; BOSANAC, M.; PANTIC, T.; KOLAROV-BJELOBRK, I.; RADIC, J.; ILIC SABO, J.; AMIDZIC, J. & GVOZDENOVIC, N. Are statins a risk factor in patients with atherosclerosis? *Int. J. Morphol 40(5)*:1236-1241, 2022.

SUMMARY: Statins inhibit cholesterol synthesis, but also have other pleiotropic effects. There are indications that they affect macrophage survival trough the regulation of apoptosis. We analyzed 50 samples of aortic wall, selected based on statins in patients' therapy (n=25, Th-S group) or statin-free therapy (n=25, Th-nonS group). Each group had 5 samples of healthy aortic tissue, 10 samples of mild and 10 samples of severe atherosclerotic changes in aortic wall. Tissue was stained with hematoxylin-eosin and immunohistochemical methods (anti-Bcl-2 antibody). Presence of Bcl2-positive macrophages (Bcl-2^+ MP) was determined semiquantitatively, and data were processed in Microsoft Excell and IMB SPSS 23 Statistics. 60 % of patients in the Th-S group had a mild increase of Bcl-2^+ MP The use of statins leads to a significantly more frequent increase in $Bcl2^+$ macrophages in the intima of the healthy aortic tissue. Analysis of all aortic samples with pathohistological diagnosis showed that statin therapy was statistically significantly more often leading to a markedly increased presence of Bcl-2^+ MP. In the media, all samples of the Th-S group have a mild increase of Bcl-2^+ MP, and in adventitia 40 % of patients. The use of statins more often leads to a markedly increased presence of Bcl-2^+ MP in aortic tissue with diagnosed mild and severe atherosclerosis. In samples of severe atherosclerosis, statins lead to a markedly increased presence of Bcl-2^+ MP in the parts of the plaque towards the intima and towards the media. Statins lead to an increased presence of Bcl-2^+ macrophages, prolong their life, both in healthy and atherosclerotic altered aortic tissue. This indicates potentiation of inflammation and damage to the aortic wall, and calls into question the positive effect of statins on the aortic wall with atherosclerosis.

KEY WORDS: Statins; BCL-2; Apoptosis; Atherosclerosis; Macrophages.

INTRODUCTION

Statins are drugs with a broad spectrum of action, primarily for reducing lipoproteins in blood, the incidence and complications of myocardial infarction, stroke, atherosclerotic changes and other diseases (Paraskevas, 2008). Their effect is reflected in the reduction of the total concentration of the LDL fraction of cholesterol in the blood. In addition to the above-mentioned role, statins also increase the concentration of HDL cholesterol, leading to their cumulative effect - overall improvement of the patient's lipid status, ie reduction of the atherosclerosis index (Bobryshev *et al*., 2016).

Macrophages (MP) play a decisive role in all phases of atherosclerotic plaque formation and progression. They stimulate the local inflammatory process and, in addition, actively ingest lipoproteins thus forming foam cells/macrophages. Accumulation of foam cells contributes to further lipid accumulation, atherosclerotic plaque growth and progression to complicated atherosclerotic plaque, which is prone to rupture. The mentioned roles of MP and foam cells make them interesting for research but also as a therapeutic target. Several principles on which MP-targeted therapy

[1] Department of Histology and Embryology, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 2100, Novi Sad, Serbia.
[2] Department of Pathology, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 2100, Novi Sad, Serbia.
[3] Institute for Cardiovascular Diseases of Vojvodina, Put doktora Goldmana 4, 21204, Sremska Kamenica, Serbia.
[4] Department of Internal Medicine, Faculty of Medicine, University of Novi Sad, Veljkova 3, 2100, Novi Sad, Serbia.
[5] Oncology Institute of Vojvodina, Put doktora Goldmana 4, 21204, Sremska Kamenica, Serbia.
[6] Center for Pathology and Histology, Clinical center of Vojvodina, Hajduk Veljkova 1, 21000, Novi Sad, Serbia.
[7] Clinic for Orthopedic Surgery and Traumatology, Clinical center of Vojvodina, Hajduk Veljkova 1, 21000, Novi Sad, Serbia.
[8] Department of Emergency Medicine, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 2100, Novi Sad, Serbia.
This research is financially supported by the Autonomous Province of Vojvodina (Project No 142-451-2543/2021-02)

Received: 2022-07-19 Accepted: 2022-08-11

could be based are considered: regulation of MP survival, prevention of MP migration into the plaque area, and modification of the plasticity of the MP inflammatory response, ie. ability to differentiate/polarize towards pro- or anti-inflammatory phenotype of macrophages (M1 or M2 phenotype) (Moore *et al*., 2013; Bobryshev *et al*., 2016). Until new drugs are available, the effect of existing drugs on atherosclerosis, progression and "lifespan" of plaque is of interest. There are many studies documenting the effects of statins on lowering blood lipoproteins, but few studies have examined the effect of statins on MP survival. These scarce data suggest that statins contribute to MP survival. Statins achieve this effect by inducing the expression of antiapoptotic factors in MP including members of the Bcl-2 group of proteins (Qin *et al*., 2012; Shearn *et al*., 2012; Wood *et al*., 2013).

Given the widespread use of statins and the variety of actions they perform, attention should be paid to and encourage research into the histological, cellular and subcellular aspects of their action in various changes. MP as cells, also of very diverse functions, deserve a special place in aortic research, and therefore it is important to examine the interaction of this type of drugs and MP. For these reasons, we conducted a study to determine whether the use of statins in therapy affects the presence of Bcl2-positive macrophages (Bcl2+ MP) in the aortic wall.

MATERIAL AND METHOD

Examined groups and sample structure. The study included 50 patients who underwent aortic surgery at the Institute for Cardiovascular Diseases of Vojvodina in Sremska Kamenica (Serbia). Based on the use of statins in the treatment of patients, two groups were formed: Th-S (n = 25 (50 %)) and Th-nonS group (n = 25 (50 %)). In each group, there were 5 samples of healthy aortic tissue (without pathohistological changes), 10 samples with changes corresponding to mild and 10 samples corresponding to severe atherosclerosis. For all patients, data were obtained from medical histories and pathohistological findings. The use of the material for the purpose of research was approved by the Ethics Committee of the Institute for Cardiovascular Diseases of Vojvodina (No. 3674-1/9).

Pathohistological processing of tissue samples. Aortic tissue was processed by standard histological technique and stained by routine histological method hematoxylin and eosin (HE). The absence of changes in the aortic wall as well as atherosclerosis was diagnosed on HE stained sections (Fig. 1). Representative tissue slides were selected for immunohistochemical staining.

Selected representative histological sections of aortic tissue were stained by immunohistochemical method, according to the manufacturer's instructions for manual protocol using the EnVisionTM FLEX System, with a primary anti-Bcl-2 antibody (Abcam 108346). The goal of immunohistochemical staining was the visualization of Bcl-2^+ MP, according to correspondence of Bcl2+ cells with morphology of MP.

Semiquantitative assesment of presence of Bcl-2^+ macrophages. For each section of aortic tissue, 5 microscopic fields were photographed, under the magnification 200x. On samples of healthy aortic tissue, the photographs included each layer of the aortic wall (intima, media and adventitia). The number of Bcl-2^+ MP was semiquantitatively estimated for each layer of the wall separately (Table I) (Fig. 1).

Table I. Semiquantitative assessment of the presence of macrophages.

Grade	Nummber of Bcl-2^+ MP in the photograph
0 (normal)	0
1 (normal)	<5
2 (mild increase)	5-10
3 (marked increase)	10-15

Tissue sections diagnosed with mild or severe atherosclerosis were photographed in specific areas of the plaque: 5 photographs - one involving part of the plaque towards T. intima ("top" of the plaque, subintimal), one involving part of the change in the media and closer to adventitia ("bottom" of the plaque), as well as 3 photographs that cover the lateral parts of the plaque (so-called "corners" of the plaque). The number of Bcl-2^++ MP in each photograph was semiquantitatively determined (Table I). As there were no relevant reference data on what could be considered normal/ expected number of Bcl-2^+ MP, we determined this based on the analysis of healthy aortic wall samples (without pathohistological changes) in patients who did not receive statins in therapy. As 0-5 Bcl-2^+ MP were observed in 93.34 % of patients, which corresponds to grades 0 and 1, these grades are considered normal, ie the presence of up to 5 Bcl-2^+ MP is not considered as increased presence, while grade 2 will be interpreted as mild, and grade 3 marked increased presence of Bcl-2^+ MP.

Statistical analysis. For statistical analysis of the data, we used IMB SPSS Statistics 23 software for t-test and x2-test.

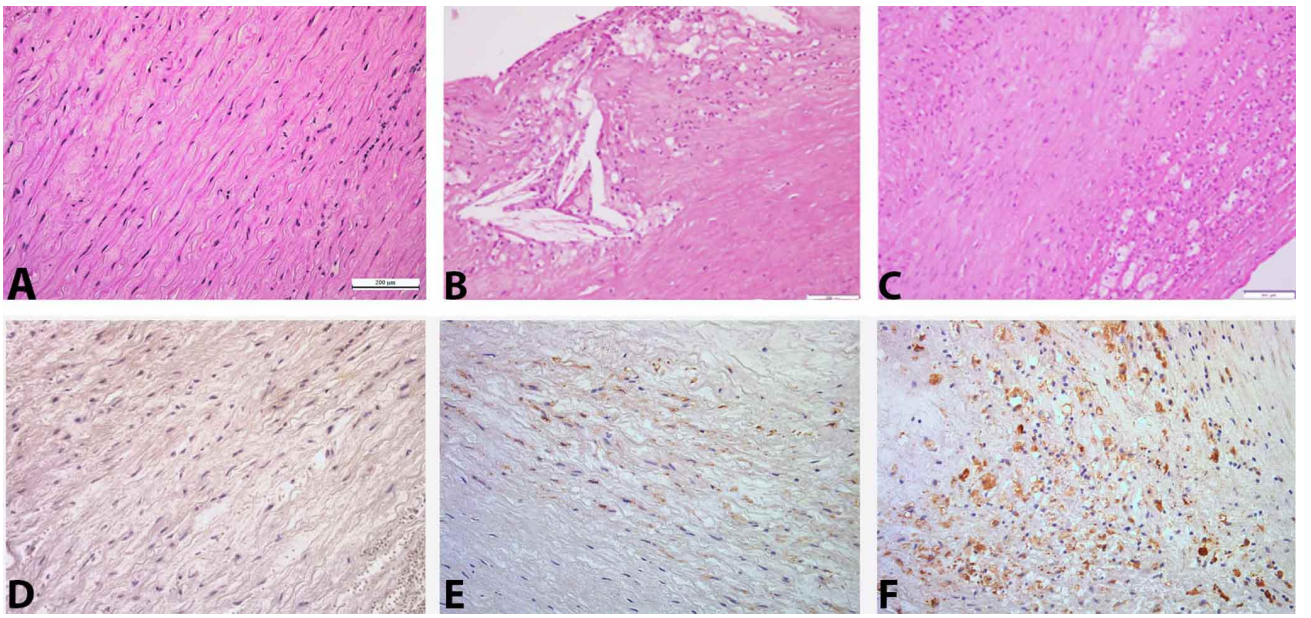

Fig. 1. Aortic wall: A - absence of pathohistological changes (HE, 200x); B – mild atherosclerosis (HE, 200x); C – severe atherosclerosis (HE, 100x); D - grades 0 and 1 -normal presence of Bcl-2$^+$ MP (BCL-2, 200x); E - grade 2 - mildy increased number of Bcl-2$^+$ MP (BCL-2, 200x) and F - grade 3 - markedly increased number of Bcl-2+ MP (BCL-2, 200x).

RESULTS

Presence of Bcl-2$^+$ MP in healthy aortic tissue and aortic tissue with pathohistological changes. Only 6.67 % of patients without statin therapy had a mild increase of Bcl-2$^+$ MP. In contrast, 60 % of patients in the Th-S group had a mild, and 20 % marked increase of Bcl-2$^+$ MP ($p < 0.01$) (Table II).

Presence of Bcl-2$^+$ MP in aortic tissue with pathohistological changes. Analysis of all aortic samples with pathohistological diagnosis showed that statin therapy was statistically significantly more often leading to a markedly increased presence of Bcl-2$^+$ MP ($p < 0.01$) (Table II).

Presence of Bcl-2$^+$ MP in healthy aortic tissue. The use of statins (Th-S) leads to a significantly more frequent increase in Bcl2$^+$ MP in the intima of the healthy aortic tissue (mild increase 40 %, $p < 0.05$; marked increas 60 %, $p < 0.01$), as opposed to only 20 % of mild increase in the Th-nonS group (Table III). In the media, all samples of the Th-S group have a mild increase of Bcl-2$^+$ MP, and in adventitia 40 % of patients ($p < 0.05$).

Table II. Presence of Bcl-2$^+$ MP in healthy aortic tissue aortic tissue with pathohistological changes.

Bcl-2$^+$ MP	Healthy aortic tissue			Aortic tissue with diagnosed pathohistological		
	Th-nonS	**Th-S**	**P**	**Th-nonS**	**Th-S**	**P**
Normal	93.4 %	20 %	p <0.01	29.5 %	23.75 %	p >0.05
Mild increase	6.67 %	60 %	p <0.01	42.5 %	40 %	p >0.05
Marked increase	0 %	20 %	p <0.01	25 %	36.25 %	p <0.01

Table III. Presence of Bcl-2$^+$ macrophages in healthy aortic tissue.

Bcl-2$^+$ MP	*T.Intima*		*T.Media*		*T.Adventitia*	
	Th-nonS	Th-S	Th-nonS	Th-S	Th-nonS	Th-S
Normal	80 %	0 %	100 %	0 %	100 %	40 %
Mild increase	20 %	40 % *	0 %	100 % *	0 %	40 % *
Marked increase	0 %	60 %†	0 %	0 %	0 %	0 %

Legend: * - statistically significant difference in level $p < 0.05$, † - statistically significant difference in level $p < 0.01$.

Presence of Bcl-2$^+$ MP in aortic tissue diagnosed with atherosclerosis. The use of statins more often leads to a markedly increased presence of Bcl-2$^+$ MP in aortic tissue with diagnosed mild and severe atherosclerosis (Table IV).

In samples of severe atherosclerosis, statins lead to a markedly increased presence of Bcl-2$^+$ MP in the parts of the plaque towards the intima and towards the media, the so-called top and bottom of the plaque ($p < 0.05$). Mild increase of Bcl-2$^+$ MP in the subintimal area of the plaque ("top") is more frequent in patients without statin therapy ($p < 0.05$). There is no statistical significance of the observed differences in the lateral areas of the plaque (Table V).

In mild atherosclerotic changes of aorta, statin therapy did not lead to differences in the presence of Bcl-2$^+$ MP.

Table IV. Presence of Bcl 2$^+$ MP in the aortic wall with diagnosed atherosclerosis.

Bcl-2$^+$ Mp	Mild atherosclerosis			Severe atherosclerosis		
	Th-nonS	**Th-S**	**P**	**Th-nonS**	**Th-S**	**P**
Normal	18 %	20%	p >0.05	40 %	20 %	p <0.01
Mild increase	54 %	44%	p <0.01	36 %	32 %	p >0.05
Marked increase	28 %	36%	p <0.05	24 %	48 %	p <0.01

Table V. Impact of statins on Bcl-2$^+$ MP in specific areas of plaque in aortas with severe atherosclerotic changes.

Bcl-2$^+$ MP	The "top" of the plaque		The "bottom" of the plaque		Plaque corners	
	Th-nonS	Th-S	Th-nonS	Th-S	Th-nonS	Th-S
Normal	30 %	40 %	60 %	20 %	33.3 %	13.4 %
Mild increase	50 % *	20 %	40 %	20 %	33.3 %	40 %
Marked increase	20 %	40 % *	0 %	60 % *	33.3 %	46.6 %

Statistically significant difference in level $p < 0.05$.

DISCUSSION

The incidence of cardiovascular diseases (CVD) as well as aortic diseases that require surgical treatment have not decreased, and even increased in recent decades (McClure *et al*., 2018; Coselli, 2018). Due to the prevalence of the CVD, a large number of drugs and supplements are prescribed, but statins predominate. Moreover, they are considered the "gold drug" or "gold standard" in the treatment of hyperlipoproteinemias that underlie many CVDs. What additionally provides statins with a central place in therapy is the wide range of pleiotropic effects (Davignon, 2004). Although the basic and numerous pleiotropic functions of statins have been described in many studies, few analyze the "survival" of macrophages under statin therapy, as well as what this actually means for the course of the disease itself and for the patient's health.

Our research for the first time provides data on the extent to which the presence of Bcl-2$^+$ MP can be considered normal. Based on the analysis of healthy aortic tissue (ones without diagnosed pathohistological changes) without the use of drugs from the statin group, we found that over 90 % of samples 0-5 Bcl-2$^+$ MP. Therefore, our study established that the range of 0-5 Bcl-2$^+$ MF can be considered as normal in the aortic wall.

In both healthy and atherosclerotic aortic tissue, statins significantly lead to increased presence of Bcl-2$^+$ MP. Data related to unaltered aortas are rare, but data on the relationship between statins and the Bcl-2 family are somewhat more common. What is contradictory in this regard is that in anticancer studies, statins stimulate apoptosis, reduce anti- and enhance the expression of proapoptotic Bcl-2 proteins (eg, reduce Bcl-2 and induce Bax). On contrary, in non-cancer studies, statins show the opposite effect. Most if not all data supporting the proapoptotic action of statins come from studies on tumor cell cultures using high doses of statins. Studies advocating the antiapoptotic role of statins are few, conducted on non-tumor tissues and with lower doses (Shearn *et al*. 2012). The results of our study support the concept of antiapoptotic action of statins, since both in unaltered and altered aortic tissue samples, statins lead to an increased presence of Bcl-2$^+$ MP in the aortic wall. Similar results were obtained by Qin *et al*. (2012) when antiapoptotic activity with induction of Bcl-2 expression in aortic tissue was demonstrated in a model of ApoE-deficient mice fed atherogenic diet and simvastatin therapy. Although they come to the same conclusions as us, it is correct to note that the study is not based on the same methodology. Namely, they indicate that

the number of macrophages in the plaque area increased under the action of simvastatin, but the induction of Bcl-2 and Bcl-xL was detected by the Western blot method and does not imply that induction is present (only) in macrophages.

In atherosclerotic changes of the aorta, lipid deposition in the subintimal region activates proinflammatory signals, attracts circulating monocytes that pass through the intima and polarizes in the direction of M1 macrophages that stimulate plaque development. Such proinflammatory macrophages further secrete proatherosclerotic cytokines, free oxygen and nitrogen radicals (Duffield, 2003). The pronounced increased presence of Bcl-2^+ MP was statistically significantly more often detected due to the use of statins (Th-S groups) in mild and severe atherosclerotic changes. These data support the claim that statins prolong life, ie. protect against macrophage apoptosis in atherosclerotic lesions, and given their proinflammatory role, statin use could even be considered a risk factor for development towards complicated lesions and plaque ruptures. This claim is based on the fact that it is known that macrophages after death, contribute to the formation of necrotic plaque nuclei and the secretion of MMP2 and MMP9 contribute to the thinning of the so-called, plaque caps, which is a feature of complicated lesions (plaques) that are prone to rupture (Kajimoto *et al*., 2009). However, such claims should be taken with a grain of salt. Hardtner *et al*. present data that statins cause regression and reduction of plaques in atherosclerosis, which is histologically manifested by a reduced number of macrophages in plaques, but the mechanism by which this is achieved has not been elucidated (Zhang *et al*., 2018; Härdtner *et al*., 2020). On the other hand, Zhang *et al.* (2018) found that pravastatin increases plaque dimensions even in the absence of a lipid-rich diet (atherogenic diet). The key mechanism according to their research, conducted on an animal model, is the change in the polarization of macrophages, in favor of the M2 phenotype (Zhang *et al*., 2018).

In our study, we also analyzed in which part of the plaque there is a greater accumulation of Bcl-2+ MP. No significant differences were observed in the aortic wall with mild atherosclerotic changes, but in the aorta with severe atherosclerotic changes, the use of statins leads to statistically significant changes. Namely, the presence of Bcl-2^+ MP in the area of the "top" and "bottom" of the plaque is more pronounced, ie. in the subintimal part and basally, towards the media. Our data are in line with the research of Bobrisev *et al*., which states that plaque growth is achieved by accumulating both macrophage populations, but that M1 accumulates predominantly in the subintimal ("top") plaque region, while anti-inflammatory, M2 are localized in the more stable area of the "bottom" of the plaque, ie towards the media. In line with this claim, the benefit of statins in severe atherosclerosis with complicated plaques is once again questioned if they prolong the life of proinflammatory macrophages in the subintimal region ("top"), as shown in our study. Identification of subspecies M1 and M2 is difficult because there are no specific markers (Bobryshev *et al*, 2016). Unfortunately, we were not in a position to differentiate which population (M1 or M2) belongs to the numerous Bcl-2^+ MP, detected in the "top" of the plaque of severe atherosclerosis and to indicate the fate of the plaque. This would be especially significant in mild atherosclerosis, since in aortas diagnosed with mild atherosclerosis without statin therapy - mild increase of Bcl-2^+ MP is significantly more often. In this way, it would be established whether the plaque develops in the direction of progression / instability or regression, regardless of statin therapy, and the treatment of patients could be approached more adequately.

ACKNOWLEDGMENTS. This research is financially supported by the Autonomous Province of Vojvodina (Project No 142-451-2543/2021-02)

VISNJIC BOJANA, A.; GOLUB, S.; MILANA, B.; PANTIC, T.; KOLAROV-BJELOBRK, I.; RADIC, J.; ILIC SABO, J.; AMIDZIC, J. & GVOZDENOVIC, N.¿Son las estatinas un factor de riesgo en pacientes con aterosclerosis?. *Int. J. Morphol., 40(5)*:1236-1241, 2022.

RESUMEN: Las estatinas inhiben la síntesis de colesterol, pero también tienen otros efectos pleiotrópicos. Hay indicios de que afectan la supervivencia de los macrófagos a través de la regulación de la apoptosis.Se analizaron 50 muestras de pared aórtica, seleccionadas en base a estatinas en tratamiento de pacientes (n=25, grupo Th-S) o en tratamiento libre de estatinas (n=25, grupo Th-nonS). Cada grupo tenía 5 muestras de tejido aórtico sano, 10 muestras de cambios ateroscleróticos leves y 10 muestras de cambios ateroscleróticos severos en la pared aórtica. El tejido se tiñó con hematoxilina-eosina y métodos inmunohistoquímicos (anticuerpo anti-Bcl-2). La presencia de macrófagos positivos para Bcl2 (Bcl-2^+ MP) se determinó semicuantitativamente y los datos se procesaron en Microsoft Excell e IMB SPSS 23 Statistics. El 60 % de los pacientes del grupo Th-S tuvo un aumento leve de Bcl-2^+ MP. El uso de estatinas conduce a un aumento significativamente más frecuente de macrófagos Bcl2^+ en la íntima del tejido aórtico sano. El análisis de todas las muestras aórticas con diagnóstico anatomopatológico mostró que la terapia con estatinas fue significativamente más frecuente desde el punto de vista estadístico, lo que condujo a una presencia marcadamente mayor de Bcl-2^+ MP. En los medios, todas las muestras del grupo Th-S tienen un leve aumento de Bcl-2^+ MP, y en adventicia en el 40 % de los pacientes. El uso de estatinas con mayor frecuencia conduce a una presencia marcadamente mayor de MP Bcl-2^+ en el tejido aórtico con aterosclerosis leve y grave diagnosticada. En muestras de

aterosclerosis severa, las estatinas conducen a una presencia aumentada de Bcl-2^+ MP en las partes de la placa hacia la íntima y hacia la media. Las estatinas conducen a una mayor presencia de macrófagos Bcl-2^+, prolongan su vida, tanto en tejido aórtico sano como aterosclerótico alterado. Esto indica la potenciación de la inflamación y el daño a la pared aórtica y pone en duda el efecto positivo de las estatinas en la pared aórtica con aterosclerosis.

PALABRAS CLAVE: Estatinas; BCL-2; Apoptosis; Aterosclerosis; Macrófagos.

Corresponding author:
Bojana Andrejic Visnjic PhD
Assistant Professor
Department of Histology and Embryology
Faculty of Medicine
University of Novi Sad
Hajduk Veljkova 3, 21000 Novi Sad
SERBIA

E-mail: bojana.andrejic-visnjic@mf.uns.ac.rs

REFERENCES

Bobryshev, Y. V.; Ivanova, E. A.; Chistiakov, D. A.; Nikiforov, N. G. & Orekhov, A. N. Macrophages and their role in atherosclerosis: pathophysiology and transcriptome analysis. *Biomed Res. Int., 2016*:9582430, 2016.

Coselli, J. S. Aortic dissection: A little help from our friends. *J. Thorac. Cardiovasc. Surg., 155(6)*:2249-50, 2018.

Davignon, J. Beneficial cardiovascular pleiotropic effects of statins. *Circulation, 109(23 Suppl. 1)*:III39-43, 2004.

Duffield, J. S. The inflammatory macrophage: a story of Jekyll and Hyde. *Clin. Sci. (Lond.), 104(1)*:27-38, 2003.

Härdtner, C.; Kornemann, J.; Krebs, K.; Ehlert, C. A.; Jander, A.; Zou, J.; Starz, C.; Rauterberg, S.; Sharipova, D.; Dufner, B.; *et al*. Inhibition of macrophage proliferation dominates plaque regression in response to cholesterol lowering. *Basic Res. Cardiol., 115(6)*:78, 2020.

Kajimoto, K.; Miyauchi, K.; Kasai, T.; Shimada, K.; Kojima, Y.; Shimada, A.; Niinami, H.; Amano, A. & Daida, H. Short-term 20-mg atorvastatin therapy reduces key inflammatory factors including c-Jun N-terminal kinase and dendritic cells and matrix metalloproteinase expression in human abdominal aortic aneurysmal wall. *Atherosclerosis, 206(2)*:505-11, 2009.

McClure, R. S.; Brogly, S. B.; Lajkosz, K.; Payne, D.; Hall, S. F. & Johnson, A. P. Epidemiology and management of thoracic aortic dissections and thoracic aortic aneurysms in Ontario, Canada: A population-based study. *J. Thorac. Cardiovasc. Surg., 155(6)*:2254-64, 2018.

Moore, K. J.; Sheedy, F. J. & Fisher, E. A. Macrophages in atherosclerosis: a dynamic balance. *Nat. Rev. Immunol., 13(10)*:709-21, 2013.

Paraskevas, K. I. Applications of statins in cardiothoracic surgery: more than just lipid-lowering. *Eur. J. Cardiothorac. Surg., 33(3)*:377-90, 2008.

Qin, W.; Lu, Y.; Zhan, C.; Shen, T.; Dou, L.; Man, Y.; Wang, S.; Xiao, C.; Bian, Y. & Li, J. Simvastatin suppresses apoptosis in vulnerable atherosclerotic plaques through regulating the expression of p(53), Bcl-2 and Bcl-xL. *Cardiovasc. Drugs Ther., 26(1)*:23-30, 2012.

Shearn, A. I. U.; Deswaerte, V.; Gautier, E. L.; Saint-Charles, F.; Pirault, J.; Bouchareychas, L.; Rucker 3rd, E. B.; Beliard, S.; Chapman, J.; Jessup, W.; *et al*. Bcl-x inactivation in macrophages accelerates progression of advanced atherosclerotic lesions in Apoe(-/-) mice. *Arterioscler. Thromb. Vasc. Biol., 32(5)*:1142-9, 2012.

Wood, W. G.; Igbavboa, U.; Muller, W. E. & Eckert, G. P. Statins, Bcl-2, and apoptosis: cell death or cell protection? *Mol. Neurobiol., 48(2)*:308-14, 2013.

Zhang, X.; Xiao, S. & Li, Q. Pravastatin polarizes the phenotype of macrophages toward M2 and elevates serum cholesterol levels in apolipoprotein E knockout mice. *J. Int. Med. Res., 46(8)*:3365-73, 2018.