Morphometric Analysis of Atherosclerotic Plaques in Human Carotid Arteries

V. S. Shishkina*,, S. V. Kashirina*, V. N. Sirotkin*, O. P. Il'inskaya*,**, and E. M. Tararak***

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 152, No. 11, pp. 577-580, November, 2011
Original article submitted October 28, 2010

Morphometric analysis of 35 biopsy specimens from patients with stable (n=10) and unstable (n=25) atherosclerotic lesions was carried out. The structure of the plaques and their connective tissue caps was studied by various methods of histological sections staining. A new morphometric approach to quantitative evaluation of atherosclerotic lesions instability is suggested. It consists in calculation of the morphological "rigidity" coefficient, due to which the plaque is characterized more accurately. The proportion of areas of the "rigid" (connective tissue and calcium salt deposition areas) to "soft" (atheronecrotic nuclei, microvessels, clots and hemorrhages) structures of the plaque is evaluated. Plaque instability (liability of a to rupture) is associated with changes in the extracellular matrix components in the cap: accumulation of collagen and reduction of elastic fiber content reducing vessel elasticity and making its locally more rigid.

Key Words: *carotid arteries; atherosclerotic plaques; lesion stability/instability; morphometry*

Acute clinical manifestations of cardiovascular diseases are associated with the development of unstable atherosclerotic lesions liable to ruptures in the main arterial walls. The mechanisms of atherosclerotic plaque instability development are different and not amply studied. The risk of plaque rupture is explained primarily by its structure: thickness of the fibrous cap and content of extracellular matrix proteins (collagen, elastin, and glycosaminoglycans), size and consistence of atheromatous nuclei, and inflammatory phenomena [4,7]. Rupture of an unstable plaque can lead to acute circulatory disorders (acute coronary syndrome and brain stroke) and death [6]. High positive correlation between atherosclerosis and increase of pulse wave velocity (PWV) has been demonstrated [13]. The PWV is determined by morphological characteristics of the vessel and other parameters [1-3]; it characterizes the elastic stress of the vascular wall and increases with increasing artery rigidity [5]. The content and proportion of collagen (responsible for the vessel strength) and elastic fibers (responsible for elasticity and stretching/compression of the vessel) largely determine biomechanics of the vascular wall [11,13]. Arterial wall with increased content of collagen fibers and reduced content of elastic fibers becomes more rigid [5]. Pathological changes in the composition of vascular matrix components are fraught with higher risk of cardiovascular complications of hypertensive nature [8,9].

Here we use morphological criteria for evaluation of vascular wall rigidity based on the morphometric analysis of carotid artery sections from patients with atherosclerosis. We hypothesized that vascular rigidity can serve as an indicator of plaque instability.

The relationship between vascular wall rigidity and plaque instability was investigated. To this end, detailed morphological analysis of biopsy specimens of carotid arteries and extracellular matrix components was carried out and the plaque rigidity coefficient was

*Institute of Experimental Cardiology, Moscow; **Biological Faculty, M. V. Lomonosov Moscow University, Russia. ***Address for correspondence:*** ShishkinaValya@mail.ru. V. S. Shishkina

0007-4888/12/1525-0642

evaluated morphometrically. These data were analyzed with consideration for the presence of signs of the atherosclerotic plaque instability.

MATERIALS AND METHODS

A total of 35 biopsy specimens of the carotid arteries were obtained during endarterectomy from patients (27 men and 8 women aged 63±9 years) with acute clinical manifestations of carotid failure. The specimens were cut into transverse sections of 5-7 mm, fixed in methanol:chloroform:glacial acetic acid (6:3:1) mixture, and embedded in paraffin. Sections (6 μ) were stained with hematoxylin and eosin for common morphological studies. Collagen fibers were detected by van Gieson method, elastic fibers by orsein staining, and acid glycosaminoglycans (GAG) by alcian blue staining. Atherosclerotic lesions were classified after H. C. Stary [12]. In each specimen, thrombi and hemorrhages, foci of calcification and vascularization, local thinning and ruptures in the cap, and the number and size of atheronecrotic nuclei were recorded [4,7]. The content of collagen and elastic fibers and GAG was evaluated after appropriate staining by a semiquantitative method using a 3-point scale (1: no staining/slight staining; 2: medium staining; 3: intense staining). Three sections were analyzed for each staining type. Morphometric analysis was carried out in photoimages using ImageJ software. Areas of the plaque occupied by "rigid" (outer cap, connective tissue of the plaque, calcium salt deposition foci) and "soft" (atheronecrotic nuclei, foci of microvessels accumulation, intramural clots and hemorrhages) morphological structures were measured in a section. The atherosclerotic plaque rigidity coefficient was calculated as the proportion of areas occupied by rigid and soft morphological structures.

The data were statistically processed by Statistica 7.0 software using nonparamateric two-way Mann–Whitney test and Fisher exact test. The differences were considered significant at $p<0.05$.

RESULTS

The greater part of biopsy specimens were type IV lesions according to Stary: complicated, multilamellar (several atheronecrotic nuclei one above another) lesions with a surface defect, hematoma, and/or thrombosis.

Histomorphological analysis of atherosclerotic lesions showed that virtually all plaques (n=32; 91%) had a thick (more than 125 μ) cap. Local thinning of the cap was detected in the majority (n=27; 77%) of lesions. These data indicated that local thinning of the cap regarded as a sign of vulnerable plaque was not the key factor for characterization of its instability. A sum of instability criteria was taken into consideration for reliable diagnosis of the plaque rupture risk. These factors were cap defects (ruptures, local thinnings, hemorrhages), hemorrhages in the plaque, thrombi, abundant vascularization and infiltration by hematogenic cells, large lipid nucleus or high lipid/fibrous proportion [7,10].

Depending on the presence/absence of instability signs, all plaques were divided into 2 groups (Table 1): with stable (n=10) and unstable (n=25) lesions

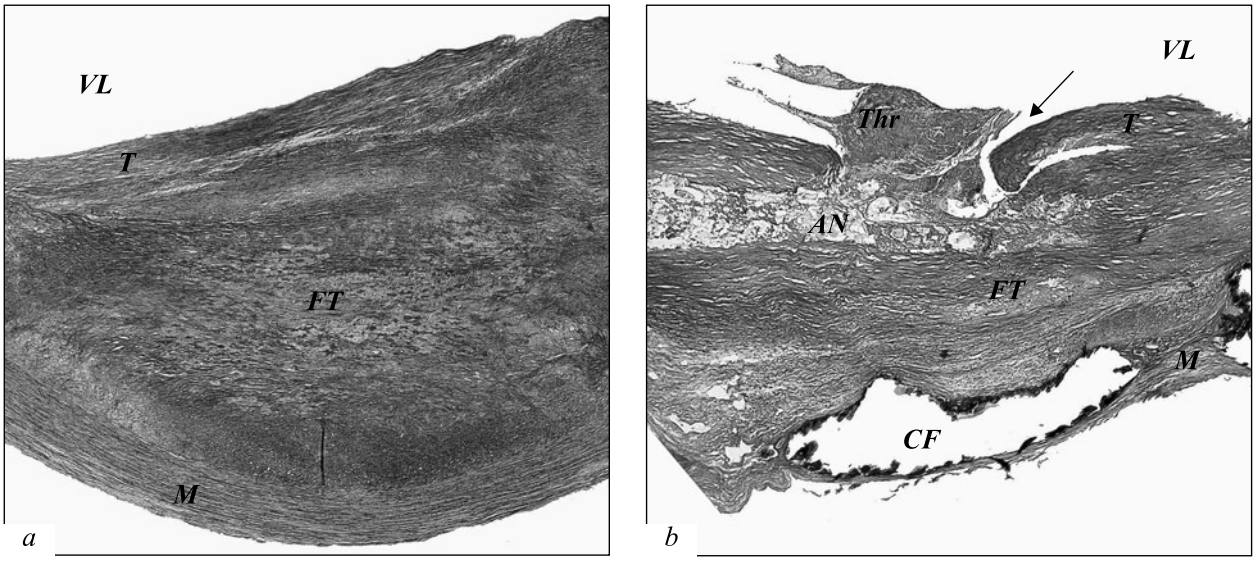

Fig. 1. Atherosclerotic lesions of human carotid arteries. van Gieson staining, ×60. *a*) stable atherosclerotic plaque with well-expressed fibrous cap and well-developed connective tissue matrix; *b*) unstable plaque with fibrous cap rupture, hemorrhage into atheromatous nucleus, and parietal thrombus in carotid artery lumen. Arrow: site of cap rupture; VL: vessel lumen; T: cap; AN: atheromatous nucleus; FT: fibrous tissue; Thr: thrombus; CF: calcification foci; M: media.

TABLE 1. Histomorphometric Parameters of Stable and Unstable Plaques in the Carotid Arteries

Parameter			Stable plaques (n=10)	Unstable plaques (n=25)	Significance
Type of lesions [12]					
type IV, atheroma			2	0	$p<0.01$
type Va, fibroatheroma			2	0	
type Vb, fibrous lesions			1	1	
type Vc, calcification			1	1	
type VI, complex lesion with surface defect, hematoma			4	23	
Rigidity coefficient ($M\pm m$)			2.0±1.5	1.1±0.7	$p<0.05$
Characteristics of plaque					
Fibrous cap	Collagen	2 points	2	0	$p=0.08$
		3 points	8	25	
Fibrous tissue lamina	GAG	2 points	2	17	$p<0.05$
		3 points	5	4	
External atheronecrotic nucleus	Thrombus		8	16	$p<0.05$
	Elastin	2 points	5	21	$p=0.08$
		3 points	5	4	
Internal atheronecrotic nucleus	Internal nucleus organized by connective tissue		6	2	$p<0.001$
	Collagen	1 point	0	1	$p=0.07$
		2 points	1	13	
		3 points	6	8	
	Elastin	1 point	0	2	$p<0.01$
		2 points	2	17	
		3 points	5	3	
	GAG	1 point	0	2	$p=0.06$
		2 points	3	17	
		3 points	4	3	

(Fig. 1). A greater variety of types was found in the group of stable *vs.* unstable plaques; 92% unstable plaques were referred to type VI lesions (Table 1). Statistical analysis of morphometric values revealed the differences between the groups (Table 1). First, the lesions in the groups differed significantly by the presence of GAG in fibrous tissue of the plaque. The content of GAG in the connective tissue and cap of unstable plaques was often significantly lower than in stable plaques. Second, organized clots and atheronecrotic nuclei were significantly more incident in stable *vs.* unstable plaques. In addition, the lesions differed significantly by the content of elastic fibers in internal atheronecrotic nuclei. Elastin staining intensity was significantly higher in the nuclei of stable plaques. These results indicated more severe degenerative changes in the vascular wall (ruptures, clots, hemorrhages) in unstable plaques.

Analysis of the collagen-elastic framework of the plaque cap showed that the content of collagen fibers was low in 20% caps of stable plaques, while in unstable plaques virtually all caps were collagen-rich. Ruptures were found in 3 unstable plaques, despite thick collagen cap; in 2 cases they were coupled with surface thrombosis (Fig. 1, *b*). It is noteworthy that staining for elastin was minimum in the caps with intense staining for collagen (data not presented). Presumably, rigidity of the plaque cap increased with increasing the content of collagen and reducing the content of elastic fibers, which makes it less stretchable and more fragile, vulnerable to ruptures under conditions of changing hemodynamic load in the vessel.

The lipid/fibrous proportion characterizing the size of lipid nucleus and fibrous tissue content [10] is often used as an additional sign for evaluation of atherosclerotic plaques instability. However, other soft areas of atherosclerotic lesion also contributing to plaque instability are neglected in this case. We estimated the atherosclerotic plaque rigidity coefficient by evaluating the proportion of areas of all the above rigid and soft morphological structures. The mean rigidity coefficient for stable and unstable plaques was 2.0±1.5 and 1.1±0.7, respectively. Statistical analysis of all rigidity coefficients for both groups of lesions was carried out using nonparametric two-way Mann–Whitney test. The results demonstrated significant differences ($p<0.05$) between stable and unstable lesions by this sign. The rigidity coefficient proposed by us most amply characterizes the atherosclerotic lesion and can be used as an additional numerical morphometric index of atherosclerotic plaque instability.

The study was partially supported by the Russian Foundation for Basic Research (grant No. 08-04013753-ofi_c) and Department of Science and Industrial Strategy of Moscow (state contract No. 8/3-280H-10).

The authors are grateful to Prof. I. P. Mikhailov, Doct. Med. Sci., Head, Dept. of Vascular Surgery of N. V. Sklifosovsky Institute of Urgent Care, and to V. V. Akhmetov, Cand. Med. Sci. for assistance.

REFERENCES

1. R. S. Karpov and V. A. Dudko, *Atherosclerosis* [in Russian], Tomsk (1998).
2. V. I. Titov, S. A. Chorbinskaya, and B. A. Belova, *Kardiologiya*, **42**, No. 3, 95-98 (2002).
3. R. Asmar, A. A. Benetos, G. London, *et al.*, *Blood Press.*, **4**, No. 1, 48-54 (1995).
4. W. Casscells, M. Naghavi, and J. T. Willerson, *Circulation*, **107**, No. 16, 2072-2075 (2003).
5. P. B. Dobrin and A. A. Rovick, *Am. J. Physiol.*, **217**, No. 6, 1644-1651 (1969).
6. B. J. Dunmore, M. J. McCarthy, A. R. Naylor, and N. P. Brindle, *J. Vasc. Surg.*, **45**, No. 1, 155-159 (2007).
7. E. Falk, P. K. Shah, and V. Fuster, *Circulation*, **92**, No. 3, 657-671 (1995).
8. E. G. Lakatta and D. Levy, *Circulation*, **107**, No. 1, 139-146 (2003).
9. E. G. Lakatta and D. Levy, *Ibid.*, **107**, No. 2, 346-354 (2003).
10. J. K. Lovett, J. N. Redgrave, and P. M. Rothwell, *Stroke*, **36**, No. 5, 1091-1097 (2005).
11. K. S. Midwood and J. E. Schwarzbauer, *Mol. Biol. Cell*, **13**, No. 10, 3601-3613 (2002).
12. H. C. Stary, *Arterioscler. Thromb. Vasc. Biol.*, **20**, No. 5, 1177-1178 (2000).
13. N. M. van Popele, D. E. Grobbee, M. L. Bots, *et al.*, *Stroke*, **32**, No. 2, 454-460 (2001).