O PAPEL DOS MARCADORES INFLAMATÓRIOS NA AVALIAÇÃO DE ALTERAÇÕES DO MIOCÁRDIO, DIAGNÓSTICOS E PROGNÓSTICOS EM PACIENTES COM ENDOCARDITE INFECCIOSA

THE ROLE OF INFLAMMATORY MARKERS IN THE EVALUATION OF MYOCARDIUM ALTERATIONS, DIAGNOSTICS AND PROGNOSIS IN PATIENTS WITH INFECTIVE ENDOCARDITIS

РОЛЬ МАРКЕРОВ ВОСПАЛЕНИЯ В ОЦЕНКЕ ИЗМЕНЕНИЙ МИОКАРДА, ДИАГНОСТИКЕ И ПРОГНОЗЕ ПРИ ИНФЕКЦИОННОМ ЭНДОКАРДИТЕ

FEDOROVA T.A.^{1*}; TAZINA S.I.²; SEMENENKO N.A.³; MAMONOV A.V.⁴; SOTNIKOVA T.I.⁵

^{1,2,3,4,5} Department of Therapy at the I.M. Sechenov First Moscow State Medical University (Sechenov University)

* Correspondence author
e-mail: ta-fedorova@mail.ru

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RESUMO

Os resultados do exame de 121 pacientes com endocardite infecciosa (EI), que receberam tratamento hospitalar no Hospital Clínico Estadual Botkin de 2008 a 2016, foram apresentados no artigo. Os dados obtidos em EI primária e secundária, com desenvolvimento de doença complicada e não complicada, foram analisados. A significância diagnóstica dos ensaios dinâmicos da proteína C-reativa altamente sensível (PCR-as), do fator de necrose tumoral α (TNF α) e da troponina I altamente sensível (TnI-as) na avaliação da atividade do processo tóxico infeccioso, gravidade da doença e complicações identificadas. Os resultados obtidos indicam o alto valor informativo do ensaio dinâmico dos níveis de TNF α , hsCRP e TnI-as em pacientes com EI. Os autores estabeleceram a correlação entre os níveis dos marcadores, a expressão das manifestações da síndrome tóxica e infecciosa, a gravidade da doença e o desenvolvimento de complicações. Uma correlação direta foi estabelecida entre o aumento dos níveis de citocinas pró-inflamatórias e o nível de TnI-as. Foi demonstrado que o aumento na concentração de troponina correspondeu às alterações morfofuncionais expressas em pacientes com IC grave. O estudo morfológico da destruição de cardiomiócitos revelou a formação de distrofia focal, desenvolvimento de fibrose focal e difusa, indicada no dano miocárdico não coronarogênico em pacientes com EI. As alterações do miocárdio foram causadas por vários fatores (hipóxia, falha da microcirculação, distúrbios da permeabilidade, etc.), e o efeito citotóxico das citocinas pró-inflamatórias, em particular, o TNF α , desempenhou um papel essencial em seu desenvolvimento. Alterações miocárdicas, juntamente com a destruição valvar, tornam-se parte importante da patogênese dos distúrbios hemodinâmicos em pacientes com EI.

Palavras-chave: endocardite infecciosa, proteína C-reativa altamente sensível, fator de necrose tumoral α , troponina I altamente sensível, alterações morfológicas do miocárdio.

ABSTRACT

The results of the examination of 121 patients with infective endocarditis (IE), who received inpatient treatment at the Botkin State Clinical Hospital from 2008 to 2016, were presented in the article. The obtained data on primary and secondary IE with complicated and uncomplicated disease development was analyzed. Diagnostic significance of the dynamic assays of the highly sensitive C-reactive protein (hsCRP), tumor necrosis factor α (TNF α), and highly sensitive troponin I (hsTnI) in the evaluation of the infective toxic process activity, disease severity, and complications were identified. The obtained results indicate the high informative value of the dynamic assay of the levels of TNF α , hsCRP, and hsTnI in patients with IE. The authors established the correlation between the levels of the markers, and the expression of toxic and infectious syndrome manifestations, severity of the disease, and development of complications. A direct correlation was established between the increase in the pro-inflammatory cytokines levels and hsTnI level. It was shown that the increase in troponin concentration corresponded to the expressed morphofunctional alterations in patients with severe HF. Morphological study of the cardiomyocyte destruction revealed the formation of focal dystrophy, development of

focal and diffuse fibrosis, indicated on the non-coronarogenic myocardium damage in patients with IE. The myocardium alterations were caused by many factors (hypoxia, microcirculation failure, permeability disorders, etc.), and the cytotoxic effect of pro-inflammatory cytokines, in particular, TNF α , played an essential role in their development. Myocardium alterations, along with the valve destruction, become an important part of the pathogenesis of hemodynamic disorders in patients with IE.

Keywords: *infective endocarditis, highly sensitive C-reactive protein, tumor necrosis factor α , highly sensitive troponin I, morphological alterations of the myocardium.*

АННОТАЦИЯ

Приводятся результаты обследования 121 больного инфекционным эндокардитом (ИЭ), находившегося на лечении в ГКБ им. С.П. Боткина с 2008 по 2016 год. Полученные данные проанализированы у больных с первичным и вторичным ИЭ, с осложненным и неосложненным течением заболевания. Определена диагностическая значимость динамического исследования высокочувствительного С-реактивного белка (hsCRP), фактора некроза опухоли α (ФНО α), высокочувствительного тропонина I (hsTnI) в оценке активности инфекционно-токсического процесса, тяжести течения заболевания, выявлении осложнений. Обнаружена связь возрастания hsTnI с изменениями маркеров воспаления, морфо-функциональными показателями миокарда и выраженностью недостаточности кровообращения. При морфологическом исследовании выявлены воспалительные и дистрофические изменения сердечной мышцы, очаговый и диффузный кардиофиброз, свидетельствующие о развитии некоронарогенного повреждения миокарда, играющего существенную роль в развитии и прогрессировании сердечной недостаточности (СН) при ИЭ.

Keywords: *инфекционный эндокардит, сердечная недостаточность, маркеры воспаления, высокочувствительный тропонин I, морфологические изменения миокарда.*

1. INTRODUCTION

At present, infective endocarditis (IE) is one of the most complicated problems in clinical medicine. Its significance is defined by the increasing rate of morbidity, difficulty in early diagnostics, high rate of disability, and mortality.

According to the published data, in England from 2008 to 2013, the morbidity rate increased monthly by 0.11 cases and reached 35 cases per month per 10 million people (Dayer *et al.*, 2015), in the USA, the morbidity rate reached 10.000-15.000 cases per year (Slipczuk *et al.*, 2013). In Russia, annually, there are more than 10.000 cases are registered (Bakuleva, 2016).

The rate of primary diagnostics of IE during the surgery remains 21-30% (Fernández Guerrero *et al.*, 2012).

The lethality remains high depending on the etiology and reaches 20-40% (Slipczuk *et al.*, 2013; Bustamante *et al.*, 2014).

One of the causes of death in patients with IE is the progressing, therapy-resistant heart failure (HF) (Fedorova *et al.*, 2014). It is often diagnosed even in patients with minor valve defects and can be determined by myocardium alterations associated with the ongoing infectious, inflammatory process. However, the character of myocardium damage and the possibilities of its

diagnostics in patients with IE remain understudied.

One of the main elements of the IE pathogenesis is the systemic inflammatory response that is accompanied by the synthesis and expression of a broad spectrum of biologically active substances, i.e., endogenous mediators of the acute inflammation phase.

C-reactive protein (CRP) is one of the most studied markers of inflammation activity. It was first described by W. Tillet and T. Francis in 1930 as a protein that was capable of reacting with the pneumococcus C-polysaccharide resulting in the formation of precipitation. It consists of 5 similar subunits with the weight of 21-23 KDa each that are noncovalently interlinked. The molecular weight of the whole protein slightly exceeds 100 000 Da (Fomin and Kozlovskaya, 2003).

CRP is synthesized in the liver under the influence of pro-inflammatory cytokines (IL-6, IL-1, and TGF α) and is characterized as a protein of the acute inflammation phase. Its concentration increases 4 – 6 hours after tissue damage and reaches its maximum in 24 – 96 hours. It was found that the activation of its synthesis determined the CRP concentration increase during the inflammation, while its clearance failure was insignificant. The period of half-life is around 19 hours regardless of its concentration in

plasma (Pepys and Hirschfield, 2003).

CRP induces phagocytosis, activates complement, neutrophils, monocytes, and macrophages. It can modulate the effects of some cytokines: effectively inhibits the effect of IL-8 on human neutrophils, reduces the mitogenetic influence of the recombinant IL-2 on mononuclear cells in peripheral blood. It is considered that CRP inhibits the immune response in the bloodstream preventing the development of potential autoimmune reaction (Turchinovich and Nazzarov, 2002).

For a long time, the level of CRP higher than 5 mg/L was considered diagnostically significant. The lower level of CRP was considered as the lack of systemic inflammatory response. However, presently, there are highly sensitive methods of the markers assays (hsCRP) that allow for the identification of the basic concentration with the zero bottom border of the threshold. It was proved that even a slight increase in CRP concentration can indicate on subclinical process of inflammation in a vessel wall and can be a risk factor for the development of acute myocardial infarction, cerebral stroke, relapse of unstable angina, restenosis after coronary artery bypass grafting or angioplasty as well as a sudden death (Grigoryan *et al.*, 2012; Osipova, 2013).

Manifold increase in the marker's level is probably associated with the presence of acute or subacute infectious inflammation (Ebihara *et al.*, 2017; Ford *et al.*, 2012; Yu *et al.*, 2013).

Cytokines play an essential role in the development of inflammation during IE. One of such cytokines is TNF α . It is a pleiotropic pro-inflammatory cytokine with 17 kDa molecular weight that performs regulatory and effector functions in the immune response and inflammation. It plays a significant role in the coordination of inflammatory response and cytokine cascade (Ostanin *et al.*, 2013). Its level in serum of healthy donors is very low: from 0 to 6 pg/ml.

The increase in TNF α in blood serum is observed in acute viral, bacterial, and parasitic infections, injuries, oncological diseases, liver cirrhosis, and many other pathological processes. High concentrations of the cytokine are found in cerebrospinal fluid in patients with disseminated sclerosis and cerebrospinal meningitis, and in synovial fluid in patients with rheumatoid arthritis.

The main producers of TNF α are the cells of the innate immune response: monocytes,

macrophages, lymphocytes, and granulocytes in blood, as well as components of microorganism cell walls, IL-1, IL-2, alpha and beta interferons (Kuznik, 2012; Simbirtsev, 2002).

TNF α is a cytokine of the "first wave" that activates phagocytosis along with IL-1 and IL-6, enhances phagocytosis, proliferation of T and B-cells, and cytotoxic lymphocytes, stimulates mononuclear phagocytes and other types of cells to produce interleukins, chemoattractants, adhesive molecules (ICAM-1, VCAM-1, etc.) and acute-phase proteins, activates fibroblasts, collagen synthesis and coagulation (Demianov *et al.*, 2013; Chelvarajan *et al.*, 2012).

TNF α promotes quick cells size increase and initiation of apoptosis. Apart from the direct anti-inflammatory effect, it exerts a broad spectrum of immune regulatory effects and influences on the metabolism (Eliseev *et al.*, 2009; Hollegaard and Bidwell, 2006).

In a number of experimental models, the cytokine showed an anti-inflammatory effect (Liu *et al.*, 1998) that could be partially determined by the induction of neutrophil apoptosis. This type of cells death allows for the elimination of potentially dangerous neutrophils from the inflammation focus and contributes to its limitation (Savill and Fadok, 2000).

Excessive production of TNF α is observed in patients with septicemic conditions and is considered to be a direct cause of septic shock. Generalization of the damaging effects is also determined by a high number of receptors to this marker and the capability of other cytokines to perform its liberation (Bochorshvili and Bochorshvili, 1997; Oberhoffer *et al.*, 2000).

There is little data on the unfavorable prognostic significance of low concentrations of TNF α and IL-6 in patients with septicemic (Graham *et al.*, 2013).

One of the most important manifestations of IE is heart failure (HF). Its symptoms are identified already in the disease onset (7.5%), and in a month after the IE onset, it is identified in all the examined patients. In 46.6% of patients, HF III-IV FC by NYHA is observed (Shevchenko, 2014; Chu *et al.*, 2008).

Circulatory failure is an unfavorable prognostic feature of IE and is considered to be the leading cause of the patients' death (Mocchegiani and Nataloni, 2009; Ramírez-Duque *et al.*, 2011). Thus, successful treatment of IE is impossible without the knowledge of pathogenetic grounds of heart pathology.

However, the issue of the mechanisms of the development of HF in patients with IE still provokes discussions.

The significance of myocardium damage in the genesis of circulatory failure was discussed in the 1950s. H.L. Correl *et al.* (1951) believed that myocardium damage plays an essential role in the development of congestive heart failure (CHF) in patients with active IE. On the contrary, E.L. Perry *et al.* (1952) stated that decompensation of heart activity was rarely caused by the myocardium damage and that, primarily, it was the result of valve dysfunction.

Presently, there is still no clear explanation. T.A. Fedorova *et al.* (2016) highlight that the alterations in the myocardium are an essential factor in the development and enhancement of the clinical symptoms of HF and hemodynamic disorders. At the same time, E.M. Idov and I.I. Reznik (2009) believe that the primary cause of congestive circulatory failure is the quick development of the structural heart defect. The discussion of this issue is primarily explained by the remaining difficulty in the diagnostics of myocardium alterations: clinical manifestations of myocardium dysfunction are quite similar to the clinical manifestations of IE, and the available laboratory and instrumental diagnostic criteria are low specific.

Numerous studies of the mechanisms of skeletal muscles contraction, conducted at the end of the 1950s and at the beginning of the 1960s, led to the discovery of a protein that was similar to tropomyosin and regulated the sensitivity of the contractile apparatus to calcium. Based on the data, obtained in 1965 by S. Ebashi and A. Kodama (1965), troponin was described, and in 1973 M.L. Greaser and J. Gergely (1973) described molecular mechanisms that induced heart contraction. These studies gave grounds for the assay of cardiac troponin in clinical practice, which remains one of the most successful diagnostic tests.

During the past decades, the study of cardiac troponin level in dynamics is taken for the "golden standard" in the diagnostics of acute myocardial infarction (Thygesen *et al.*, 2012). The researchers also developed a scale of the increased concentrations of the marker that reflected, first, subclinical pathology of the myocardium associated with its structural (non-ischemic) damage, then, stable diseases of the coronary arteries, then, unstable angina and, finally, myocardial infarction. Moreover, it was discovered that there was a high number of

pathologies that were not associated with myocardial ischemia, but associated with the increased levels of highly sensitive troponin (hsTnI).

It was shown that high levels of hsTnI, remaining for 6 years, are the predictors of IHD and HF with normal or reduced ejection fraction (EF).

The marker levels were higher in patients with HF III-IV FC and unfavorable disease outcome (Januzzi *et al.*, 2012; Liguori *et al.*, 2014).

It is known that the increase in hsTnI is observed in 31-80% of patients with the systemic inflammatory response syndrome, sepsis and septic shock (Ammann *et al.*, 2001). It is considered that this is determined by the active metabolism of cardiomyocytes that require increased coronary blood flow. Anemia, tachycardia and microcirculation disorders, associated with the sepsis, contribute to the development of unbalance between the myocardium requirements in oxygen and its delivery (Kyung *et al.*, 2017; Levy *et al.*, 2005; Yucel *et al.*, 2008).

The aim of the study was to evaluate the significance of the markers of inflammation (hsCRP, TNF α) and myocardium damage (hsTnI) in the diagnostics, prognosis, and progression of the heart failure in patients with IE.

2. MATERIALS AND METHODS.

The material of the present study was the data obtained during the examination of 121 patients with IE that received inpatient treatment in Botkina State Clinical Hospital from 2008 to 2016. Clinical diagnosis IE was based on the modified criteria of DUKE. Patients with acute coronary pathology were excluded from the study.

The authors evaluated the age-related and social structure of the pathology, peculiarities of the clinical picture, the data obtained by laboratory and instrumental tests, and the disease outcome.

The special program included the identification of the concentration of highly sensitive C-reactive protein (hsCRP) in serum by the method of immunoturbidimetry «CRP hs FS» («Diasys Diagnostic Systems») using automatic biochemical analyzer KoneLab 20 (Finland),

tumor necrosis factor α (TNF α) by the method of enzyme-linked immunoassay using the test system alfa-TNF-IFA-Best (CJSC «Vector-Best», Russia), highly sensitive troponin I (hsTnI) by the method of chemiluminescent microparticle immunoassay (CMIA) using automatic analyzer ARCHITECT i2000SR (Abbott Laboratories, USA). These parameters were measured in dynamics: at admission, in 3 weeks and in 6 months.

All the patients had transthoracic echocardiography (TTE) and Doppler imaging performed on ultrasound apparatus Technos mpX (ESAOTE, Italy), transesophageal access was used when indicated. The authors measured in dynamics the size of heart chambers, left ventricular ejection fraction (LVEF), interventricular septum thickness, left ventricular posterior wall thickness, the degree of valve regurgitation, lung hypertension, size and localization of vegetations and the presence of endocardial complications.

The materials for the morphological study were right atrial appendage myocardium biotates that were taken at the beginning of the surgery. During the autopsy, ventricle myocardium was also examined. Biotates were treated in 10% neutral formalin and then fixed in paraffin. Paraffin blocks were made that were used for preparing paraffin sections mounted on glass slides. The histologic specimen of myocardium was stained with hematoxylin and eosin.

Depending on the variant of IE development, all the patients were divided into two groups: group I included 57 patients with primary IE and group II included 64 patients with secondary IE.

Statistical processing of the obtained data was performed by a statistical software package IBM SPSS Statistics 23.0.

The type of quantitative features distribution was identified visually (during histogramming) and/or by Kolmogorov-Smirnov test. At the normal distribution of the quantitative features, their description was performed by the arithmetical mean (M) $\pm$ SEM (m). At nonparametric distribution, the log of this concentration that was closer to normal was used instead of the concentration of the feature.

Qualitative parameters were presented in shares (percent), by the ordinal scale and/or the fact of the feature presentation. The hypothesis on the equality of two independent samples was

checked by two-sample Student's t-test that was corrected by statistically significant Levene test for dispersion homogeneity in case of normal distribution. At non parametric data distribution, the authors used the Mann-Whitney U test. To compare the mean values of the paired samples, the authors used the t-test for two paired samples. At abnormal distribution, the authors used Wilcoxon's test. The comparison of frequency features in independent samples was performed by Pearson's test χ^2 . The difference in paired frequency features (values before and after) was estimated by McNemar test. Correlation analysis of the obtained data was performed with the calculation of correlation coefficient (r). The critical level of statistically significant difference was taken as $p < 0.05$.

3. RESULTS.

The authors examined 121 patients with IE that received inpatient treatment in Botkin State Clinical Hospital from 2008 to 2016. There were 61.2% (CI: 52.7% - 69.1%) of males and 38.8% (CI: 30.9% - 47.3%) of females. The patients' age varied from 16 to 87 years old, one-third of the patients were elderly (28.9% CI: 21.8% - 37.0%), 4.1% of them (CI: 1.8% - 8.2%) were older than 80.

The identification of primary infectious focus was complicated by the late admission of patients to the hospital and was performed in 64.5% of cases (CI: 56.1% - 72.2%). The most popular pathway of bacterial infection was the injection of narcotic drugs (24.8% CI: 18.1% - 32.6%) and medical manipulations (19.1% CI: 15.2% - 21.7%). Foci of chronic infection and odontogenic interference were the causes of valve apparatus infection in an equal number of cases (7.4% CI: 4.0% - 12.6%).

In 57 patients (47.1% CI: 38.8% - 55.6%), primary infective endocarditis (PIE) was diagnosed, in 64 patients (52.9% CI: 44.4% - 61.2%) – secondary infectious endocarditis (SIE).

In the majority of patients, the development of SIE was associated with arterial sclerotic valve disease (17.4% CI: 11.8% - 24.4%). The share of rheumatic heart disease was 8.3% (CI: 4.7% - 13.8%), myxomatous degeneration of the cusps – 6.6% (CI: 3.5% - 11.6%), of the replaced valves – 5.8% (CI: 2.9% - 10.5%).

Bacteriologic blood assay was performed

in 91.9% of patients. In 94.3% of cases, it was performed along with antibacterial therapy. Among the infectious agents that caused IE, *Staphylococcus aureus* (22.3% CI: 16.0% - 29.9%) prevailed, enterococci were also identified (9.9% CI: 5.8% - 15.7%). Polymicrobial highly virulent associations that are primarily met in socially disabled patients were registered in 5.0% (CI: 2.4% - 9.4%). Streptococcal flora was identified only in 1.7% of cases (CI: 0.5% - 4.5%).

According to the data obtained by TTE, the prevailing pathology was mitral (33.1% CI: 25.5% - 41.3%) or tricuspid (23.1% CI: 16.7% - 30.8%) valve damage. The share of aortic valve disease decreased (21.5% CI: 15.2% - 29.0%). In 26 patients (21.5% CI: 15.2% - 29.0%), the infectious process involved two valves.

In 75.2% (CI: 67.4% - 81.9%) of cases, cardiac rhythm disorders were observed. Severe disorders of rhythm and conduction prevailed (38.2% CI: 32.4% - 45.3%), paroxysmal (12.4% CI: 7.8% - 18.7%) or permanent (10.4% CI: 8.0% - 14.6%) forms of atrial fibrillation were less common. In 19.0% of patients (CI: 13.1% - 26.2%), supraventricular extrasystole of high gradation was revealed.

Nearly all the patients expressed the symptoms of circulatory inefficiency (98.3% CI: 95.5% - 99.5%). HF II (37.2% CI: 29.3% - 45.6%) or III (44.6% CI: 36.4% - 53.1%) FC were registered more often. In patients with SIE, HF progression was more severe.

The choice of antibacterial drugs at the inpatient facility was based on the recommendations of the European Society of Cardiology on the prevention, diagnostics, and treatment of the IE (the newly revised version of 2015). All the patients had combined antibacterial therapy indicated. 77% (CI: 69.2% - 83.3%) received semisynthetic or inhibitor-protected penicillins in combination with gentamicin and/or fluoroquinolones. The therapy included cephalosporins of the III-Vth generation (48% CI: 39.6% - 56.4%), vancomycin (35% CI: 27.0% - 43.0%), rifampicin (24.8% CI: 18.1% - 32.6%) or carbapenems (16.5% CI: 11.1% - 23.4%). These drugs were also indicated in combination with aminoglycosides and/or fluoroquinolones. In 18 patients (14.9% CI: 9.7% - 21.5%), positive response was observed after daptomycin indication. During the treatment of IE, caused by fungi, fluconazole was indicated. The treatment of circulatory inefficiency was performed by conventional standards.

At the patients' admission to hospital, the

level of TNF α was elevated in 100.0% of patients (CI: 92.0% - 99.9%) with IE and, on average, was equal to 24.99 ± 0.77 pg/ml (norm – 0-6 pg/ml).

In patients with PIE, the concentration of the cytokine increased to 26.23 ± 1.20 pg/ml (Figure 1). The level of TNF α correlated with the activity of toxic and infectious manifestations: fever, leukocytosis, elevated ESR ($r=0.490 - 0.529$) ($p<0.05$). The highest TNF α level was registered in patients with IE, caused by *Staphylococcus* spp., the lowest – by *Enterococcus* spp. When immune complex and thromboembolic complications were observed, the concentration of the marker significantly exceeded the values that are registered in patients with uncomplicated disease progression (27.10 ± 1.16 pg/ml, 26.66 ± 0.97 pg/ml, 23.3 ± 1.14 pg/ml, respectively, $p<0.05$). Injection drug user had the level of the cytokine significantly higher (29.45 ± 2.05 pg/ml) than in other patients (23.30 ± 1.03 pg/ml) ($p=0.009$). In patients that abuse alcohol, the marker concentration was significantly lower (24.45 ± 2.97 pg/ml). In the most severely sick patients that required intensive care and that died within the first weeks of hospitalization, the concentration of TNF α was 5 times higher than normal values and was equal to 29.67 ± 4.33 pg/ml.

Figure 1. The dynamics of the levels of TNF α in patients with IE

In patients with SIE, the level of TNF α was also elevated in all the examined patients at the admission (100.0% CI: 92.0% - 99.9%). However, it increased slower than in the group I and was equal, on average, to 23.70 ± 0.96 pg/ml (Figure 1). The maximum concentration of the cytokine was observed in patients with IE, caused by *Staphylococcus* spp., minimum – by *Corinebacter*. In patients with immune complex and thromboembolic complications, the

concentration of the cytokine also exceeded the concentration of the cytokine observed in patients with uncomplicated disease development (26.56 ± 4.4 pg/ml, 24.49 ± 3.09 pg/ml and 22.43 ± 1.61 pg/ml, respectively), but the difference was statistically insignificant. In the majority of patients with rhythm disorders (74.1% CI: 57.7% - 86.2%), the concentration of the marker exceeded 21.0 pg/ml. The highest concentration of TNF α was registered at paroxysmal (26.73 ± 7.43 pg/ml) and permanent (25.21 ± 9.54 pg/ml) forms of atrial fibrillation, as well as at combined arrhythmias (27.42 ± 8.25 pg/ml). Similar to the group I, injection drug users had TNF α concentration higher (28.68 ± 5.39 pg/ml) than in other patients (23.47 ± 0.97 pg/ml). In patients that abuse and do not abuse alcohol, the levels of the cytokine were similar (23.62 ± 2.03 pg/ml and 23.72 ± 1.08 pg/ml, respectively). In patients, who died in late hospitalization period, the average concentration of the marker was equal to 25.18 ± 2.18 pg/ml, in patients that died within half a year after the discharge from the hospital, it was equal to 27.29 ± 2.18 pg/ml, which was 4 times higher than average values.

It should be noted that the concentration of TNF α lower than 19 pg/ml was associated with an unfavorable prognosis for the disease development in both groups. All these patients had severe disease development and high mortality rate.

In 3 weeks of the therapy, the decrease of TNF α was observed nearly in half of the survived patients (48.1% CI: 31.9% - 64.7%). However, all these patients (100.0% CI: 87.2% - 99.9%) had elevated concentration of the marker, which, on average, was equal to 24.86 ± 1.42 pg/ml in patients with PIE and 24.44 ± 1.10 pg/ml at SIE (Figure 1).

The lack of positive dynamics or a further increase in TNF α during the therapy was registered in both groups during the long-term (more than 2 weeks) fever, enhancing leukocytosis, lymphopenia, recurrent staphylococcus infection, abscesses, and unfavorable disease outcome.

In 6 months of the observation, half of the survived patients (57.1% CI: 29.0% - 81.6%) had the level of TNF α decreased. It was more expressed in patients with PIE (by 4.81 ± 2.88 pg/ml) than in patients with SIE (by 1.48 ± 1.01 pg/ml). However, the normalization of the marker's level was not observed in patients (Figure 1). In the majority of the patients, half of

them had surgeries, high levels of the cytokine corresponded to the laboratory remission and satisfactory condition.

At the patients' admission to the hospital, the level of hsCRP was elevated in 100.0% of patients with IE (CI: 97.0% - 100.0%) and was equal, on average, 72.93 ± 6.05 mg/L (norm 0-5 mg/L).

The marker's concentration identification in patients with PIE revealed its increase to 88.16 ± 9.50 mg/L (Figure 2). Higher protein values, exceeding the norm by 15-20 times, were registered in patients younger than 40 years old with pyretic fever (114.3 ± 22.4 mg/L), leukocytosis (100.1 ± 15.8 mg/L), thrombocytopenia (87.5 ± 17.6 mg/L), splenomegaly (96.3 ± 16 mg/L). Maximum values of hsCRP were observed in patients with PIE, caused by *S. aureus* and *Str. bovis* (107.4 ± 22.3 mg/L and 128.7 ± 20.2 mg/L, respectively).

Figure 2. Dynamics of the levels of hsCRP in patients with IE

In patients with thromboembolic and immune complex complications, intracardial abscesses, fistulas or septic shock, the concentration of the marker was significantly higher than in patients with uncomplicated disease development ($p < 0.05$).

The highest level of the protein was registered in 6 patients (27.3% CI: 13.9% - 45.4%) that died in the late hospitalization period and was equal to 120.73 ± 29.38 mg/L.

SIE, as well as PIE, was characterized by the increasing level of hsCRP in all the examined patients. However, average marker levels were significantly lower and were equal to 54.17 ± 6.19 mg/L ($p = 0.005$) (Figure 2).

Maximum levels were observed in

patients with hectic fever (45.4 ± 6.5 mg/L), leukocytosis - more $15 \times 10^9/L$ (49.9 ± 13.7 mg/L), elevated ESR (40.8 ± 7.2 mg/L). Significantly higher concentration of the peptide was registered in patients with IE of enterococcal etiology (75.4 ± 3.2 mg/L), thromboembolic and intracardial complications (45.8 ± 7.5 mg/L and 55.6 ± 12.5 mg/L, respectively).

At the admission to the hospital, injection drug users had hsCRP level significantly higher than in other patients: 117.62 ± 21.82 mg/L in patients with PIE and 55.89 ± 14.05 mg/L in patients with SIE. Alcohol abusers had lower levels of the protein (84.07 ± 15.86 mg/L and 20.06 ± 2.96 mg/L, respectively).

In 3 weeks of the therapy, the concentration of hsCRP was identified in 6.2% of patients with PIE and 61.4% of patients with SIE. PIE was characterized by more expressed positive dynamics of the marker ($p=0.004$) (Figure 2).

Remaining high and increasing levels of the protein during the treatment was observed in patients with the relapsing fever ($p=0.016$), leukocytosis ($p<0.001$), elevated ESR ($p<0.001$), recurrent *S. aureus* or *Enterococcus* spp. bacteriemia

In patients with PIE, there was correlation dependence between hsCRP levels and rhythm disorders ($r=0.203$) ($p=0.046$) and thromboembolic complications ($r=0.223$) ($p=0.042$). In patients with SIE, a similar tendency was observed. However, the correlation was insignificant.

In 6 months of the observation, the level of hsCRP decreased, on average, by 65.53 ± 12.03 mg/L in 94.1% of patients with PIE ($p<0.001$) and by 42.94 ± 9.38 mg/L in 95.0% of patients with SIE ($p<0.001$). Average marker levels did not differ significantly in the studied groups and were equal to 11.66 ± 3.47 mg/L and 11.62 ± 4.054 mg/L, respectively. High concentrations of the protein correlated with the activity of infectious and toxic manifestations.

At the admission to the hospital, the level of hsTnI was elevated in more than half of the patients (58.2% CI: 45.9% - 69.7%) with IE and varied from 3.6 to 219.60 pg/ml (norm - 12 pg/ml). Because of the significant positive asymmetry of the marker distribution, the authors analyzed the logarithm of its concentration. Natural logarithm (ln) of the hsTnI concentration was equal to 3.14 ± 0.21 (23.10 pg/ml).

PIE was characterized by the increase in

the protein level to 2.49 ± 0.25 (12.06 pg/ml) in 38.5% (CI: 23.4% - 55.7) of patients (Figure 3).

Figure 3. The dynamics of the levels of $\ln(\text{hsTnI})$ in patients with IE. Troponin means log with CI 95%.

Maximum levels of hsTnI were registered in patients with disorders of rhythm and conduction (21.33 pg/ml; $\ln=3.06 \pm 0.48$) ($p=0.047$), focal alterations on EKG (patients with IM were not included into the study) (30.57 pg/ml; $\ln=3.42 \pm 0.48$) ($p=0.002$), CH IV FC (31.19 pg/ml; $\ln=3.44 \pm 0.97$).

A statistically significant correlation between the level of hsTnI and the levels of leukocytes, lymphocytes, and ESR was not observed.

The highest level of the marker was registered in patients that died within the first 2 weeks of the hospitalization (44.70 pg/ml; $\ln=3.81 \pm 0.7$).

At the admission to the hospital, the level of hsTnI in patients with SIE was elevated in 75.9% (CI: 60.3% - 87.3%) of patients 2 times more often than in patients with PIE. The marker concentration exceeded the values in patients in group I and was equal to 41.68 pg/ml ($\ln=3.72 \pm 0.30$) ($p=0.030$) (Figure 3). High neutrophil count ($r=0.414$) ($p=0.026$) and lymphopenia ($r=-0.380$) ($p=0.042$) correlated with higher levels of the protein. In 94% (CI: 89.5% - 97.1%) of patients with the elevated marker level, dilation of the left heart chambers was observed; reduction of EF was registered 3 times more often; disorders of the rhythm and conduction were registered 5 times more often. Like in patients with PIE, higher levels of hsTnI were registered in patients with the depression of ST segment and negative T segment on ECG (47.46 pg/ml; $\ln=3.86 \pm 0.33$), CH IV FC (79.04 pg/ml;

$\ln=4.37\pm0.79$) and those that died (105.64 pg/ml ; $\ln=4.66\pm1.25$).

In 3 weeks of treatment, the level of hsTnI remained elevated in 67.9% of patients with SIE 2 times more often than in patients with PIE (36.0%) and was significantly higher than in the reference group (35.52 pg/ml and 8.85 pg/ml, respectively) ($p=0.001$). The lack of positive dynamics or increase in the protein level correlated with the fever relapse, increases in neutrophil count and ESR. In patients with rhythm and conduction disorders, the marker level was 2.7 times higher in patients with SIE (39.65 pg/ml) than in patients with PIE (14.58 pg/ml). The levels of hsTnI were elevated in 88.2% of patients with SIE with relapsing depression of ST segment and negative T segment on ECG (35.52 pg/ml) by 1.3 times more often and by 2 times higher than in the reference group (66.7%, 17.99 pg/ml). The progressing of circulatory insufficiency of I FC corresponded to the significant increase in the marker concentration by 1.99 pg/ml ($p=0.005$). Maximum levels of the marker (239.85 pg/ml) were observed in patients with SIE that died because of enhancing HF and active infectious process.

In 6 months of the observation, the level of the marker significantly decreased in patients in both groups ($p=0.046$) and ($p=0.003$), respectively. Normalization of the marker level was observed in all the survived patients with PIE and in half of the sick patients with SIE.

The authors compared the results of the study on the inflammation factors (TNF α , hsCRP) and hsTnI in patients at different stages of IE development. At the admission to the hospital, the patients showed significant correlations between the levels of CRP and TNF α ($r=0.265$) ($p=0.007$). In the most serious patients with high activity of an infectious process, the correlation was observed between hsTnI and hsCRP ($r=0.650$) ($p<0.001$), hsTnI and TNF α ($r=0.700$) ($p<0.001$).

In 3 weeks of the therapy, the activity of infectious and toxic syndrome reduced, especially in patients with PIE. The level of hsTnI decreased in patients with PIE and increased in patients with SIE (Figure 4 A, B).

In 6 months, a one-way tendency to the decrease of all the parameters was observed. It was less expressed for TNF α (Figure 4 A, B).

A)

B)

Figure 4. The dynamics of the levels of the inflammation factors and hsTnI in patients with PIE (A) and SIE (B)

Morphological examination of the myocardium biopsies revealed a significant amount of damaged cardiomyocytes with structural alterations, cells dissociations and wave-like deformation of the muscle fibers associated with the stroma edema. Pero iron hematoxylin staining of the sarcoplasm stained these structures black (Figure 5).

Figure 5. Contracture damage in cardiomyocytes. Rego staining, x400

Along with the contractive alterations, focal damages were observed: myocytolysis as areas of lysed sarcoplasm. Cross and longitudinal sections in cardiomyocytes cytoplasm also showed dystrophic alterations and nuclear pycnosis (Figure 6). As the process progressed, the nucleus degraded and hydropic or vacuole degeneration developed. These events had a diffuse character. Longitudinal sections of cardiomyocytes showed degeneration of different degree (Figure 7).

Figure 6. Myocytolysis and dystrophic alterations in nuclei. A. Sarcoplasm lysis. B. Nuclei pycnosis. Hematoxylin and eosin staining, x400.

Figure 7. Diffusion vacuole dystrophy in cardiomyocytes. Hematoxylin and eosin staining, x200.

The ongoing inflammatory process contributed to the development of diffuse infiltration stroma of the myocardium primarily by polymorphonuclear leukocytes, destruction of cardiomyocytes, and focal alterations of necrotic character (Figure 8).

Figure 8. Diffuse infiltration of myocardium stroma by the inflammation cells (A), destruction of cardiomyocytes (B), minor necrosis foci (C). Hematoxylin and eosin staining x200.

Inflammatory and dystrophic processes resulted in the development of focal or diffuse cardiosclerosis. In patients with diffuse cardiosclerosis, fibrous foci transformed into the areas that consisted of mature connective-tissue elements that substituted cardiomyocytes (Figure 9).

Figure 9. Diffuse cardiosclerosis (A). Hematoxylin and eosin staining x100.

4. DISCUSSION.

The aim of the present study was to investigate the dynamics of the inflammatory markers (hsCRP, $\text{TNF}\alpha$), myocardium damage (hsTnl) and to evaluate morphofunctional alterations in the myocardium in 121 patients with IE.

The analysis of $\text{TNF}\alpha$ concentration showed that it was elevated in all the patients with IE, correlated with the expression of toxic and infectious manifestations and increased in patients with complicated development of the disease. This data agrees with the results of other studies and data, obtained from the patients

with IE and sepsis (24, 25), and reflects the behavior of this marker as a pro-inflammatory cytokine.

At the same time, J.G. Graham *et al.* (2013) showed an unfavorable prognosis significance of low concentrations of TNF α and IL-6 in patients with septic processes. The present study showed that prognostically unfavorable were the initially low levels of TNF α , lower than 19 pg/ml. In all these patients, severe development of the disease and a high rate of lethality was observed. The obtained results can indicate on the disorders in cytokine cascade and, as a result, on insufficient inflammatory response with further unfavorable prognosis.

The majority of scientists believe that the infection causing agents of endocarditis promote the increase in the marker level. For example, J.S. Kim *et al.* (2012) studied the synthesis of TNF α and IL-1 β in mice infected with *Streptococcus mutans*. They showed that significant increase in cytokine synthesis was observed in 2-4 hours after the infection, and maximum concentration was achieved in a day due to the activity of the systems ERK/p38/JNK and NF- κ B that were activated via TLR2 and TLR4 receptors. A.M. Edwards *et al.* (2012) identified extracellular proteins (Eap) in the vessels endothelium that binded with *S.aureus* and activated TNF α synthesis. Further, the cytokine contributes to the interaction of the pathogen and Eap, which supports inflammatory reaction.

Similar data were presented by the scientists from Arkansas University that investigated the influence of *Coxiella burnetii* on cytokine status. The researchers came to the conclusion that low virulent types of pathogens promote the increase in TNF α and IL-6 levels. At the same time, highly virulent strains promote the decrease of cytokines levels (Graham *et al.*, 2013).

T. Tsaganos *et al.* (2013) reported the diverse effect of the pathogens on the concentration of TNF α (2013). The study was focused on the methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) influence on the production of cytokines by macrophages in patients with IE. It was established that MSSA stimulated the synthesis of TNF α more actively than MRSA due to different effect on the TLR4-receptors.

The results of the present study showed that the increase in the cytokine level was observed in patients with all the identified

pathogens, but there were some differences in the concentration depending on their type and variant of IE. Thus, the maximum concentration of TNF α was registered in patients with PIE and SIE caused by *Staphylococcus* spp, and the lowest concentration – by *Enterococcus* spp in group I and *Corinebacter* in group II.

The experiment showed that the association between long-term (more than 3 days) circulation of TNF α in high concentrations and structural damage of endothelium (Banda and Takahashi, 2014). Endothelium damage and progressing adhesion of platelets leads to the activation of clots formation in small diameter peripheral vessels, causing ischemia, and contribute to the development of microcirculatory disorders and organ dysfunctions (Kuznik, 2012).

TNF α actively takes part in the process of quick enlargement of cells and initiation of apoptosis. Apart from direct pro-inflammatory effect, it exerts a wide range of immune regulating effects and influences on the metabolism (Eliseev *et al.*, 2009; Hollegaard and Bidwell, 2006).

The present study results showed that the highest concentrations of the cytokine were observed in patients with rhythm disturbances, which could indicate on myocardium structural damage.

Evaluation of TNF α in dynamics showed a tendency towards the decrease of the marker concentration in 3 weeks in half of the patients with IE. However, the marker concentration remained high.

The lack of positive dynamics or a further increase in TNF α during the therapy was registered in both groups in patients with long-term (more than 2 weeks) fever, developing leukocytosis, lymphopenia, recurrent *Staphylococcal* infection, abscesses, and unfavorable disease outcome.

In 6 month of the observation, despite the observed decrease, the levels of the cytokine remained high in patients with the favorable clinical picture and lack of other elevated markers of inflammation. On the one hand, this dynamics can indicate on the persisting inflammatory process, which explains the higher risk of IE relapse in these patients. But on the other hand, this can indicate on the changed role of the mediator from the pro-inflammatory to endothelial and myocardial dysfunction, which corresponds to the prevalence of the heart failure symptoms, rhythm and conduction disorders in the clinical

picture in patients that do not have signs of inflammation.

CRP is one of the well-studied and widely used markers in clinical practice. In 2000, C.C. Lamas and S.J. Eykyn proposed to introduce the CRP level more than 100 mg/L in DUKE-diagnostics of IE (Lamas and Eykyn, 2000).

The studies showed that the level of the peptide was the highest in patients with IE caused by *Streptococcus pneumoniae* or *Staphylococcus aureus*, acute and complicated disease development, valve abscess and that require surgery (Heiro *et al.*, 2005; Tornos *et al.*, 2011; Verhagen *et al.*, 2008). Remaining high or increasing levels of CRP before a surgery indicates on the high risk of the development of post-operative complications and unfavorable prognosis (Okada *et al.*, 2014).

T.A. Fedorova *et al.* (2010) proved the significance of CRP level monitoring for the evaluation of the effectiveness of the ongoing anti-bacterial therapy for its timely correction and indication of surgery.

Multivariant analysis showed that a high level of the marker was an independent predictor of death in patients with IE (Tyurin, 2012; Elbey *et al.*, 2013).

It was shown that a high level of CRP in patients with HF III-IV FC was an unfavorable prognostic factor in patients with IE with a replaced valve (Elbey *et al.*, 2013).

These data do not agree with the results published by C.W. Yu *et al.* in 2013. The analysis of 6 major studied allowed the authors to conclude that CRP is a low informative marker in diagnostics of IE (Yu *et al.*, 2013).

M. A. Gurevich *et al.* (2001, 2006) noted that long-term treatment led to the adaptation to the systemic chronic inflammatory response. Thus, the diagnostic significance of the marker decreased. According to the researchers, the prevalence of pathological immune changes in the clinical picture can lead to a decrease of the marker concentration.

The present study showed that at the admission to the hospital, all the patients with IE had elevated levels of hsCRP. Significantly higher levels were observed in with PIE. Average protein levels were equal to 88.16 ± 9.50 mg/L in patients with PIE and 54.17 ± 6.19 mg/L in patients with SIE, which was lower than the diagnostically significant levels proposed by C.C. Lamas and S.J. Eykyn. The correlation was revealed between the marker and expression of toxic and

infectious manifestations and complications. In patients with complex immune complications, the levels of hsCRP were also high, unlike the results that were published by M.A. Gurevich *et al.* (2001, 2006).

It was noted that the pathogen that caused IE influenced on the concentration of the protein. Thus, the highest levels were registered in patients with PIE caused by *Staphylococcus aureus* or *Streptococcus bovis* and with SIE of enterococci etiology.

The dynamics of the changing level of hsCRP was more expressed than TNF α , which again indicates the possibility of a different role played by the cytokine, apart from the inflammation marker. The decrease of hsCRP in 3 weeks and 6 months was more expressed in the first group. The remaining high or increasing levels of the protein in 3 weeks during the anti-bacterial therapy corresponded to the relapsing fever, leukocytosis, increase in ESR, recurrent bacteremia with *Staphylococcus aureus* or *Enterococcus* spp. Thus, the remaining high levels of hsCRP, primarily, indicate on the activity of the inflammation process and IE.

hsTnI is a marker of myocardium damage. The majority of the studies were focused on acute myocardium damage and heart failure. There are few studies on the changes in hsTnI levels in patients with septic processes and IE.

P. Ammann *et al.* (2001) showed that the increase in hsTnI level was observed in 31-80% of patients with systemic inflammatory response syndrome, sepsis, and septic shock. It is explained by the active metabolism of cardiomyocytes that require enhanced coronary circulation. The associated anemia, tachycardia and microcirculation disorders promote the development of unbalance between the myocardium requirements in oxygen and its delivery (Kyung *et al.*, 2017; Levy *et al.*, 2005; Yucel *et al.*, 2008).

Some studies indicate the increase in hsTnI levels in patients with IE (Burton *et al.*, 2013; Tsenovoy *et al.*, 2009; Watkin, 2009).

According to A.B. Stancoven *et al.* (2011), the increase in the marker concentration was observed in 93% of patients with IE and correlated with unfavorable disease outcome.

The study of Z.A. Thoker *et al.* (2016) showed that the elevated marker levels were observed in 35% of acute patients, 77.8% of them died in the future.

The lethality rate was higher in patients

with increasing hsTnI and NT-pro-BNP (69%) markers than in patients with only one increased marker (29%) (Shiue *et al.*, 2010).

M.G. Snipsøyr *et al.* (2016) consider the markers hsTnI and NT-pro-BNP as the most promising markers for further investigation of IE.

The present study showed an increase in hsTnI levels in half of the patients with IE. Maximum levels of the marker were registered in patients with rhythm and conduction disorders, ischemic alterations on ECG (patients with AMI were not included in the study), HF IV FC in both groups. These results confirmed the structural damage of the myocardium in the majority of patients with IE. Maximum levels of hsTnI in patients that died within the first two weeks of the hospitalization indicate on the prognostic role of this marker.

The lack of statistically significant correlation between the level of hsTnI and the expression of the inflammation process (leukocyte and lymphocyte count, ESR) in patients with PIE can indicate on the development of the myocardium damage even at an unexpressed infectious process.

The prevalence of the elevated level and a higher concentration of markers in patients with SIE than with PIE can be explained by initial heart damage and development of HF in these patients with the mentioned disease.

In 3 weeks of the disease development, the levels of hsTnI remain elevated in the majority of patients with IE, also prevailing in patients with SIE, which indicates on the remaining process of the myocardium damage despite the reduction of toxic and inflammatory manifestations.

At the same time, it was noted that the lack of positive dynamics or increase in the protein level correlated with the relapse of fever, increase in neutrophil count, and ESR. Thus, remaining, increasing or chronic inflammatory and septic process lead to the increase in hsTnI, which indicates on the ongoing active myocardium damage. These patients have FC HF increasing and unfavorable prognosis.

It was revealed that progressing of circulatory insufficiency, I FC corresponded to the significant increase in the marker level by 1.99 pg/ml.

These results indicate on the prognostic role of the dynamic changes of hsTnI levels.

In 6 months, the marker level significantly decreased in both groups, but its normalization

was observed in all the patients with PIE, in half of the patients with SIE, it can indicate on the higher FC HF and more expressed myocardium damage in group II.

Interesting results were obtained when the levels of the inflammation factors were compared (TNF α , hsCRP) and hsTnI. Significant correlations between the levels of TNF α and hsCRP, registered at the patients' admission to the hospital, were consistent and interconnected with the expression of toxic and infectious manifestations. The authors also identified correlations between the inflammation factors (TNF α , hsCRP) and hsTnI, which indicated on the myocardium damage depending on the expression of the inflammatory process.

The study of the morphological picture of the myocardium biotates confirmed the theory on the damage of not only endocardium but also myocardium. A significant amount of the damaged cardiomyocytes with contractive alterations and the stroma edema with the diffuse deformation of muscle fibers, as well as focal alterations (myocytolysis), were revealed. The disease outcome results in the formation of focal or diffuse cardiosclerosis. This morphological data confirm the obtained results on the dynamic alteration of hsTnI levels.

5. CONCLUSION.

The obtained results indicate on high informative value of the dynamic assay of the levels of TNF α , hsCRP, and hsTnI in patients with IE. The authors established the correlation between the levels of the markers, and the expression of toxic and infectious syndrome manifestations, severity of the disease, and development of complications. A direct correlation was established between the increase in the pro-inflammatory cytokines levels and hsTnI level. It was shown that the increase in troponin concentration corresponded to the expressed morphofunctional alterations in patients with severe HF.

Morphological study of the cardiomyocyte destruction revealed the formation of focal dystrophy, development of focal and diffuse fibrosis, indicated on the non-coronarogenic myocardium damage in patients with IE. The myocardium alterations were caused by many factors (hypoxia, microcirculation failure, permeability disorders, etc.), and the cytotoxic effect of pro-inflammatory cytokines, in particular, TNF α , played an essential role in their

development. Myocardium alterations, along with the valve destruction, become an important part of the pathogenesis of hemodynamic disorders in patients with IE.

6. ACKNOWLEDGMENTS

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