Clin. Cardiol. 27, 154–158 (2004)

Increased Blood Coagulation and Platelet Activation in Patients with Infective Endocarditis and Embolic Events

N. Semnur Buyukasýk, m.d., Mehmet Ileri, m.d.,* Aysin Alper, m.d.,† Kubilay Senen, m.d.,* Ramazan Atak, m.d.,* Ismet Hisar, m.d.,* Ertan Yetkin, m.d.,* Hasan Turhan, m.d.,* Deniz Demirkan, m.d.*

Department of Internal Medicine, Ankara Numune Hospital; *Department of Cardiology, Türkiye Yüksek Ihtisas Hastanesi; †Department of Chest Surgery, Atatürk Center for Chest Disease and Chest Surgery, Ankara, Turkey

Summary

Background: Inflammation-induced procoagulant changes and alterations in platelet activity appear to play an important role in thromboembolic complications of infective endocarditis (IE).

Hypothesis: The aim of this study was to investigate systemic coagulation activity, fibrinolytic capacity, and platelet activation in patients with IE with and without embolic events by measuring the plasma levels of prothrombin fragment 1+2 (PF1+2), thrombin-antithrombin III complex (TAT), plasminogen activator inhibitor-1 (PAI-1), beta-thromboglobulin (β-TG), and platelet factor 4 (PF4), respectively.

Methods: The study included 76 consecutive patients (female = 55, male = 21, mean age 26 years, range 8–64 years) with definite IE according to the Duke criteria; of these, 13 (17.1%) had embolic events.

Results: Plasma concentrations of PF1+2 (3.2 ± 1.3 vs. 1.7 ± 0.7 and 1.4 ± 0.7 nmol/l, $p < 0.001$, respectively) and TAT (7.3 ± 1.5 vs. 2.9 ± 1.2 and 2.2 ± 1.1 ng/ml, $p < 0.001$, respectively) were elevated in patients with embolic events compared with patients without embolic events and control subjects. Similarly, patients with embolic events had increased plasma levels of β-TG (63.3 ± 10.9 vs. 33.1 ± 11.6 and 19.1 ± 10.6 ng/ml, $p < 0.001$, respectively) and PF4 (106.0 ± 28.7 vs. 50.3 ± 16.7 and 43.0 ± 15.8 ng/ml, $p < 0.001$, respectively) compared with those without embolic events and the control group. Embolic patients also had higher PAI-1 levels than nonembolic patients and healthy subjects (14.4 ± 6.4 vs. 8.6 ± 5.9 and 5.4 + 4.3 ng/ml, $p = 0.002$, respectively).

Conclusion: Patients with IE and with subsequent thromboembolism have increased systemic coagulation activation, enhanced platelet activity/damage, and impaired fibrinolysis. The resulting imbalance produces a sustained hypercoagulable state, which contributes to the increased risk of thromboembolic events in this particular group.

Key words: infective endocarditis, thromboembolism, coagulation activation, platelet activity

Introduction

Thromboembolic events remain a relatively common and serious complication of infective endocarditis (IE), occurring in 20 to 43% of cases.[1–3] Septic emboli from the vegetations in heart valves have been the most frequently suggested mechanism to explain stroke in IE.[4] However, the results of several echocardiographic studies, which attempt to correlate the formation of embolic events in IE with size and morphologic characteristics of valvular vegetations, have been inconsistent.[2, 5, 6] Recently, some investigators have reported 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 alterations in platelet activity appear to play a major role in this setting.[10] The interrelationships between coagulation status, platelet activity, and risk of embolic events have not been studied previously in a large patient population with definite IE.

Prothrombin fragment 1+2 (PF1+2) is a peptide byproduct of the conversion of prothrombin to thrombin, the pivotal

Address for reprints:

Dr. Mehmet Ileri
Samur sokak, 30/10, Kurtuluş
Ankara, Turkey
e-mail: maraspoli2000@yahoo.com

Received: July 1, 2002
Accepted: April 9, 2003

event in the initiation of the coagulation cascade. Thrombin-antithrombin III complex (TAT) is also measured as a marker of in vivo thrombin generation.[10] Plasminogen activator inhibitor-1 (PAI-1) is regarded as a major determinant of fibrinolytic activity in human plasma. Its synthesis by endothelial cells, hepatocytes, and fibroblasts is enhanced under conditions such as local hemostasis and in the presence of endotoxin or cytokines. Beta-thromboglobulin (β-TG) and platelet factor 4 (PF4) are released from alpha granules of platelets and reflect in vivo platelet activation/damage. Hence, the aim of this study was to investigate systemic coagulation activity, fibrinolytic capacity, and platelet activation in patients with IE with and without embolic events by measuring the plasma levels of PF1+2, TAT, PAI-1, β-TG, and PF4, respectively.

Methods

The study included 76 consecutive patients (female = 55, male = 21, mean age 26 years, range 8–64 years) with definite IE according to the Duke criteria,[11] who were referred to our clinic between January 1996 and April 1999. Infective endocarditis was diagnosed if one of the following criteria existed: (1) histopathologic evidence of 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 immunologic phenomena. Patients with a history of thromboembolic events before hospital admittance, those who had prosthetic valve devices, or those who were treated with anticoagulants and antiplatelets were excluded from the study. Patients with a history of cerebral hemorrhage or an uncertain diagnosis of an embolic event were not included. Thirty-four age- and gender-adjusted healthy subjects served as the control group. The patients were prospectively followed in our clinic by a team of cardiologists, infectious disease specialists, cardiac surgeons, and neurologists. The mean duration of hospital stay was 44 days. The study protocol was approved by our institutional ethics committee. 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; 2 popliteal artery and 1 femoral artery) embolic event during the in-hospital follow-up period. The remaining 63 patients with IE had no embolic events. Diagnosis of an embolic event was based on physical examination, cerebral computed tomography, peripheral Doppler ultrasonographic investigation, or angiography. In all cases, cerebral embolus was diagnosed by an experienced neurologist who otherwise was not involved in this study. Microvascular emboli such as cutaneous microinfarctions or immune complex phenomena were not regarded as embolus.

Transthoracic two-dimensional and color flow Doppler echocardiography was performed in all patients using ultrasound equipment (Toshiba SSH160A, Tokyo, Japan) with commercially available 2.5 MHz transducers. Within 3 days of initiation of antimicrobial therapy and after a 6-h fasting period, transesophageal echocardiography was carried out in the left lateral decubitus position with the use of 5-MHz phased array transducer. All studies were recorded on super VHS videotape for subsequent independent review and analysis by two echocardiographers blinded to the clinical status of the patients. In case of disagreement, a third examiner was consulted. Patients with any thrombus in the left atrial cavity or appendage by transesophageal examination were excluded. The presence of vegetations and their characteristics were evaluated according to transesophageal examinations. 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.[12] The lesion had to be detectable throughout the complete cardiac cycle. Diffuse valvular irregularities or valvular thickening were not regarded as a vegetation.

Simple hematological parameters including hematocrit, erythrocyte sedimentation rate, activated partial thromboplastin time (APTT), platelet counts, and fibrinogen concentrations of patients were tested routinely at hospital admission. At hospital admission and before any embolic events had occurred, peripheral venous blood samples of both patients and control subjects for measuring hemostatic markers were drawn between 8 and 10 A.M. with 21 g vacuum tube phlebotomy needles into 3.8% 1:9 trisodium citrate containing tubes without venous stasis. Plasma was immediately obtained by centrifugation of the blood at 3,000 × gravity for 15 min and then stored in several aliquotes at −70˚C until assayed.

Plasma TAT (Enzygnost TAT micro enzyme immunoassay, Behringwerke AG, Marburg, Germany) and PF1+2 (Enzygnost PF1+2 micro enzyme immune assay, Behringwerke AG) concentrations as the markers of in vivo thrombin generation were measured by solid phase sandwich enzyme-linked immunosorbent assay method. Measurements of plasma β-TG and platelet factor 4 (PF4) levels as indices of platelet activation/damage were taken using commercial enzyme-linked immunosorbent assay kits (Diagnostica Stago, Asnières, France). Specific measurement of PAI-1 levels reflecting fibrinolytic activity was performed with an immunofunctional assay as described previously.

Statistical Analysis

Results were expressed as mean ± standard deviation. Comparison of clinical and echocardiographic variables between the patients with IE with and without embolic events was performed with the use of Student's *t*-test for numerical variables and chi-square test for categorical data. All patients with IE with and without embolic events as well as control subjects were compared for TAT, PF1+2 , PAI-1, and P-selectin concentrations with the use of Mann-Whitney U test. A p value < 0.05 was considered to be significant.

TABLE I Clinical and echocardiographic characteristics of patients with infective endocarditis with and without embolic events

	Embolic event (+) (n = 13)	Embolic event (−) (n = 63)	p Value
Age (years)	28	24	NS
Male/female	4/9	17/46	NS
Hematological variables			
Hematocrit (%)	35.3 ± 4.1	34.4 ± 5.2	NS
Erythrocyte sedimentation rate (mm/h)	98	76	NS
Platelet count ($X10^{-9}$/l)	265.5 ± 62.4	245.6 ± 56.4	NS
APTT (s)	26.8 ± 4.0	31.5 ± 6.3	NS
Fibrinogen (g/l)	6.1 ± 1.3	4.2 ± 1.5	NS
Causative microgrames			
Viridans streptococci (%)	5 (38.4)	22 (34.9)	NS
Group D streptococci and enterococci (%)	2 (15.4)	9 (14.2)	NS
Other streptococci (%)	1 (7.6)	4 (6.3)	NS
Staphylococci (%)	3 (23.1)	14 (22.2)	NS
Other microorganism (%)	1 (7.6)	7 (11.1)	NS
Undetermined (%)	1 (7.6)	7 (11.1)	NS
Vegetation detected (%)	11 (84.6)	51 (80.9)	NS
Location of vegetation			
Mitral (%)	6 (54.5)	27 (52.9)	NS
Aortic (%)	3 (27.2)	19 (37.2)	NS
Mitral and aortic (%)	1 (9.1)	3 (5.8)	NS
Tricuspid (%)	1 (9.1)	2 (3.9)	NS
Vegetation characteristics			
Size (cm)	1.4 ± 0.4	1.0 ± 0.3	0.03
Mobile/immobile	3/8	14/49	NS
Pedunculated/sessile	1/10	7/56	NS

Abbreviations: APTT = activated partial thromboplastin time, NS = not significant.

Results

Clinical and echocardiographic characteristics of the patients with and without embolic events are shown in Table I. Among the 76 patients with IE included in the study, 13 (17.1%) had embolic events. Measurements of hematological variables and causative microorganisms did not differ significantly between patients with IE with and without embolic events. The localization of vegetations was similar in the two groups of patients with and without embolic events; mitral valve was the most common site of involvement (54.5 vs. 52.9%, $p > 0.05$) in both. Although there was a trend toward a higher rate of vegetations detected in those with embolic events, this did not reach statistical significance (84.6 vs. 80.9%, $p > 0.05$). Significantly larger vegetations were observed in patients with than in those without embolic events (1.4 vs. 1.0 cm, $p = 0.03$). Size, mobility, and attachment characteristics of the vegetations were similar between the embolic and nonembolic patients.

As shown in Table II, the mean plasma concentrations of PF1+2 (3.2 ± 1.3 vs. 1.7 ± 0.7 nmol/l and 1.4 ± 0.7 nmol/l, $p < 0.001$, respectively) and TAT (7.3 ± 1.5 vs. 2.9 ± 1.2 ng/ml and 2.2 ± 1.1 ng/ml, $p < 0.001$, respectively) were elevated in patients with compared with those without embolic events and control subjects. Similarly, patients with embolic events had increased plasma levels of β-TG (63.3 ± 10.9 vs. 33.1 ± 11.6 and 19.1 ± 10.6 ng/ml, $p < 0.001$, respectively) and PF4 (106.0 ± 28.7 vs. 50.3 ± 16.7 and 43.0 ± 15.8 ng/ml, $p < 0.001$, respec-

TABLE II Hemostatic variables in patients with infective endocarditis with and without embolic events and control subjects

Variables	Embolic event (+) (n = 13)	Embolic event (−) (n = 63)	Control (n = 34)
PF1+2 (nmol/l)	3.2 ± 1.3 [a]	1.7 ± 0.7	1.4 ± 0.7
TAT (ng/ml)	7.3 ± 1.5 [b]	2.9 ± 1.2	2.2 ± 1.1
β-TG (ng/ml)	63.3 ± 10.9 [c]	33.1 ± 11.6	19.1 ± 10.6
PF4 (ng/ml)	106.0 ± 28.7 [d]	50.3 ± 16.7	43.0 ± 15.8
PAI-1 (ng/ml)	14.4 ± 6.4 [e]	8.6 ± 5.9	5.4 ± 4.3

[a] $p < 0.001$ compared with embolic (−) patients and control.
[b] $p < 0.001$ compared with embolic (−) patients and control.
[c] $p < 0.001$ compared with embolic (−) patients and control.
[d] $p < 0.001$ compared with embolic (−) patients and control.
[e] $p = 0.002$ compared with embolic (−) patients and control.
Abbreviations: PF 1+2 = prothrombin fragment 1+2, TAT = thrombin-antithrombin III complex, β-TG = beta-thromboglobulin, PF4 = platelet factor 4, PAI-1 = plasminogen activator inhibitor type-1.

tively) compared with those without embolic events and the control group. Embolic patients also had higher PAI-1 levels than both nonembolic patients and healthy subjects (14.4 ± 6.4 vs. 8.6 ± 5.9 and 5.4 + 4.3 ng/ml, p = 0.002, respectively). No significant differences were found between the levels of PF1+2, TAT, β-TG, PF4, and PAI-1 in patients without embolic events and control subjects (p > 0.05 for all).

Discussion

Fragmentation of valvular vegetations as a result of turbulent blood flow within the cardiac chambers is supposed to lead to systemic emboli in patients with IE.[4] However, the results of echocardiographic studies do not clearly support the simple embolus hypothesis that heart valve vegetations are the sole mechanism leading to embolic events in these patients. Data regarding the prognostic implication of vegetation characteristics such as shape, mobility, and size for the occurrence of thromboembolic events are conflicting.[2, 5, 6] We have studied 76 consecutive patients with definite IE according to Duke criteria and correlated the thromboembolic events with the morphologic characteristics of the vegetations examined by transesophageal echocardiography. Although there was a trend toward a higher rate of vegetations detected in embolic patients, this did not reach statistical significance in our relatively large population. It was not surprising that there was a significant correlation between larger vegetations and thromboembolic events. However, there was a large overlap (1.4 ± 0.4 vs. 1.0 ± 0.3 cm) of the size of vegetations in patients with and without embolic events, together with a lack of significant differences in their morphologic characteristics such as location, mobility, and attachment properties. This rendered the structural findings of the vegetations unhelpful as determinants for risk calculation of further thromboembolism for individual patients.

Other mechanisms, which have been suggested in the literature as possible causes for systemic embolism in bacterial infections, are activation of coagulation system and endothelial cell injury. Recent investigations have demonstrated that systemic bacterial infections constitute an independent risk factor for embolic events even in the absence of cardiac involvement.[7–9, 13] Previous reports have stated that severe bacterial infections change the prostocycline–thromboxane ratio in the direction of thrombosis and induce production of cytokines, interleukin-1, and tumor necrosis factor, which may lead to activation of blood coagulation and platelet aggregation.[14, 15] Activation of the coagulation system, which contributes to the formation of endocardial vegetations, is an important feature in the course of bacterial endocarditis.[16–18] Buiting *et al.* showed the procoagulant activity of endocardial vegetations and blood monocytes in rabbits with *Streptococcus sanguis* endocarditis.[19] It has been demonstrated that phagocytosis of bacteria by monocytes on the vegetational surface might account for the expression of tissue factor activity, which in turn activates the coagulation system locally in bacterial endocarditis.[20] Kupferwasser *et al.* have also reported that the presence of other humoral factors, such as antiphospholipid antibodies, which were detectable in a substantial number of patients with IE (14.3%), influencing the coagulation system at several stages, adds significantly to the risk of embolic events in IE.[21] Patients with elevated levels of antiphospholipid antibodies showed higher levels of PF1+2, PAI-1, von Willebrand factor, and lower levels of activated protein C than those with normal levels of these antibodies. Data of the present study add another important piece to the puzzle of the pathogenesis of thromboembolic complications in bacterial endocarditis. For the first time, we have demonstrated in a large population that patients with IE and with subsequent embolizations had increased systemic coagulation activity compared with those without emboli and healthy individuals. Because patients with embolic events before hospital admission as well as those with prosthetic heart valves were excluded from the study, it is likely that elevated coagulation markers reflecting a hypercoagulable state are causative for and not the consequence of established thromboembolic events in IE. It is interesting that PF1+2 and TAT levels were not elevated significantly in patients with IE but without thromboembolism compared with healthy controls. This points to the important aspect of the situation that there was a systemic coagulation activation which may occur independently of IE but may be of importance for the clinical course, especially in terms of thromboembolic events. Therefore these infection-associated humoral factors may be significantly associated with the increased risk of thromboembolic events in IE. Endothelium is now recognized to play a potentially active role in regulating blood coagulation. Although normally nonthrombogenic, it may express tissue factor when injured or stimulated by a limited number of substances including endotoxin, interleukin-1, and tumor necrosis factor.[22] Recently, we have shown the evidence of endothelial dysfunction in patients with IE with embolic events.[23] With induction of a proadhesive and prothrombotic surface, this may lead to activation blood coagulation and subsequent thrombus formation in this particular group of patients.

Besides the coagulation markers, we have also measured plasma PAI-1 levels in the study group. Plasminogen activator inhibitor-1 is an important regulator of fibrinolytic activity in human plasma.[24] It also represents a function of endothelial release and activation, although platelet granules also contain PAI-1, which may complicate plasma measures. High plasma levels of PAI-1 have been found in conditions associated with increased thromboembolic risk, such as recurrent deep vein thrombosis, postoperative period, malignant tumors, septicemia, and coronary artery disease.[25–27] High PAI-1 levels in embolic patients represent decreased fibrinolytic activity and contribute to the prothrombotic state in this group.

Another important finding of this study was the fact that patients with IE with subsequent embolizations had significantly higher β-TG and PF4 levels, reflecting enhanced platelet activation and/or damage. Circulating platelets have critical and complex contributions to the initiation of hemostasis, coagulation, and arterial thrombogenesis. With induction of a proadhesive surface, presence of previously suggested endothelial injury in this subgroup of patients may lead to a subsequent

distortion of the endothelial–platelet axis and thrombus formation. Investigators have also demonstrated that certain bacteria and other microorganisms can cause complete and irreversible platelet aggregation in platelet-rich plasma.[28, 29] Platelet-bacterial interactions that result in platelet activation have been shown to proceed through several mechanisms, including activation of the complement system on the platelet surface by endotoxin components and direct lytic destruction of platelet membrane by hemolysins elaborated by certain bacteria. Isolates from individuals with subacute bacterial endocarditis were reported to have a greater tendency toward aggregating platelet-rich plasma spontaneously than strains not associated with endocarditis.[30] Putting these data together, platelet activation might play an additional role in thromboembolic complications of IE.

Conclusion

Data of the present study demonstrate that patients with IE with subsequent thromboembolic events have increased systemic coagulation activation, enhanced platelet activity/damage, and impaired fibrinolysis. The resulting imbalance produces a sustained hypercoagulable state which contributes to the increased risk of thromboembolic events in this particular group of patients.

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