Blood Levels of Inflammatory and Destructive Biomarkers in Coronary Atherosclerosis of Different Severity

Yu. I. Ragino, A. M. Chernjavski*, Ya. V. Polonskaja, S. Yu. Tchimbal*, D. A. Redjkin*, E. V. Semaeva*, E. V. Kashtanova, and M. I Voevoda

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 5, pp. 520-523, May, 2010
Original article submitted April 1, 2009

In male patients with coronary atherosclerosis without acute coronary syndrome, the levels of inflammatory-destructive biomarkers of atherosclerotic plaque instability depended on the severity and dissemination of coronary atherosclerosis. The highest levels of C-reactive protein and matrix metalloproteinase 3 were found in men with atherosclerotic involvement of all three main coronary arteries, primarily their middle and distal segments, and in men with predominance of low-grade stenoses (<50%) of coronary arteries in areas of atherosclerotic plaques.

Key Words: *coronary atherosclerosis; biomarkers of atherosclerotic plaque instability; highly sensitive C-reactive protein; metalloproteinases*

Atherothrombosis, a pathomorphological basis of acute coronary syndrome (ACS), is triggered in 70-80% cases by violation of endothelium integrity at the site of ulceration/destruction of the cap of vulnerable unstable atherosclerotic plaque. This plaque typically has thin or locally thinned cup with endothelium destruction and inflammatory-cell infiltration with monocytes/macrophages and T cells and loose lipid core with necrotic foci [7,13-15]. The key role in destabilization of the atherosclerotic plaque is played by inflammatory and destructive changes induced by activated monocytes/macrophages producing inflammatory mediators and cytokines, including IL-1β, IL-6, IL-8, TNF-α, *etc*. [1,2,7,14,15]. Along with inflammatory cytokines, activated macrophages produce destructive matrix metalloproteinases (MMP) inducing degradation of extracellular matrix of the plaque cap [2,5,7]. These data were confirmed by clinical studies detecting high levels of some inflammatory-destructive biomarkers, in particular IL-6, highly-sensitive C-reactive protein (CRP), MMP-3, and MMP-9, in patients with ACS [3-5], therefore they are now considered by many authors as biomarkers of atherosclerotic plaque instability [2-5,10,11].

Another aspect of the problem of instability of atherosclerotic plaques is the role of the relationship between their size and degree of arterial stenosis [6,8,9,13]. Analysis of coronary angiograms performed one week before the acute event showed that in 60-70% patients with ACS the causal atherosclerotic plaques narrowed the artery lumen by less than 70% (often by <50%) [6,9,13]. Further studies in this direction are stimulated by the conclusion that cracks and ruptures of plaque caps leading to atherothrombosis can occur in instable plaques producing and not producing arterial stenosis.

Here we studied the relationship between the levels of inflammatory-destructive biomarkers of plaque instability with the degree of stenosis, dissemination,

Institute of Therapy, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk; *E. N. Meshalkin Research Institute of Circulatory Pathology, Federal Agency for High-Technological Medical Care, Novosibirsk, Russia. ***Address for correspondence:*** ragino@mail.ru. Yu. I. Ragino

and primary localization of atherosclerotic foci in coronary arteries (coronary angiography data) in male patients with coronary atherosclerosis without ACS.

MATERIALS AND METHODS

The study included 96 male patients aging 42-70 years (mean age 56.1±1.2 years) with coronary atherosclerosis verified by selective coronary angiography performed on Advantex LC/LP angiography apparatus (General Electric) with effort angina without ACS. The patients were admitted at E. N. Meshalkin Research Institute of Circulatory Pathology for coronary bypass surgery.

Seventy-two patients (75%) had a history of myocardial infarction (not less than 6 months before the study). The history of CHD varied from 1 to 40 years (mean 7.1±1.3 years). Class II, III, and IV effort angina was diagnosed in 14 (14.5%), 67 (70%), and 15 (15.5%) patients, respectively. Coronary angiography according to ACC/AHA Guidelines for coronary angiography (1999) [12] revealed atherosclerotic lesion of one of three main branches of coronary arteries (anterior descending and right or left circumference arteries) in 17 patients (18%); in 30 (31%) and 49 (51%) patients atherosclerotic lesions were found in two and three branches, respectively. The predominant degree of coronary artery stenosis was 25-50% of lumen diameter in 14 patients (15%), 60-80% in 36 patients (37%), and 90-100% in 46 patients (48%). In 25 patients (26%), atherosclerotic foci were primarily located in the proximal segments (first segments) of main branches of coronary arteries, predominant localization of atherosclerotic foci in the middle (second) and distal (third) segments was observed in 27 (28%) and 17 (18%) patients, respectively, and no predominant localization of atherosclerotic foci (total localization) was found in 27 patients (28%).

In all patients, the blood was drawn from the ulnar vein after overnight fast (12 h after the last meal). Serum levels of CRP (Biomerica test systems), TNF-α, IL-1β, IL-6, IL-8, destructive MMP-3 and MMP-9 and tissue inhibitors of MMP (TIMP-1; BCM Diagnostics test systems) were measured by ELISAs on Multiscan EX ELISAs analyzer.

The data were processed statistically by comparative and correlation methods and One-Way ANOVA with Dunnett test for multiple comparison using SPSS for Windows software.

RESULTS

Analysis of changes in blood levels of inflammatory and destructive biomarkers in patients with coronary atherosclerosis depending on the number of involved coronary arteries (Table 1) revealed the following tendencies. Blood concentrations of biomarkers decreased with increasing the number of involved arteries from 1 to 3: IL-1β by 1.3 times, TNF-α by 2.4 times, and IL-8 by 2.4 times. For some biomarkers of atherosclerotic plaque instability, CRP and stromolysin MMP-3, opposite tendency was observed: their blood concentrations increased with increasing the number of involved arteries by 1.9 and 1.6 times, respectively.

Similar tendencies were found during the analysis of changes in blood levels of inflammatory and destructive biomarkers in patients with coronary atherosclerosis depending on predominant localization of atherosclerotic foci in coronary arteries (Table 2). Thus, in patients with predominant localization of atherosclerotic plaques in proximal segments of the main coronary arteries, blood concentrations of TNF-α

TABLE 1. Blood Levels of Inflammatory and Destructive Biomarkers in Male Patients Coronary Atherosclerosis Depending on the Number of Involved Coronary Arteries (Right, Anterior Descending, Circumference) Coronary Angiography Data (*M±m)*

Parameter	Number of involved coronary arteries			$p<0.05$
	1 (n=17)	2 (n=30)	3 (n=49)	
IL-1β, pg/ml	2.4±0.2	2.1±0.2	1.8±0.1	3-1
TNF-α, pg/ml	6.4±0.2	5.2±0.3	2.7±0.2	3-1,2
IL-6, pg/ml	9.3±1.0	11.4±1.3	9.3±1.1	
IL-8, pg/ml	59.4±5.2	34.4±3.0	25.2±3.0	1-2,3
CRP, μg/ml	5.9±0.3	7.3±0.5	11.4±0.7	3-1,2
TIMP-1, ng/ml	490.6±27.7	464.0±25.2	430.1±18.2	
MMP-3, ng/ml	15.7±1.2	21.8±2.0	24.8±2.1	3-1
MMP-9, ng/ml	430.0±15.5	428.2±21.2	424.1±32.4	

TABLE 2. Blood Levels of Markers of Inflammation and Destruction in Male Patients with Coronary Atherosclerosis Depending on Predominant Localization of Atherosclerotic Foci in Coronary Arteries: Coronary Angiography Data (*M±m)*

Parameter	Segments ($^1/_3$ length) of coronary arteries				p<0.05
	1 proximal (n=25)	2 middle (n=25)	3 distal (n=17)	4 total (n=25)	
IL-1β, pg/ml	1.7±0.1	1.9±0.2	2.0±0.2	1.8±0.1	
TNF-α, pg/ml	4.8±0.3	4.6±0.3	3.5±0.2	3.7±0.3	1-3
IL-6, pg/ml	8.3±0.7	9.9±0.8	9.8±0.8	9.9±0.9	
IL-8, pg/ml	57.9±4.3	26.9±2.2	24.0±2.0	26.9±2.0	1-2,3,4
CRP, μg/ml	4.2±0.3	8.0±0.5	8.1±0.5	6.1±0.5	1-2,3
TIMP-1, ng/ml	468.9±37.4	463.2±30.6	449.7±39.5	441.1±29.6	
MMP-3, ng/ml	14.8±1.1	28.0±2.1	27.5±2.2	28.1±2.0	1-2,3,4
MMP-9, ng/ml	487.5±32.4	387.9±31.2	398.5±27.5	421.0±28.6	

and IL-8 were higher than in patients with predominant localization of plaques in distal segments by 1.4 and 2.4 times, respectively. An opposite tendency was observed for biomarkers of atherosclerotic plaque instability, CRP and stromolysin MMP-3: higher blood levels of these biomarkers were found in patients with predominant involvement of middle (by 1.9 times) and distal segments (by 2 and 1.8 times, respectively). Close relationship between the levels of CRP and destructive MMP-3 and MMP-9 was reported in many recent studies concerning the problem of atherosclerotic plaque instability and ACS [4,5,10].

Analysis of the relationship between blood levels of inflammatory-destructive biomarkers of atherosclerotic plaque instability and predominant degree of stenosis/occlusion of involved coronary arteries (Table 3) revealed their co-directed changes. For instance, blood concentrations of inflammatory cytokines, CRP, and destructive MMP in patients with low degree (25-50%) of coronary artery stenosis were higher than in patients with severe stenosis (90-100%): IL-1β by 1.3 times, TNF-α by 1.7 times, IL-8 by 1.5 times, CRP by 1.3 times, and MMP-3 and MMP-9 by 1.4 and 1.5 times, respectively. These findings attest to higher level of biomarkers of atherosclerotic plaque instability in patients with less severe stenoses of coronary arteries at the sites of atherosclerotic foci. These results agree with earlier observations and conclusions that the greater is the clear space above the atherosclerotic plaque, the higher is blood flow and the greater is the area of its contact with the surface of fibrous cap of the plaque, and hence, the higher is the risk of its tear/rupture [8-10]. This clinical-angiographic paradox can be determined by certain factors [13,14]. First,

TABLE 3. Blood Levels of Markers of Inflammation and Destruction in Male Patients with Coronary Atherosclerosis Depending on Predominant Severity of Stenosis/Occlusion of Involved Coronary Arteries. Coronary Angiography Data (*M±m)*

Parameter	Degree of stenosis/occlusion of coronary arteries			p<0.05
	1 (25-50%; n=14)	2 (60-80%; n=36)	3 (90-100%; n=46)	
IL-1β, pg/ml	2.4±0.2	1.8±0.1	1.8±0.1	1-2,3
TNF-α, pg/ml	5.0±0.3	3.1±0.3	3.0±0.2	1-3
IL-6, pg/ml	9.9±0.9	9.5±0.9	10.0±1.0	
IL-8, pg/ml	41.9±4.0	28.8±2.0	28.1±2.1	1-2,3
CRP, μg/ml	6.8±0.3	5.6±0.3	5.2±0.2	1-2,3
TIMP-1, ng/ml	441.0±35.5	448.9±29.8	450.6±28.4	
MMP-3, ng/ml	24.3±2.2	22.2±2.1	17.1±1.5	1-3
MMP-9, ng/ml	470.2±24.0	401.4±22.9	316.0±22.5	3-1,2

plaques slightly stenosing the arterial lumen are more numerous than plaques heavily stenosing the arteries; second, the myocardium around plaques heavily stenosing the artery is enriched with collateral vessels, which protects it from clinical manifestations of acute occlusion; third, the cap of the plaques slightly stenosing the arterial lumen can be more vulnerable for cracking/tearing/rupture due to more extensive area of its contact with active blood flow factors.

The dependency of changes in blood levels of biomarkers of atherosclerotic plaque instability on the degree of stenosis, dissemination and predominant localization of atherosclerotic foci in coronary arteries reviewed by us in between-group comparisons were confirmed by detected correlations between the levels of CRP, MMP-3, and MMP-9 coronary angiographic characteristics of atherosclerotic lesion of coronary arteries (primarily significant Spearmen *r* and Kendall *r* coefficients of non-parametric distribution; $p<0.01$).

Thus, we revealed some relationships between the levels of inflammatory-destructive biomarkers of atherosclerotic plaque instability and coronary atherosclerosis severity and distribution in male patients with coronary atherosclerosis without ACS. The highest levels of C-reactive protein and matrix metalloproteinase 3 were found in men with atherosclerotic involvement of all three main coronary arteries, primarily their middle and distal segments, and in men with predominance of low-grade stenoses (<50%) of coronary arteries at the sites of atherosclerotic plaques.

The study was supported by the Program of the President of the Russian Federation (grant No. MD-539.2007.7) and Novosibirsk City Council (grant No. 22-08).

REFERENCES

1. Yu. I. Ragino, A. M. Cherjyavski, Ya. V. Polonskaja, *et al., Byull. Eksp. Biol. Med*., **144**, No. 11, 500-503 (2007).
2. Yu. I. Ragino, A. M. Chernjavski, Ya. V. Polonskaja, *et al., Kardiologiya*, No. 9, 62-67 (2007).
3. E. J. Armstrong, D. A. Morrow, and M. S. Sabatine, *Circulation*, **113**, No. 6, e72-75 (2006).
4. E. J. Armstrong, D. A. Morrow, and M. S. Sabatine, *Ibid.*, **113**, No. 7, e152-155 (2006).
5. E. J. Armstrong, D. A. Morrow, and M. S. Sabatine, *Ibid.*, **113**, No. 9, e382-385 (2006).
6. M. Asakura, Y. Ueda, O. Yamaguchi, *et al., J. Am. Coll Cardiol*., **37**, No. 5, 1284-1288 (2001).
7. J. C. Fruchart, *Handbook of Dyslipidemia and Atherosclerosis.* France, University of Lille (2003), Pt. 1. pp. 1-65.
8. J. A. Goldstein, D. Demetriou, C. L. Grines, *et al., N. Engl. J. Med.*, **343**, No. 13, 915-922 (2000).
9. D. G. Katritsis, J. Pantos, and E. Efstathopoulos, *Coron. Artery Dis.,* **18**, No. 3, 229-237 (2007).
10. M. Naghavi, E. Falk, H. S. Hecht, *et al., Am. J. Cardiol*., **98**, No. 2A, 2H-15H (2006).
11. M. Naghavi, P. Libby, E. Falk, *et al*., *Circulation*, **108**, No. 14, 1664-1672 (2003).
12. P. J. Scanlon, D. P. Faxon, A. M. Audet, *et al., J. Am. Coll Cardiol*., **33**, No. 6, 1756-1824 (1999).
13. P. K. Shah, *High-Risk Atherosclerotic Plaques: Mechanisms, Imaging, Models, and Therapy*. Ed. L. M. Khachigian, New York (2005), pp. 1-19.
14. P. K. Shah and B. Sharifi, *Biochemistry of Atherosclerosis*, Ed. S. K. Cheema, New York (2006), pp. 455-563.
15. R. Waksman and P. W. Seruys, Handbook of the Vulnerable Plaque. London (2004), pp. 1-48.