Regional Left Atrial Coagulation and Fibrinolytic Activities in Patients With Mitral Stenosis

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SUMMARY

Systemic thromboembolism is a major complication of mitral stenosis (MS), especially in those patients having atrial fibrillation (AF). Recent evidence has suggested that regional left atrial coagulation activity may be increased in MS and may contribute to the pathophysiology of left atrial thrombus. However, the relation of left atrial coagulation activity to factors that predispose to left atrial thrombus formation is unknown. Also, the relations between left atrial and systemic coagulation activity, fibrinolysis, and platelet activation remain unresolved.

Left atrial and peripheral venous levels of fibrinogen, antithrombin III, factor VII and factor VIII for coagulation, D-dimer, tPA and PAI-I, plasmin and antiplasmin for fibrinolysis, and platelet factor 4 and vWF for platelet activation, and endothelial dysfunction were measured in 46 patients with MS and normal clotting times who were undergoing percutaneous mitral valvuloplasty.

Left atrial tPA, plasmin, PAI-I, antiplasmin, PF4, and vWF levels exceeded the corresponding peripheral venous levels ($P < 0.05$) in patients with MS, being more significant in the AF subgroup. There were no significant differences between left atrial and peripheral venous levels of fibrinogen, D-dimer, factor VII, and factor VIII within the patient group ($P > 0.05$).

The results suggest that there are significant variations in the indices of coagulation, fibrinolytic system and platelet activation, and endothelial dysfunction between left atrial and peripheral venous blood samples of patients with MS that may be due to limited spill-over from the left atrium to the systemic circulation. (Jpn Heart J 2004; 45: 779-788)

Key words: Atrium, Coagulation, Fibrinolysis, Platelet activation, Mitral stenosis

SYSTEMIC haemostatic abnormalities have been found in cross-sectional studies of patients with mitral stenosis (MS) and atrial fibrillation (AF).1,2 Whether blood coagulation activation in patients with MS and sinus rhythm (SR) involved is controversial. Both normal3 and increased4-6 peripheral venous levels of some of the thrombin generation markers like fibrinopeptide A and B, throm-
bin/ antithrombin III and prothrombin fragment 1+2 have been reported in the literature. In the present study, we investigated plasma levels of biochemical markers for coagulation activation, platelet activity, and fibrinolysis in the peripheral venous blood and left atria of 46 consecutive patients with rheumatic MS, either in SR or in AF, who were undergoing percutaneous mitral balloon valvuloplasty.

**METHODS**

**Patients:** Forty-six consecutive patients (8 males, 38 females) with symptomatic rheumatic MS (New York Heart Association functional class II and III) who underwent percutaneous mitral valvuloplasty were enrolled in the study. Their clinical characteristics are summarized in Table I. Eighteen patients had chronic AF, and twenty-eight had SR before valvuloplasty. None of the patients had previously undergone commissurotomy or percutaneous mitral valvuloplasty. An age-matched control group consisted of 20 healthy volunteers (5 males, 15 females; mean age, 35 ± 8 years). Written informed consent was obtained from all subjects before blood sampling.

The exclusion criteria were as follows; patients > 70 years old, recent venous thrombosis or a history of systemic embolism, left atrial thrombus, previous anticoagulant treatment, surgery, acute infectious or inflammatory disease, ongoing rheumatic activation, malignancy, unstable vascular disease, renal or hepatic insufficiency, diabetes mellitus, hypertriglyceridemia or obesity, significant mitral regurgitation, aortic valve disease, and left ventricular dysfunction in the echocardiographic study. Taking aspirin was not an absolute contraindication, but it had to be discontinued 3 days before the beginning of the study.

**Echocardiography:** All patients underwent M-mode, two-dimensional, and Doppler echocardiography at the study entry with a commercially available Vingmed System Five (Norway) and a 2.5-3.5 MHz transducer to assess left atrial diameter, left ventricular systolic and diastolic dimensions, mitral valve area, transmitral pressure gradient, and pulmonary artery pressure. Left atrial and ventricular dimensions were measured by M-mode echocardiography in the parasternal long-axis view. Left atrial anteroposterior diameter was determined with the standard M-mode criteria. Mean transmitral diastolic gradients and pulmonary artery pressures were calculated by Doppler. Mitral valve area was calculated according to the pressure half-time method. The presence of mitral regurgitation was evaluated by Doppler color flow mapping.

Transesophageal echocardiography (TEE) was performed on the day of percutaneous mitral valvuloplasty before obtaining blood samples in all patients to detect intracardiac thrombus and the degree of left atrial spontaneous echo con-
Contrast. After application of xylocaine spray to the back of the pharynx, a 5 MHz transducer (Toshiba Model REF 510 PB, Japan) was passed into the oesophagus. Adequate views of the left atrial cavity and appendage were obtained in all patients. Left atrial thrombus was excluded by the absence of a clearly defined intracavitary mass that was acoustically distinct from the underlying endocardium. Patients with a left atrial or appendage thrombus were excluded. Maximum and minimum areas, ejection fraction, and inward and outward velocities of the left atrial appendage were also calculated.

**Valvuloplasty procedure:** Patients were sedated with 10 mg of diazepam (IM) about half an hour before being transferred to the catheterization laboratory. In the catheterization laboratory, introducer sheaths were inserted into the right and left femoral veins and left femoral artery. A 6F polymer pigtail catheter was inserted into the left ventricle for pressure measurement and ventriculography. A 7F Cournand catheter was inserted via a femoral approach to measure right heart and pulmonary capillary wedge pressures. The left ventricle/pulmonary wedge pressure gradient was calculated. Left ventriculography was performed in a right anterior oblique projection with the use of a nonionic contrast (Iohexol 350 mg/mL) and the severity of any observed mitral regurgitation was graded on a scale of 1 to 4 according to standard criteria. After an atrial transseptal puncture with a Brockenborough needle, an 8F transseptal catheter was advanced into the left atrium and the mitral valve was dilated with an Inoue balloon catheter (Toray Medical Industries). No thromboembolic event occurred in any patient during or after the valvuloplasty procedure.

**Blood sampling:** Prevalvuloplasty blood samples were drawn from an antecubital vein by careful venipuncture in a 21G sterile syringe without stasis at 08.00-10.00 AM after the TEE procedure and were immediately transferred to an evacuated tube containing 3.2% sodium citrate.

Blood samples from patients with MS were collected through introducer sheaths during the mitral valvuloplasty procedure, and at least twice the dead space volume was withdrawn. Immediately after transseptal puncture, central prevalvuloplasty samples were withdrawn from all 46 patients through the transseptal catheter positioned in the body of the left atrium and through the right femoral venous sheath for the measurement of the haematological parameters.

In control patients, peripheral venous blood samples were collected through a needle puncture after the initial 3 mL of blood was discarded. Femoral venous blood samples were not considered in either patients or in controls because no difference was revealed between antecubital and femoral approaches in previous studies.

**Assay procedures:** After the collection of blood samples, the plasma fractions were obtained by centrifugation for 10 minutes at 2000 g and stored at -40°C until
further analysis. Plasma fibrinogen was assayed by the modified Clauss assay. D-dimer (DD) was measured with a turbidimetric method strengthened by latex. Antithrombin III (ATIII) levels were assayed by the chromogenic method (functional activity). Factor VII (FVII), factor VIII (FVIII), and plasminogen activator inhibitor-1 (PAI-1) were measured using an automatic coagulometer (BCS system, Dade-Behring, Germany). Tissue plasminogen activator (tPA), plasmin, and antiplasmin were assayed by an enzyme-linked immunosorbent assay (ELISA) (Biopool, Sweden). Von Willebrand factor (vWF) concentration was measured using a ristocetin cofactor activity platelet agglutination method. Blood samples for platelet factor-4 (PF4) were transferred to the laboratory on ice and kept for 30 minutes at -2 and -8°C. Samples for PF4 were centrifuged at 3000 rpm for 30 minutes at -4°C and platelet poor plasma fractions were obtained and stored at -40°C until assayed. PF4 levels were measured by ELISA (Stago, Paris, France).

**Data analysis:** Data are expressed as the median ± SD and were analysed with standard statistical tests. All statistical calculations were performed with Statistical Package for Social Sciences (SPSS) for Windows (SPSS Inc., Chicago, Illinois). All patients with MS either in SR or AF versus the control group were compared using Kruskal-Wallis variance analysis. If any difference was found among these three groups, the groups were compared with the Mann-Whitney U test using the Bonferroni correction. The Mann-Whitney U test was also used to compare the patients grouped according to the rhythm. A P value < 0.05 was considered statistically significant for all analyses.

**RESULTS**

Forty-six patients with rheumatic MS (28 in SR, 18 in AF) were included in the study. The control group and the patient subgroups were not statistically different with respect to age and gender (P > 0.05). The left atrial dimensions and pulmonary artery pressures were significantly higher in the patient group (P < 0.05). There were no significant differences between the left atrial dimensions, left ventricular ejection fractions, pulmonary artery pressures, mean diastolic mitral gradients, mitral valve areas, and left atrial appendage inward velocities (P > 0.05) in the AF and SR groups, although the maximum and minimum areas of the left atrial appendage were significantly higher in the AF group (P < 0.05). Ejection fraction and left atrial appendage outward velocity were significantly higher in the SR group (P < 0.05) (Table I).

Hematologic parameters in the patient subgroups and control subjects are listed in Tables II, III, and IV.
Peripheral venous versus regional left atrial blood samples in the patient group: The fibrinogen, ATIII, and FVII concentrations in the left atrial and peripheral venous samples of the SR group were identical; only the FVIII concentrations were lower in the left atrium \( (P < 0.05) \). The fibrinogen and FVIII concentrations in the left atrium and peripheral venous samples of the AF subgroup were identi-
cal, however, the ATIII and FVII concentrations in the left atrium of the AF group were statistically different from those in the peripheral venous blood \( (P < 0.05) \) (Table II).

The DD, plasmin, and antiplasmin concentrations in the left atrium and peripheral venous samples were identical, however, the tPA and PAI-I concentrations were statistically higher in the left atrium of the SR group \( (P < 0.05) \). The tPA, PAI-I, plasmin, and antiplasmin concentrations were increased in the left atrium in the AF group \( (P < 0.05) \) (Table III).
Peripheral venous and regional left atrial platelet counts, mean platelet volume, and PF4 and vWF concentrations were identical in the SR group (P > 0.05). Similarly, all these variables were also identical in the AF subgroup, except vWF concentration, which was statistically higher in the left atrium (P < 0.05) (Table IV).

**Peripheral venous blood samples of the patients and controls:** The patients demonstrated significantly higher concentrations of fibrinogen, AT-III, and FVII than matched healthy controls (P < 0.05). FVII concentrations in peripheral blood were higher in SR than in AF, while the FVIII concentrations were higher in the SR subgroup (P < 0.05) (Table IV).

Although the DD and tPA concentrations were significantly elevated in patients with AF than the other two groups (P < 0.05), no differences were evident in peripheral venous PAI-I, plasmin, and antiplasmin levels as a whole (P > 0.05) (Table III).

In patients with MS, platelet count, mean platelet volume, and PF4 and vWF concentrations were all significantly higher than controls (P < 0.05). The platelet count, mean platelet volume, and vWF concentrations in the AF subgroup were statistically higher than in the SR subgroup (P > 0.05) (Table IV).

**Regional left atrial blood samples of the patients:** The fibrinogen and ATIII concentrations in the left atrium were identical in the SR and AF subgroups, but a significant increase in FVII concentration in the SR subgroup and a similar increase in FVIII concentration in the AF subgroup were observed (P < 0.05) (Table II).

The DD, PAI-I, plasmin, and antiplasmin concentrations in the left atrium of the SR and AF subgroups were identical, but the tPA concentration in the left atrium of the AF subgroup was significantly higher (P < 0.05) (Table III).

PF4 and vWF levels, which are markers of platelet activation, were significantly elevated in peripheral venous blood samples of patients with MS (P < 0.05). However, there were also significant differences between peripheral venous and left atrial blood samples of patients with AF and SR (P < 0.05) (Table IV).

**DISCUSSION**

We investigated regional left atrial and peripheral venous coagulation, fibrinolytic activity, and platelet activation in patients with MS either in SR or in AF. The study produced three main findings. First, coagulation, fibrinolysis, and thrombogenesis are activated in patients with MS. Second, increased regional left atrial coagulation and fibrinolytic system activities are evident mainly in patients with MS and AF. Finally, increased left atrial coagulation activity was not reflected in peripheral venous blood samples, especially in the AF subgroup.
Not many investigators have focused on blood coagulation in patients with MS and SR. Elevated peripheral venous levels of DD, ATIII, and fibrinogen have been reported, however, Peverill, et al claimed that coagulation was increased mainly in the left atrium of patients with MS in SR. Our results indicated increased intravascular fibrin turnover in peripheral venous and left atrial blood samples of patients with MS, expressed by the increased levels of DD and ATIII. Moreover, significantly lower FVII, but normal FVIII levels, in the left atrium of patients with AF suggest activation in the extrinsic pathway and increased consumption.

Elevated levels of PAI-I found mainly in the left atrial blood samples of patients both in SR and AF suggested fibrinolytic dysfunction. Patients in SR probably have similar clinical aspects as patients with AF to promote a thrombotic state. Although impaired fibrinolytic function as evidenced by elevated PAI-I in plasma is a common finding in patients with thrombotic disease, the fibrinolytic system function in patients with AF is controversial. Roldan, et al demonstrated an increase in PAI and a decrease in tPA levels, suggesting impaired fibrinolysis in patients with chronic AF. Marin, et al reported increased PAI-I, but normal tPA levels, in MS with SR and concluded there was defective fibrinolytic activity. Mitusch, et al reported stimulation of fibrinolysis in patients with AF revealed by elevated plasma tPA levels, but they found no difference in PAI-I. In our study, left atrial tPA and plasmin levels were elevated in patients with AF, reflecting increased fibrinolytic activity, but only tPA was elevated in peripheral blood. Moreover, PAI-I and antiplasmin, which are responsible from the control of this system, were only elevated in the left atrium of patients, both in SR and in AF, suggesting fibrinolytic dysfunction. Patients in SR probably have similar clinical characteristics as patients with AF with respect to promoting a thrombotic state.

The large multimeric glycoprotein, vWF, mediates platelet adhesion to the vascular subendothelium and is essential for the formation of platelet thrombi in flow that is characterized by high shear stress. In a previous study, using vWF to detect endothelial injury, this phenomenon was found to appear in patients with MS and AF, contributing to the pathophysiology of coagulation activation. Moreover, vWF concentrations in peripheral blood were found to be significantly higher and to contribute to thrombotic tendency in MS. Finally, Goldsmith, et al demonstrated that endocardial cell surface damage was more commonly seen in the left atrial appendages of patients with MS and found increased vWF levels in the left atrium. In our study, vWF levels were mainly elevated in the left atrium of patients with MS and AF, suggesting the precise site of injury.

A shortened platelet survival time was documented in patients with MS, suggesting activated platelet activity. Increased PF4 levels were reported in
rheumatic and nonrheumatic AF and also in patients with decreased atrial velocity.\textsuperscript{21-23} In our study, PF4 levels tended to increase significantly in the left atrium of patients with MS either in SR or in AF, representing markedly increased platelet activity within the left atrium.

**Conclusion:** We attempted to evaluate regional left atrial coagulation and fibrinolytic activities and platelet activation in patients with MS, normal blood clotting times, and no left atrial thrombus. Our results suggest that increased regional left atrial coagulation and fibrinolytic activities and platelet activation occur in the presence of MS. Moreover, increased left atrial fibrinolytic activity and prothrombotic state are more evident, especially in the subgroup of patients with AF that is not reflected in peripheral venous blood samples and this association contributes to understanding the pathophysiology of left atrial thrombus formation in MS.

**REFERENCES**


