Increased levels of soluble adhesion molecules, E-selectin and P-selectin, in patients with infective endocarditis and embolic events

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Aims Inflammation-induced procoagulant changes and endothelial cell activation appear to play an important role in thromboembolic complications of infective endocarditis. Hence, the aim of this study was to compare the plasma levels of soluble adhesion molecules E- and P-selectin in infective endocarditis patients with and without embolic events, and healthy subjects.

Methods and Results The study group consisted of 76 consecutive patients (mean age=26 years old, range from 8 to 64 years) with definite infective endocarditis according to the Duke criteria. Thirteen of the patients (17.1%) had embolic events. Transoesophageal echocardiographic examinations were performed on all patients within 3 days of initiation of antimicrobial therapy. Although there was a trend towards a higher rate of vegetations detected in those with embolic than in those without, this did not reach statistical significance (84.6% vs 80.9%, \( P > 0.05 \)). Significantly larger vegetations were observed in patients with embolic events compared to those without embolic events (1.4 cm vs 1.0 cm, \( P = 0.03 \)). The mean plasma concentrations of P-selectin were elevated in patients with embolic events as compared to both patients without embolic events and control subjects (58.69 ± 7.49 ng . ml\(^{-1}\) vs 29.65 ± 5.69 ng . ml\(^{-1}\), \( P < 0.001 \) and 58.69 ± 7.49 ng . ml\(^{-1}\) vs 25.82 ± 5.38 ng . ml\(^{-1}\), \( P < 0.001 \)). Similarly, the patients with embolic events had increased plasma levels of E-selectin compared to those without embolic events and the control group (73.15 ± 11.47 ng . ml\(^{-1}\) vs 42.84 ± 8.77 ng . ml\(^{-1}\), \( P < 0.001 \) and 73.15 ± 11.47 ng . ml\(^{-1}\) vs 34.23 ± 5.92 ng . ml\(^{-1}\), \( P < 0.001 \)).

Conclusion Determination of these membrane activation molecules may provide useful markers with which to identify patients at high thromboembolic risk from infective endocarditis.

Key Words: Infective endocarditis, embolic events, E-selectin, P-selectin.

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Introduction

Thromboembolic events represent a relatively common and serious complication in infective endocarditis, and which occur in 20% to 43% of cases, with the majority as cerebral emboli\(^1\)\(^-\)\(^3\). It is proposed that the emboli in infective endocarditis are caused by fragmentation of valvular vegetations as a result of turbulent blood flow within the cardiac chambers\(^4\). However, the results of several echocardiographic studies, which attempted to correlate the formation of an embolic event in infective endocarditis with the morphological characteristics of a valvular vegetation, have been controversial\(^2\)\(^-\)\(^5\)\(^,\)\(^6\).

Some recent studies have demonstrated that systemic bacterial infections, even in the absence of cardiac involvement, represent an independent risk factor for systemic thromboembolism\(^7\)\(^-\)\(^9\). Inflammation-induced procoagulant changes and endothelial cell activation appear to play a major role in this setting\(^10\). Recently, adhesion molecules such as E- and P-selectin were found to shed from cell surfaces into the circulation. These soluble forms can be detected by enzyme-linked immunoassays and may reflect their expression on activated or injured endothelial cells and platelets\(^11\),\(^12\). Hence, the aim of this study was to compare plasma levels of soluble adhesion molecules P- and E-selectin in infective endocarditis patients with and without embolic events, and in healthy subjects.
Methods

The study group consisted of 76 consecutive patients (female=55, male=21, mean age=26 years old, ranged from 8 to 64 years) with definite infective endocarditis according to the Duke criteria[13], who were referred to our hospital between January 1996 and April 1999. In all patients, the diagnosis was made if one of the following criteria existed: (1) histopathological evidence of infective endocarditis; (2) persistent positive blood cultures, excluding other potential sources of bacteremia, and a new regurgitant murmur or predisposing heart disease; and (3) negative or intermittently positive blood cultures plus fever in the presence of a new regurgitant murmur and microvascular or immunological phenomena. Patients with a history of thromboembolic events before hospital admittance or those being treated with anticoagulants or with prosthetic valve devices were excluded from the study group to avoid confounding by anticoagulation treatment. Patients with a cerebral haemorrhage or uncertainty about the diagnosis of an embolic event were not included in the study. The control group consisted of 34 age- and sex-adjusted healthy subjects. The patients were prospectively followed in our clinics by a team composed of cardiologists, infectious disease specialists, cardiac surgeons and neurologists, involved in the management of the patients. The mean duration of hospital stay was 44 days. The study protocol was approved by our institutional ethics committee and all patients and control subjects gave informed consent.

Thirteen patients were diagnosed to develop either a cerebral (n=7), pulmonary (n=2), renal (n=1) or major peripheral (n=3; two popliteal arteries and one femoral artery) embolic event during the in-hospital follow-up period. The remaining 63 patients with infective endocarditis did not have any embolic events. The diagnosis of an embolic event was based on physical examination, cerebral computer tomography, a peripheral Doppler-ultrasonographic investigation or angiography. The diagnosis of a cerebral embolus was, in all cases, made by an experienced neurologist who otherwise was not involved in this study. Microvascular emboli, such as cutaneous microinfarctions and immune complex phenomena, were not regarded as embolus.

Transthoracic and transoesophageal echocardiographic examinations were performed on all patients within 3 days of antimicrobial therapy initiation using commercially available ultrasound units. For transthoracic studies, 2·5 MHz transducers were used. A 5 MHz phased array transducer was used for transoesophageal studies. The transoesophageal examinations were performed in the left lateral decubitus position and after a 6 h fast. All investigations were carried out without any complications. The presence of vegetations and their characteristics were evaluated according to transoesophageal echocardiography by two independent investigators blinded to the clinical diagnosis. The localization and maximal diameter of the vegetations were determined. A valvular vegetation was defined as an oscillating or fixed mass associated with a valve or its supporting apparatus, distinct in echogenic structure and with motion independent from the remainder of the involved leaflet[14]. The lesion had to be detectable throughout the complete cardiac cycle. Diffuse valvar irregularities or valvular thickening were not regarded as a vegetation.

Coagulation and haematological parameters, including haematocrit, erythrocyte sedimentation rates, activated partial thromboplastin time (aPTT), platelet counts and fibrinogen concentrations of patients were tested routinely at hospital admission. To measure E- and P-selectin levels, peripheral venous blood samples were taken from both patients and control subjects at hospital admission before any embolic events had occurred. Blood samples were drawn into 3·8% 1·9 trisodium citrate containing tubes without venous stasis between 0800 h and 1000 h using 21 g vacuum tube phlebotomy needles. Plasma was immediately obtained by centrifugation of the blood at 3000 g for 15 min and then stored in several aliquots at −70 °C until assayed. The P- and E-selectin measurements, as a marker of platelet and endothelial cell activation/damage, were performed using commercial ELISA (sandwich enzyme linked immunosorbent assay) kits.

Results are expressed as mean ± SD. Comparison of clinical and echocardiographic variables between the infective endocarditis patients with and without embolic events was performed with the use of the Student t-test for numerical variables and the chi-square test for categorical data. All infective endocarditis patients with and without embolic events and control subjects were compared for P- and E-selectin concentrations with the use of the Mann–Whitney U test. A P-value <0·05 was considered to be significant.

Results

Clinical and echocardiographic characteristics of the patients with and without embolic events are shown in Table 1. Among the 76 infective endocarditis patients included in the study, 13 (17·1%) had embolic events. Measurements of haematology variables and causative microorganisms did not differ significantly between infective endocarditis patients with and without embolic events. The location of vegetations were similar in the two groups of patients with and without embolic events and the mitral valve was the most common site of involvement (54·5% vs 52·9%, P>0·05) in both of them. Although there was a trend towards a higher rate of vegetations detected in those with embolic events compared to the non-embolic group, this did not reach statistical significance (84·6% vs 80·9%, P>0·05). Significantly larger vegetations were observed in patients with embolic events as compared to patients without embolic events (1·4 cm vs 1·0 cm, P=0·03). Size, mobility and attachment characteristics of the vegetations on echocardiography were similar between the embolic and non-embolic patients.
The plasma concentrations of P- and E-selectin in patients with and without embolic events, and control subjects are shown in Figs 1 and 2. The mean plasma concentrations of P-selectin were elevated in patients with embolic events as compared with both the patients without embolic events and control subjects (58.69 ± 7.49 ng . ml⁻¹ vs 29.65 ± 5.69 ng . ml⁻¹, P<0.001 and 58.69 ± 7.49 ng . ml⁻¹ vs 25.82 ± 5.38 ng . ml⁻¹, P<0.001). Similarly, the patients with embolic events had increased plasma levels of E-selectin compared to those without embolic events and the control group (73.15 ± 11.47 ng . ml⁻¹ vs 42.84 ± 8.77 ng . ml⁻¹, P=0.001 and 73.15 ± 11.47 ng . ml⁻¹ vs 34.23 ± 5.92 ng . ml⁻¹, P<0.001). No significant difference was found between the levels of P- and E-selectin in patients without embolic events and control subjects (29.65 ± 5.69 ng . ml⁻¹ vs 25.82 ± 5.38 ng . ml⁻¹ and 42.84 ± 8.77 ng . ml⁻¹ vs 34.23 ± 5.92 ng . ml⁻¹, P>0.05, respectively).

Discussion

It has long been known that thromboembolic events are common and severe complications of infective endocarditis[1-7]. The incidence of embolic events complicating infective endocarditis has remained constant in recent decades despite the improvements in antibiotics and a tendency towards earlier surgical interven-

tions[11,15,16]. Septic emboli from vegetations in heart valves may explain stroke in infective endocarditis[18]. However, echocardiographic findings do not clearly support the simple embolus hypothesis, that heart valve vegetations are the sole mechanism leading to embolic events in endocarditis patients. Data are conflicting with regard to the prognostic implications of vegetation characteristics such as shape, mobility and size. Some investigators have reported that the presence of vegetations on echocardiograms predicted a higher incidence of embolic complications, whereas others have found no association[25,6]. In our relatively large study population, although there was a trend towards a higher rate of vegetations detected in embolic patients, this did not reach statistical significance. Similarly, location, mobility and attachment characteristics of vegetations were also identical in embolic and non-embolic patients. The only echocardiographic parameter differing between the two groups was the mean size of vegetations, which was higher in those with embolic events.

Other mechanisms which have been suggested in the literature could cause systemic embolism in bacterial infections are activation of the coagulation system and endothelial cell injury. Some recent investigations have demonstrated that systemic bacterial infections, even in the absence of cardiac involvement, represent an independent risk factor for embolic events[7-10]. Inflammation induced procoagulant changes and endothelial cell activation/injury favouring thrombosis were suggested.
Figure 1 Plasma concentrations of E-selectin in endocarditis patients with and without embolic events, and control subjects.

Figure 2 Plasma concentrations of P-selectin in endocarditis patients with and without embolic events, and control subjects.
as an explanation in this setting. The inter-relationships between endothelial damage, alterations in platelet activation and the occurrence of embolic events have not been previously investigated in a large patient population with definite infective endocarditis.

In previous reports, it was stated that infections, especially severe bacterial infections, change the prostacycline-thromboxane ratio in the direction of thrombosis and induce the production of cytokines, interleukin-1 and the tumour necrosis factor, which may lead to the activation of endothelial cells and increase blood coagulation and platelet aggregation\[17,18\]. Soluble forms of P-selectin have been shown to be secreted from activated platelets and endothelial cells\[11,12,19\]. Thus selectin adhesion molecules, especially E-selectin are important early mediators of endothelial dysfunction in settings in which an inflammatory response occurs. It is important to note that infective endocarditis differs from other infectious diseases owing to the presence of cardiac vegetations that represent an additional independent risk factor for an embolic event. In our study, the infective endocarditis patients with subsequent embolizations had increased levels of E-selectin as compared to both those without embolies and healthy individuals. This increase may reflect the endothelial dysfunction in these patients, with induction of a pro-adhesive and pro-thrombotic surface causing a subsequent distortion of the endothelial-platelet axis and thrombus formation. In a recent report\[20\], the investigators found that of the endothelial-platelet axis and thrombus formation.

Prothrombotic surface causing a subsequent distortion these patients, with induction of a pro-adhesive and

Conclusion

The findings of our study demonstrated that infective endocarditis patients with embolic events had increased levels of E- and P-selectin as compared to those without embolization and healthy subjects. It is reasonable to assume that determination of these membrane activation molecules may provide useful markers to identify patients at high thromboembolic risk from infective endocarditis.

References