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Plasma concentrations of plasmin-α_2-antiplasmin complexes (PAP) and D-dimers in patients with acute myocardial infarction

Stężenie osoczowe kompleksów plazmina-α_2-antyplazmina (PAP) i D-dimerów u chorych z ostrym zawałem serca

Władysław Sinkiewicz[1,2], Robert Bujak[2], Jan Błażejewski[2], Ewa Żekanowska[3], Jacek Budzyński[4], Joanna Dudziak[2]

[1]Department of the Clinical Bases of Physiotherapy, Collegium Medicum, UMC Toruń, Poland
[2]Department of Cardiology, Regional Hospital, Bydgoszcz, Poland
[3]Department of Pathophysiology, Collegium Medicum, UMC Toruń, Poland
[4]Clinic of Gastroenterology, Angiology and Internal Diseases, Collegium Medicum, UMC Toruń, Poland

Abstract

Background: *Acute myocardial infarction (AMI) is, in most cases, caused by a sudden closure of the coronary artery by a clot. Elevated levels of plasmin-α_2-antiplasmin (PAP) complexes and D-dimers indicate active coagulation and fibrinolysis, which is secondary to coagulation. The purpose of the study was to evaluate the processes of coagulation and plasma fibrinolysis by measuring the concentrations of PAP complexes and D-dimers in AMI patients before starting fibrinolytic and anticoagulant treatment.*

Material and methods: *70 patients with ST-elevation AMI were enrolled in the study. The mean age of the patients was 54.2 ± 7.7 years. The control group consisted of 25 healthy subjects selected according to age and sex.*

Results: *In the AMI group the concentrations of both PAP complexes and D-dimers were significantly higher ($p < 0.0001$) than in the control group. A highly significant positive correlation ($r = 0.52$; $p < 0.0001$) was found between concentrations of PAP complexes and D-dimers. Positive correlations were established between concentrations of D-dimers and fibrinogen ($r = 0.29$; $p < 0.02$) and between concentrations of D-dimers and patient age ($r = 0.27$; $p < 0.05$). A negative correlation was identified between the concentration of PAP complexes and body mass index ($r = –0.24$; $p < 0.05$).*

Conclusions: *The significantly higher concentration of PAP complexes and its close correlation with D-dimers indicate a considerable activation of plasminogenesis in the AMI patient*

Address for correspondence: Assoc. Prof. Władysław Sinkiewicz
Oddział Kardiologii z Zakładem Diagnostyki Kardiologicznej
ul. Ujejskiego 75, 85–168 Bydgoszcz, Poland
tel./faks (+48 52) 371 92 48, e mail: bizkard@by.onet.pl
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group and support the theory of fibrinolysis secondary to coagulation. The data constitute further evidence in support of the fundamental role of both systems in the pathogenesis of myocardial infarction. (Folia Cardiol. 2005; 12: 370–376)

PAP complexes, D-dimers, myocardial infarction

Introduction

Acute myocardial infarction (AMI) is, in nearly all cases, caused by a sudden closure of the coronary artery by a clot formed on the atheromatous plaque, destabilised because of ulceration, dissection or rupture [1]. The ruptured plaque comes into contact with blood flow and triggers the clot formation on its surface, which can completely close the artery lumen. Many authors have regarded disorders in this system as an important risk factor in the development of ischaemic heart disease and atherosclerosis, with its grave complications [2, 3]. On the basis of angiography, de Wood et al. [4] diagnosed total closure of the coronary vessel by the clot in 87% of AMI patients with Q-wave myocardial infarction (QMI) within 6 hours of the onset of symptoms. In non-Q-wave myocardial infarction total artery closure within 24 hours was observed in 26% of patients. Other authors [5], in autopsy examinations, found the clot in the coronary vessel in 74% of patients who died of myocardial infarction within 6 hours of the onset of symptoms.

The mechanism of vessel closure in acute coronary episodes, particularly unstable angina pectoris and myocardial infarction, is similar. This indicates the importance of haemostasis disorders not only in the pathogenesis of the onset and progression of atherosclerosis, but also in acute complications of this disease [6].

The plasmin generation may be investigated directly by examining the formation process or indirectly by assessing the results of its action on fibrinogen and fibrin. The amount of plasmin formed is assessed by means of the concentration of plasmin-α_2-antiplasmin complex (PAP). An elevation of PAP-complex concentrations is found in diseases such as ischaemic heart disease, diabetes mellitus, in patients after surgery and in neoplasmatic diseases [7–10]. Plasmin, acting on meshed fibrin, forms fragments of varying sizes, the smallest of which are D-dimers. An elevated concentration of D-dimers may reflect activation of both coagulation and fibrinolysis, which is secondary to coagulation.

Several authors have examined PAP complexes and D-dimers in hypercoagulable states [11–14], but only a few have determined the concentration of specific haemostatic markers before fibrinolytic or anticoagulant treatment in AMI [15, 16]. Tataru et al. [13] identified higher concentrations of D-dimers in patients with more advanced arteriosclerosis. This supported the hypothesis that the concentration of D-dimers is dependent on the amount of fibrin in clots present in atheromatous plaques. In contrast, Bayes-Genis et al. [12] analysed concentrations of PAP complexes and D-dimers in patients with unstable angina following myocardial infarction, *de novo* unstable angina pectoris and unstable angina with progressive symptoms. All the patients had increased concentrations of PAP complexes and D-dimers in comparison to the control group consisting of healthy subjects. The highest increase in PAP complex concentration was found in the group with post-infarction angina pectoris, the reason for which was claimed to be the type of clot found in angiography in acute myocardial infarction. The clot contained considerable fibrin, whereas clots in two other types of unstable angina pectoris consisted mainly of platelets. The results of this study strengthened the hypothesis that thrombus plays a crucial role in the development of acute coronary episodes.

The aim of our study was to evaluate the coagulation and plasma fibrinolysis processes by measuring both PAP-complex and D-dimer concentrations in AMI patients before essential treatment.

Material and methods

70 patients with ST elevation AMI, 56 men and 14 women, aged between 37 and 67 years (mean 54.2 ± 7.7 years) were enrolled in the study. The diagnosis of AMI was based on a typical history, changes in electrocardiograms and evidence of higher levels of troponin I and fraction CK-MB. The period between the onset of myocardial infarction symptoms and blood sampling was 6 hours on average. The following were excluded from the study: patients over 70 years of age, those admitted to hospital with cardiogenic shock or after resuscitation, patients diagnosed with diabetes mellitus, neoplastic disease or chronic diseases that could

affect the body's homeostasis, patients with hyperlipidaemia treated pharmacologically and patients taking oral anticoagulants. The control group consisted of 25 healthy subjects selected according to sex and age: women with a negative history of ischaemic heart disease and men with a negative history and negative results from a cardiological exercise test on a moving track according to Bruce's protocol.

Blood for examination was sampled from patients, their written consent having previously been obtained, immediately after admission to hospital and diagnosis of acute myocardial infarction and before essential treatment (fibrinolytic/anticoagulant or invasive), although an initial dose of oral aspirin (325 mg) had been administered as part of the immediate first aid. Some patients required administration of sublingual glyceryl trinitrate or intravenous morphine. The blood was sampled from the veins of the cubital area at minimal venostasis and placed in a plastic tube containing 3.2% sodium citrate with a blood:citrate ratio of 9:1. The blood sample was placed in a centrifuge and rotated at 4°C for 20 minutes at 3,000 rotations per minute. The low platelet plasma obtained was portioned and frozen at –70°C until examination. Blood sampling in the control group was conducted in the morning (between 7 and 9 a.m.), after 30 minutes' rest.

The concentration of plasmin-α_2-antiplasmin complexes was measured with the Enzygnost PAP test produced by Behring Marburg (normal range: 120–700 ng/ml). D-dimer concentration was measured with BioMerieux VIDA apparatus and the immunoenzymatic method with a fluorescent reading (normal range: 70–500 ng/ml). PAP and D-dimers were measured using the immunoenzymatic method ELISA (Enzyme Linked Immunosorbent Assay). Fibrynogen concentration was measured with the Hemolab Fibrinomat test produced by BioMerieux (normal range 2.0–4.0 g/l). The body mass index (BMI) was calculated for each patient, defined as the ratio of body mass in kg to height in square meters: BMI = b.m./height^2.

Statistical analysis

In the statistical analysis for the various tests, the values of basic statistics were determined, such as mean value, standard deviation, parametric index of changeability, minimal value in test and maximum value in test. Because of a significant asymmetry in some tests, median, low and high quartiles were additionally calculated. Verification of the compatibility hypothesis was performed using the Shapiro-Wilks and classic χ^2-Pearson tests. For the parameters not derived from a population with a normal distribution, the Mann-Whitney U test was carried out to compare two tests. When parametric tests were used, the classic Student's T test was applied to compare tests. When the variance equality hypothesis was rejected, the parametric test was used to compare two means, according to Welch's version. The correlation index of Spearman's range was determined for the parameters studied. The results were drawn up using Microsoft® Access 2.0 for Windows and StatSoft® Statistica 5.0 for Windows. The informed consent of each patient was obtained. The Regional Ethics Committee of Medical University, Bydgoszcz gave its assent to the study.

Results

A comparison of the examined parameters of the fibrinolysis system between the AMI patient and control groups is shown in Table 1.

In AMI patients a highly significant positive correlation ($r = 0.52$; $p < 0.0001$) was found between D-dimer concentrations and PAP complexes (Fig. 1).

Positive correlations were observed between the concentration of D-dimer and fibrinogen ($r = 0.29$; $p < 0.02$) and between the concentration of D-dimer and patient age ($r = 0.27$; $p < 0.05$) (Fig. 2, 3).

Table 1. The comparison of concentrations of PAP complexes and D-dimers between patients with acute myocardial infarction and the control group

Tabela 1. Porównanie stężenia kompleksów PAP i D-dimerów u chorych z zawałem serca i pacjentami z grupy kontrolnej

Parameter [unit]	Myocardial infarction				Control group				p
	x ± SD	Median	Low quartile	High quartile	x ± SD	Median	Low quartile	High quartile	
PAP [ng/ml]	871 ± 788	705	388	1007	259 ± 131	218	189	285	0,0001
D-dimers [ng/ml]	594 ± 983	354	254	513	223 ± 109	210	136	303	0,0001

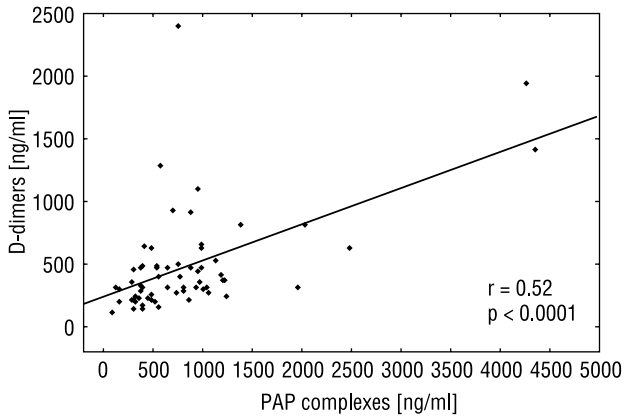

Figure 1. Correlation between D-dimer concentration and PAP complexes in patients with acute myocardial infarction

Rycina 1. Korelacja stężenia D-dimerów i kompleksów PAP u chorych z zawałem serca

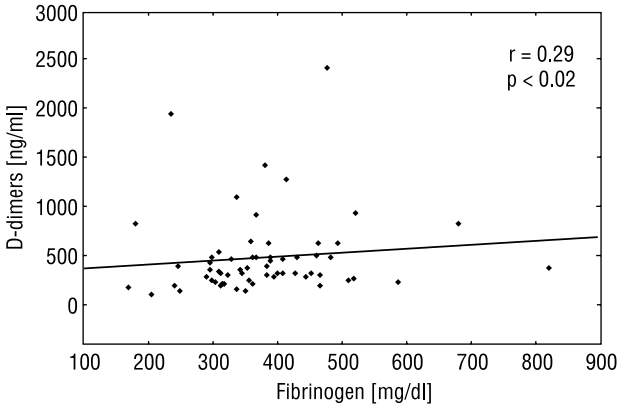

Figure 2. Correlation between D-dimer concentration and fibrinogen in patients with acute myocardial infarction

Rycina 2. Korelacja stężenia D-dimerów i fibrynogenu u chorych z zawałem serca

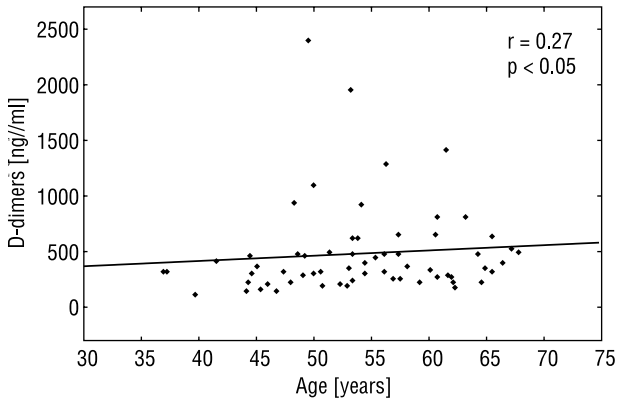

Figure 3. Correlation between D-dimer concentration and the age of patients with acute myocardial infarction

Rycina 3. Korelacja stężenia D-dimerów i wieku u chorych z zawałem serca

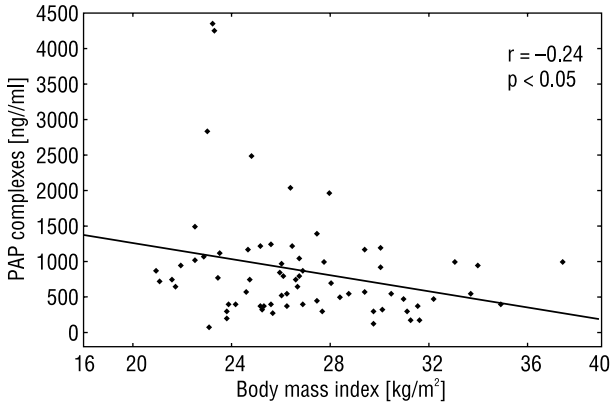

Figure 4. Correlation between concentration of PAP complexes and body mass index in patients with acute myocardial infarction

Rycina 4. Korelacja stężenia kompleksów PAP i wskaźnika masy ciała u chorych z zawałem serca

A significant negative correlation was found between the concentration of PAP complexes and BMI ($r = -0.24$; $p < 0.05$) (Fig. 4).

Discussion

Rigorous selection was enforced in our studies of coagulation and plasma fibrinolysis systems. This enabled us to qualify to the study patients diagnosed with ischaemic heart disease without any of the disorders that could influence the fibrinolysis system parameters. The evaluation of concentrations of PAP complexes and D-dimers during the early hours of myocardial infarction was not dependent on the influence of fibrinolytic drugs and anticoagulants on haemostasis, thus enabling us to present its state at the moment of the onset of acute myocardial infarction. The reliability of the measurements of the plasma fibrinolysis system parameters was increased by the relatively large number of groups of patients enrolled in the study.

The results of our study showed considerably elevated concentrations of PAP complexes and D-dimers in patients with acute myocardial infarction. This indicates the intensified process of plasma fibrinolysis in an early phase of myocardial infarction as a defence reaction of the organism to coagulation activation and clot formation in the coronary arteries. These data are consistent with the results obtained by other authors [15, 16]. Similar results were reported by Bayes-Genis et al. [12] in patients with unstable angina.

The concentrations of these parameters did not influence the clinical treatment of myocardial infarc-

tion in our patients. Nevertheless, as other authors also report, the usefulness of measuring PAP-complex and D-dimer concentrations would appear to have important implications for follow-up studies on ischemic heart disease in this group of patients [17, 18]. According to Cushman et al. [19] and Sakkinen et al. [20], elevated concentrations of PAP complexes and D-dimers may indicate subclinical atherosclerosis in patients over 65 years of age and make it possible to predict myocardial infarction in these patients in future.

In our studies, we found a highly positive correlation between concentrations of PAP complexes and D-dimers. The correlation confirms the relationship between these parameters, as published by Lopez et al. [11], Bayes-Genis et al. [12] and Sakkinen et al. [20], and shows the legitimacy of the choice of these parameters to evaluate the activity of both fibrinolysis and coagulation systems. D-dimer concentration was also positively correlated with the concentration of fibrinogen. This supports the hypothesis of other authors that D-dimer concentration depends on the amount of fibrin in clots in atherosclerotic lesions [13]. The positive correlation between D-dimers and patient age is consistent with data presented by other authors [11, 21]. D-dimer concentration increases with age and may be an indicator of more advanced atherosclerosis. This correlation was confirmed by the elevated level of D-dimers in people with spreading atherosclerotic lesions [19].

A negative correlation was established in our studies between PAP complexes and BMI. This suggests weaker plasminogenesis in patients with a higher BMI. A similar relationship was observed by Sakkinen et al. [20] in her studies. Moreover, she also showed negative correlations between PAP complexes and other components of the insulin-resistant syndrome, PAI-1 antigen and insulin.

On the basis of prospective studies, it is known that people with low basic fibrinolytic plasma activity (linked with a high concentration and low activity of t-PA antigen), are more often exposed to later myocardial infarction than patients with a low concentration of antigen and high t-PA activity [19, 22, 23]. If the endogenous method of fibrinolysis activation by t-PA in patients with myocardial infarction is malfunctioning, as other authors report [17, 24], the activation may be triggered by plasminogen activation with kallikrein and factor XII. Regardless of the means of plasma fibrinolysis activation, the lytic action of this system in patients with myocardial infarction is insufficient, though Verheught et al. [18] observed that the clot may in some cases undergo spontaneous lysis. The development of myocardial infarction indicates the advantage of the coagulation process; the clearly increased fibrinolytic plasma activity, the body's defence reaction, is not sufficient to dissolve the clot obstructing the lumen of the coronary artery.

Our studies, conducted with the use of modern laboratory methods of measuring concentrations of PAP complexes and D-dimers, confirm the fundamental role of systems, coagulation and fibrinolysis, in the pathogenesis of myocardial infarction.

Conclusions

1. High concentrations of PAP complexes indicate considerable plasminogenesis activation in an early phase of myocardial infarction.
2. A significantly increased D-dimer concentration and a high positive correlation between this and PAP complexes indicate activation of coagulation and plasma fibrinolysis.
3. Age and an increased BMI might be factors which influence the balance between the coagulation and plasma fibrinolysis systems in AMI patients.
4. Follow-up studies on the same group of patients is required for assessment of the prognostic value for the further course of ischaemic heart disease of measuring PAP-complex and D-dimer concentrations in AMI patients.

Streszczenie

Wstęp: *Ostry zawał serca jest spowodowany nagłym zamknięciem światła tętnicy wieńcowej przez skrzeplinę. Powstaniu skrzepliny może sprzyjać względna przewaga układu krzepnięcia nad układem fibrynolizy osoczowej. W momencie powstania skrzepliny dochodzi do wtórnej aktywacji procesu fibrynolizy osoczowej. Kompleksy PAP powstają w wyniku reakcji plazminy i* α_2*-antyplazminy i są czułym i powszechnie uznanym wskaźnikiem plazminogenezy* in vivo. *D-dimery są najmniejszymi fragmentami powstającymi w wyniku działania plazminy na usieciowaną fibrynę, a ich podwyższone stężenie świadczy zarówno o aktywacji krzepnięcia, jak i wtórnej do krzepnięcia aktywacji fibrynolizy. Celem pracy była ocena procesu krzepnięcia*

i fibrynolizy osoczowej poprzez oznaczenie stężenia kompleksów PAP i D-dimerów w surowicy pacjentów z ostrym zawałem serca z uniesieniem odcinka ST przed zastosowaniem leczenia zasadniczego.

Materiał i metody: *Badaniom poddano 70 chorych (56 mężczyzn, 14 kobiet) z ostrym zawałem serca. Średnia wieku badanych wynosiła 54,2 ± 7,7 roku. Grupę kontrolną stanowiło 25 osób zdrowych, dobranych pod względem płci i wieku.*

Wyniki: *W grupie chorych z ostrym zawałem serca stwierdzono znamiennie wyższe ($p < 0,0001$) stężenie kompleksów PAP, istotnie wyższe ($p < 0,0001$) stężenie D-dimerów oraz wysoką dodatnią korelację ($r = 0,52$; $p < 0,0001$) pomiędzy stężeniem kompleksów PAP i D-dimerów.*

Wnioski: *Znamiennie wyższe stężenie kompleksów PAP wskazuje na znaczną aktywację plazminogenezy w grupie chorych z ostrym zawałem serca, zaś istotnie wyższe stężenie D-dimerów i ich wysoka dodatnia korelacja z kompleksami PAP przemawia za wyraźną aktywacją krzepnięcia i wtórną do wykrzepiania aktywacją procesu fibrynolizy osoczowej. Dane te są kolejnym dowodem potwierdzającym zasadnicze znaczenie obydwu układów w patogenezie zawału serca.* (Folia Cardiol. 2005; 12: 370–376)

kompleksy PAP, D-dimery, zawał serca

References

1. Roberts R., Morris D., Pratt C.M., Alexander R. In: Schlant R.C., Alexander R.W. ed. Pathophysiology, recognition and treatment of acute myocardial infarction and its complications. The Heart, arteries and veins. McGraw-Hill. 1994; 1107–1184.
2. Ross R. The pathogenesis of atherosclerosis. In: Braunwald E. ed. Heart disease. A textbook of cardiovascular medicine. W.B. Saunders Company 1997; 1105–1125.
3. van der Bom J.G., Bots M.L., Haverkate F. et al. Activation products of the haemostatic system in coronary, cerebrovascular and peripheral arterial disease. Thromb Haemost. 2001; 85: 234–239.
4. De Wood M.A., Spores J., Notske R. et al. Prevalence of total coronary occlusion during the early hours of transmural myocardial infarction. N. Engl. J. Med. 1980; 303: 897–902.
5. Davies M.J., Thomas A. Thrombosis and acute coronary artery lesions in sudden cardiac ischemic death. N. Engl. J. Med. 1984; 310: 1137–1140.
6. Antman E.M., Braunwald E. Acute myocardial infarction. In: Braunwald E. ed. Heart disease. A text-book of cardiovascular medicine. W.B. Saunders Company 1997; 1184–1288.
7. Paramo J.A., Orbe J., Fernandez J. Fibrinolysis/proteolysis balance in stable angina pectoris in relation to angiographic findings. Thromb. Haemost. 2001; 86: 636–639.
8. Aso Y., Fujiwara Y., Tayama K., Takebayashi K., Inukai T., Takemura Y. Relationship between soluble thrombomodulin in plasma and coagulation or fibrinolysis in type 2 diabetes. Clin. Chim. Acta. 2000; 301: 135–145.
9. Do Q.B, Goyer C., Chavanon O., Couture P., Denault A., Cartier R. Hemodynamic changes during off-pump CABG surgery. Eur. J. Cardiothorac. Surg. 2002; 21: 385–390.
10. Chojnowski K., Wawrzyniak E., Trelinski J., Niewiarowska J., Cierniewski C. Assessment of coagulation disorders in patients with acute leukemia before and after cytostatic treatment. Leuk. Lymphoma 1999; 36: 77–84.
11. López Y., Paloma M.J., Rifon J., Cuesta B., Páramo J.A. Measurement of prethrombotic markers in the assessment of acquired hypercoagulable states. Thromb. Res. 1999; 93: 71–78.
12. Bayés-Genís A., Guindo J., Oliver A. et al. Elevated levels of plasmin-α_2-antiplasmin complexes in unstable angina. Thromb. Haemost. 1999; 81: 865–868.
13. Tataru M.C., Heinrich J., Junker R. et al. D-dimers in relation to the severity of arteriosclerosis in patients with stable angina pectoris after myocardial infarction. Eur. Heart. J. 1999; 20: 1493–1502.
14. Montes R., Páramo J.A., Anglés-Cano E., Rocha E. Development and clinical application of new ELISA assay to determine plasmin-α_2-antiplasmin complexes in plasma. Br. J. Haematol. 1996; 92: 979–985.
15. Brenner B., Francis B.W., Fitzpatrick P.G. et al. Relation of plasma D-dimer concentrations to the coronary artery reperfusion before and after thrombolytic

treatment in patients with acute myocardial infarction. Am. J. Cardiol. 1989; 63: 1179–1184.
16. Lawler C.M., Bovill E.G., Strump D.C., Colled D.J., Mann G., Tracy R.P. Fibrin fragment D-dimer and fibrinogen bb peptides in plasma as markers of clot lysis during thrombolytic therapy in acute myocardial infarction. Blood 1990; 76: 1341–1348.
17. Vaziri N.D., Kennedy S.C., Kennedy D., Gonzales E. Coagulation, fibrinolytic, and inhibitory proteins in acute myocardial infarction and angina pectoris. Am. J. Med. 1992; 93: 651–657.
18. Verheugt F.W.A., ten Cate J.W., Sturk A. Tissue plasminogen activator activity and inhibition in acute myocardial infarction and angiographically normal coronary arteries. Am. J. Cardiol. 1987; 59: 1075––1079.
19. Cushman M., Lemaitre L.N., Kuller H. et al. Fibrinolytic activation markers predict myocardial infarction in the elderly. The Cardiovascular Health Study. Arterioscler. Thromb. Vasc. Biol. 1999; 19: 493–498.
20. Sakkinen P.A., Cushman M., Psaty B.M. et al. Relationship of plasmin generation to cardiovascular disease risk factors in elderly men and women. Arterioscler. Thromb. Vasc. Biol. 1999; 19: 499–504.
21. Cushman M., Kuller L., Psaty B. et al. D-dimer level in prediction of incident cardiovascular disease events in the elderly. Circulation. 1996; 93 (Suppl. I): I–457.
22. Lowe G.DO., Yarnell J.W.G., Sweetnam P.M., Rumley A., Thomas H.F., Elwood P.C. Fibrin D-dimer, tissue plasminogen activator, plasminogen activator inhibitor and the risk of major ischaemic heart disease in the Caerphilly Study. Thromb. Haemost. 1998; 79: 129–133.
23. Cortellaro M., Confrancesco E., Boscheti C. et al. For the PLAT Group: Increased fibrin turnover and high PAI-1 activity as predictors of ischemics events in atherosclerotic patients - a case control study. Arterioscler. Thromb. 1993; 13: 1412–1417.
24. Ockelford P., Smith J., Johns A. Fibrinolytic capacity in ischaemic heart disease. Thromb. Haemost. 1989; 62: 398.