ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH INFECTIVE ENDOCARDITIS AFTER HEART VALVE REPLACEMENT

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Abstract

The Systemic Inflammatory Response Syndrome (SIRS) after heart valve replacement in patients with infective endocarditis (IE) leads to the endothelial dysfunction. However, surprisingly little is known about the specific role of the endothelium and cell adhesion molecules in the pathogenesis of IE and SIRS after cardiac surgery. In this study we enrolled 110 consecutive Caucasian (Russian) individuals who underwent heart valve replacement surgery due to IE. Open heart surgery with cardiopulmonary bypass was performed in all cases. We measured plasma concentrations of soluble L, P, E-selectins, MMP-1, MMP-2, TIMP-1, TIMP-2 levels and von Willebrand factor activity at the different time points after cardiac surgery. Statistically significant differences between the concentrations of MMP-1 and MMP-2, TIMP-1 and TIMP-2, E-selectin and clinical groups of patients were identified.

Keywords: *endothelial dysfunction, infective endocarditis, SIRS, cardiac surgery*

1. INTRODUCTION

Infective endocarditis (IE) is caused by microbial infection of the endothelial lining of intracardiac structures. Usually it is caused by persistent bacteremia, affects heart valve leaflets, but may involve also mural endocardium, chordal structures, myocardium, and pericardium [11] A variety of organ systems may be adversely affected in patients with IE. Although advances have improved the diagnostic accuracy for IE, morbidity and mortality remain remarkably high [12]. Although the annual incidence of IE is relatively low (2-4 cases per 100,000 in the US and 15-60 cases per 1,000,000 in the world), the treatment of this pathology is currently a difficult task for clinicians. Despite a substantial progress in early detection and surgical techniques was achieved during the last decades, the prognosis of patients with IE remains poor [17]. IE is a difficult and complex disease. The variety of clinical manifestations and complications, as well as the serious prognosis, makes it mandatory that IE patients need to be treated in experienced hospitals with a collaborative approach between different specialists, involving cardiologists, infectious disease specialists, microbiologists, surgeons, and frequently others [14].Currently, case fatality rates range from 10% in cases of IE caused by *Streptococci* to 30-40% in cases of acute IE due to *S.aureus* [5, 21]. It has also been reported that this value may be up to 70% in certain high-risk populations [17]. Furthermore, IE is responsible for from 18% to 40% of all acquired heart disease cases in the Siberian populations [1]. Much of our knowledge regarding the pathogenesis of infective endocarditis is based on studies of the virulence of Staphylococcus aureus, which has become the most frequent cause of IE nowadays. However, independently of the underlying cause of endocarditis, the pathogenesis involves the damage and disturbance of endothelial function and the formation of associated “vegetation”. Surprisingly little is known about the specific role of the endothelial dysfunction in the pathogenesis of endocarditis [4]. Bacteria that have entered the blood stream must be able to adhere to the damaged endothelium, exposed extracellular matrix or areas of fibrin deposition. Early diagnosis and aggressive treatment are the critical components of a successful management strategy [18]. Systemic inflammatory response syndrome (SIRS) may lead to the endothelial dysfunction of small vessels. For some reason, when SIRS does not resolve quickly, patients can develop widespread organ dysfunction; including lung, liver, and kidney injure [7]. Multiple organ dysfunction syndrome (MODS) increases the level of mortality of patients up to 80% [2]. Cardiopulmonary bypass (CPB) in patients who underwent open heart surgery has been associated with activation and injury of endothelial cells, probably responsible for the systemic inflammatory response syndrome [16].

However, surprisingly little is known about the specific role of the endothelium in the pathogenesis of endocarditis and systemic inflammatory response syndrome after cardiac surgery today.

2. MATERIALS AND METHODS

In the present study we enrolled 110 consecutive Caucasian (Russian) individuals who underwent heart valve replacement surgery due to IE between 2009 and 2013 at the Kemerovo Cardiology Centre (Russian Federation). All the IE cases were verified histologically and according to the modified Duke criteria (at least 1 major and 1 minor criteria or 3 minor criteria were fulfilled) [9], and all patients with IE underwent antibiotic therapy in the acute phase during the first admission to their district hospital. Antibiotic therapy and treatment of all concomitant diseases were also performed during the preoperative period at the Kemerovo Cardiology Centre. More than 90% of all the individuals were residents of Kemerovo and neighboring settlements, and the alien and aboriginal populations were excluded from the study. The study was approved by the local ethical committee and written informed consent was provided by all the participants after a full explanation of the study was given to them.

One hundred and ten patients, including 71 (65.00%) male and 39 (35.00%) female were enrolled in the study. The mean age of the study population was 48.5 (45.81-51.18) years.

Native valve IE was diagnosed in 72.3% (n=80) of subjects, prosthetic valve IE was found in the rest 27.7% (n=30) of individuals. No cases of device IE were identified in our investigation. Of note, 12.7% (n=14) of patients had native valve IE and heart valve prostheses simultaneously. In our patients, IE most frequently affected the mitral valve (39.10%, n=43), aortic valve (19.10%, n=21), or both (18.20%, n=20). Tricuspid valve was affected in 17.30% (n=19) of cases, whereas both mitral and tricuspid valves were infected only in 4.50% (n=5). So, IE was left-sided in 76.40% (n=84) and right-sided in 17.30% (n=19) of patients whilst in the rest 4.50% (n=5) both left and right sides were affected. In addition, drug addiction was revealed in 17.30% (n=19) of cases. Our patients also had certain risk factors and predisposing diseases such as hepatitis C (7.20%, n=8), hepatitis B (1.80%, n=2), chronic renal failure (13.50%, n=15), chronic obstructive pulmonary disease (2.70%, n=3), pneumonia (8.10%, n=9), pyelonephritis (11.70%, n=13), coronary heart disease (9.00%, n=10), and pulmonary hypertension (24.30%, n=27). Case fatality rate was 3.60% (n = 4).

A total of 110 adults with IE who underwent heart valve replacement surgery was divided into a group without severe SIRS (n = 36), a group with severe SIRS (n = 49) and a group with severe SIRS and MOD (n = 25).

For this classification, we used the following scales: Sepsis-Related Organ Failure Assessment (SOFA), European System for Cardiac Operative Risk Evaluation (EuroSCORE), Acute Physiology and Chronic Health Evaluation II (APACHE II), criteria for SIRS (Bone R. C., 1992) (Table 1).

Table 1. Criteria of the study population classification

Criteria	Group of patients		
	Without severe SIRS	With SIRS	With severe SIRS and MOD
SOFA, scores	0	1 – 3	> 4
APACHE II, scores	0 – 5	6 – 11	>12
Criteria for SIRS (Bone R. C.), scores	0 – 2	2	2 – 4
Fluid balance	positive	negative	very negative
The need for norepinephrine	non	single injection	prolonged

SvO^2, %	70 (68; 79)	65 (60; 67)	60 (58; 67)
EuroSCORE, scores	0 - 2	3 - 5	> 6

We measured plasma concentrations of soluble L, P, E-selectins, matrix metalloproteinase 1 (MMP1), MMP2, tissue inhibitor of matrix metalloproteinase 1 (TIMP1), TIMP2 levels and von Willebrand factor (fvWillebrand) activity. Concentrations of all these molecules were measured by enzyme-linked immunosorbent assay (ELISA) (Bender MedSystems, Austria; American Diagnostica, USA).

Blood was collected at the three time points: on the day before the surgery, and on the first and seventh days after the surgery.

All results were analyzed using Statistica software, version 6.0 (StatSoft Inc., USA). Statistical analysis of clinical data was performed using Mann-Whitney U-test and Wilcoxon test. We expressed central tendency as medians (ME) and quartile deviation (25Q and 75Q). The qualitative data were examined by the χ^2 test. A level of $P<0.05$ was considered to indicate statistical significance.

3. RESULTS

The levels of selectins, MMPs, TIMPs and fvWillebrand activity in the serum are listed in Tables 2, 3, 4 and 5, respectively.

The levels of MMP-1 and MMP-2 at all the time points of analysis were significantly higher in group without severe SIRS (Tables 2) than in two groups with SIRS, and there were significant differences between them ($P<0.05$)

Table 2. Results of the analysis of MMP-1 and MMP-2 in groups of the study population, ng/ml

Variable	Day	Group of patients		
		Without severe SIRS	With SIRS	With severe SIRS and MOD
MMP-1	Before operative	6.15 (2.26; 10.65)	3.86 (2.48; 8.71)	2.89 (0.82; 4.65)
	1-st postoperative	5.95 (2.14; 9.10)	2.50 (1.54; 3.98)	3.73 (1.92; 6.00)
	7-th postoperative	9.38 (5.40; 11.29)	5.23 (2.22; 10.58)	5.15 (1.92; 5.66)
MMP- 2	Before operative	11.90 (7.11; 17.00)	8.88 (4.87; 33.13)	5.94 (3.58; 30.74)
	1-st postoperative	13.39 (8.60; 26.10)	6.06 (3.02; 28.74)	6.69 (5.54; 9.04)
	7-th postoperative	33.22 (28.50; 41.63)	26.90 (8.74; 52.00)	11.60 (9.10; 26.49)

Levels of MMP-1 and MMP-2 in two groups with SIRS did not differed at all time points. Nevertheless, concentration of MMP-2 in patients two first group was higher ($P<0.05$) in comparison with the patients with SIRS and MOD (Table 2).

At the same time the levels of TIMP-1 and TIMP-2 in the in group without severe SIRS were significantly lower (Tables 3) at the patients groups with SIRS and SIRS and MOD ($P<0.05$).

Table 3. Results of the analysis of TIMP-1and TIMP-2 in groups of the study population, ng/ml

Variable	Day	Group of patients		
		Without severe SIRS	With SIRS	With severe SIRS and MOD
TIMP-1	Before operative	9.62 (8.52; 10.54)	12.36 (11.08; 15.17)	11.52 (9.41; 12.24)
	1-st postoperative	12.04 (10.91; 13.21)	13.49 (11.43; 15.65)	14.98 (14.60; 20.10)
	7-th postoperative	11.05 (8.69; 12.91)	13.65 (12.12; 15.19)	14.54 (12.33; 16.05)
TIMP-2	Before operative	1.13 (8.21; 1.24)	9.61 (7.91; 12.58)	13.39 (12.32; 15.72)
	1-st postoperative	0.99 (0.8; 1.11)	10.68 (8.15; 14.25)	12.68 (11.58; 21.34)
	7-th postoperative	1.01 (0.81; 1.29)	10.17 (6.66; 14.48)	10.51 (9.86; 18.92)

Among all patients with SIRS, TIMP-2 concentrations were higher on the day before the surgery in group with SIRS and MOD ($P<0.05$). Any other statistically significant differences between groups with SIRS were not revealed.

The levels of E-selectin in all the days of analysis were significantly higher in group with severe SIRS and MOD (Tables 4) and had statistically significant differences in group without severe SIRS ($P<0.05$). Moreover, significant differences were found between the two groups with SIRS regarding the concentrations of E- selectin at the all three time points ($P<0.05$), whereas no any significant differences were found between first two groups ($P>0.05$).

Table 4. Concentrations of endothelial activation and cell adhesion molecules biomarkers, pg/ml

Variable	Day	Group of patients		
		Without severe SIRS	With SIRS	With severe SIRS and MOD
E- selectin	Before operative	50.38 (34.42; 59.05)	55.55 (42.70; 77.75)	137.75 (62.00; 176.15)
	1-st postoperative	44.92 (32.32; 55.90)	53.24 (41.10; 86.42)	153.60 (89.86; 188.60)
	7-th postoperative	38.81(29.66; 56.15)	43.26 (7.34; 73.65)	87.67(45.10; 120.20)
P- selectin	Before operative	213.00 (88.23; 458.00)	356,15 (73.69; 414.00)	122.4 (46.26; 280.70)
	1-st postoperative	259.70 (66.35; 486.00)	366.75 (78.88; 453.40)	124.00 (65.67; 254.70)
	7-th postoperative	342.60 (70.34; 453.00)	266.10 (76.96; 420.90)	174.47 (59.47; 362.40)
L-selectin	Before operative	3.41 (2.81; 3.45)	3.50 (3.23; 3.84)	4.23 (3.65; 4.83)
	1-st postoperative	2.90 (2.67; 3.36)	3.37 (3.25; 3.57)	3.40 (3.17; 4.17)

	7-th postoperative	3.21 (2.84; 3.47)	3.50 (3.07; 4.10)	4,01 (3.21; 4.46)

Individuals with severe SIRS and MOD had significantly higher concentrations of L-selectin on the day before surgery and seven days after the surgery (Tables 4). We have revealed statistically significant differences between group with severe SIRS and MOD and the first two groups ($P<0.05$).

No significant differences in the levels of P- selectin between groups (all $P>0.05$) were observed throughout the observation (Tables 4)

There were no statistically significant differences concerning the activity of fvWillebrand (Tables 5) in patients during any of the time points.

Table 5. Activity of fvWillebrand in patients with IE, %

Day	Group of patients		
	Without severe SIRS	With SIRS	With severe SIRS and MOD
Before operative	35.75(17.33; 67.60)	55.61 (35.85; 102,60)	92.8 (79.69; 97.02)
1-st postoperative	118.40 (94.90; 157.20)	156.90 (116.75; 213,00)	147.80 (113.00; 206.90)
7-th postoperative	89.64 (69.52; 130.30)	166.15 (110.70; 201,30)	132.45 (88.88; 145.91)

4. DISCUSSION

The literature includes many references to inflammation and inflammatory response syndrome (SIRS) in open heart surgery. However, perioperative systemic SIRS remains a catastrophe in cardiac surgery and adequate patient screening is still lacking [13]. Infective endocarditis (IE) is an infection of a heart valve or other cardiac structure at a site of endothelial damage [12]. The activation of the acute phase reaction during CPB is an extremely complex process. It occurs at different times and has various triggers: the surgical trauma itself, blood contact with the non-physiological surfaces of the extracorporeal circuit, endotoxemia and ischemia [15]. The development of adhesion bonds, either among cells or among cells and components of the extracellular matrix, is a crucial process. These interactions are mediated by some molecules collectively known as adhesion molecules. Cell adhesion molecules are ubiquitously expressed proteins playing a central role in controlling cell migration, proliferation, survival, and apoptosis. Besides their key function in physiological maintenance of tissue integrity, these molecules play an eminent role in various pathological processes such as cardiovascular disorders and regulation of the inflammatory response. Selectins take part in interactions between leukocyte and vascular endothelium (leukocyte rolling, arrest, firm adhesion, migration). Experimental data and pathologic observations support the assumption that pathogenic microorganisms attach to vascular endothelial cells or sites of vascular injury initiating intravascular infections [8].Systemic inflammatory reactions is also associated with increased levels of soluble adhesion molecules. Higher levels of adhesion molecules are briefly expressed and return to normal within a few hours but they to be responsible for the dysfunction of multiple organ systems observed in the postoperative period [15]. A relationship between adhesion molecule expression and SIRS with MOD has also been we are shown: higher concentrations of L-selectin and E- selectin recorded in patients who had organ dysfunction after cardiac surgery. In our study associated organ dysfunction with increased levels adhesion molecules registered in day before surgery and seven days after the surgery that does not meet the published data on the course of inflammation in the postoperative period. However, in patients with IE this condition may be defined the progressions of the underlying disease and endothelial dysfunction as a result of systemic inflammation of infectious genesis.

Endothelial injury at the microcirculatory level after ischemia and reperfusion occurs. Matrix metalloproteinases (MMPs) play essential physiologic roles in numerous processes ranging from development to wound repair [20]. Unfortunately, given the broad substrate specificity of the MMP family as a whole, aberrant degradation of extracellular matrix proteins can result in destructive disease. More recent data has highlighted that MMPs play much more elaborate physiologic and pathophysiologic roles than simple matrix protein cleavage [14]. MMPs and their inhibitors (tissue inhibitor of metalloproteinases; TIMPs) are important enzymes in the control of extracellular matrix remodelling. Continued mechanical ventilation during CPB reduces serum levels of MMPs and their inhibitor TIMP-1. Some researchers suggest importance of levels MMPs in aggravation of lung injury after CPB [3]. Only one study described the mutual interaction between the dysfunctional endothelium and develops mitral valve endothelial to mesenchymal transition and myofibroblastic activation [19]. Therefore, our hypothesis about an important role of levels MMPs and TIMPs in the formation of endothelial dysfunction and the development of systemic inflammatory response confirmed. We have shown in our study, the relationship between low levels of MMP and high levels of TIMP are associated with the development of the SIRS with MOD. Nevertheless, these results should be used with caution in patients with MOD non-infective endocarditis.

Von Willebrand factor (fvWillebrand) is a large, multimeric glycoprotein mediated platelet adhesion at sites of endothelial damage. Earlier it was demonstrated that fvWillebrand binds to and promotes the surface adhesion of S. aureus, prompting this effort to identify the fvWillebrand adhesion and indicates its role in the pathogenesis IE [10]. The activity of Willebrand factor as biomarkers of the development of multiple organ dysfunction in infectious and noninfectious systemic inflammatory response syndrome [6]. Willebrand factor is an acute phase protein and its plasma level increases in systemic inflammation. When released from endothelial cells and platelets, the native multimeric glycoprotein is mostly present in the ultralarge form which may have a major clinical significance under proinflammatory conditions, who may activate endothelial cells and platelets simultaneously. In our study, the increase in the activity of the factor was first postoperative day in all patients. We are significant differences in the levels between the groups identified.

Therefore, endothelium-associated parameters showed excellent predictive ability for perioperative SIRS. The prediction of routine parameters could be further improved by inclusion endothelium-associated parameters.

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