Clin. Cardiol. 23, 587–590 (2000)

Increased Platelet Activation and Endothelial Dysfunction in Patients with Atrial Fibrillation Immediately Following Percutaneous Balloon Mitral Valvuloplasty

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Summary

Background: Immediately following percutaneous balloon mitral valvuloplasty (PBMVP), patients have a 3% risk of systemic thromboembolism.

Hypothesis: We hypothesized that this may in part be due to an increase in hypercoagulability (as indicated by abnormal coagulation, platelet activation, and endothelial dysfunction) in such patients.

Methods: We measured indices of platelet activation [soluble P-selectin (sPsel), ELISA], endothelial dysfunction [von Willebrand factor (vWf), ELISA], and coagulation (fibrinogen, modified Clauss) in 16 patients (15 women, mean age 59 ± 10 years) with chronic atrial fibrillation admitted for PBMVP, and 16 healthy age- and gender-matched controls. Blood samples were obtained as follows: (1) peripheral venous samples prior to PBMVP, immediately following PBMVP, and 24 h after PBMVP; and (2) arterial samples prior to and immediately following PBMVP.

Results: Patients with mitral stenosis and chronic atrial fibrillation demonstrated significantly higher mean levels of vWf [148 (SD 24) vs. 102 (SD 37); *t*-test, $p < 0.001$] and plasma fibrinogen [4.2 (SD 0.8) vs. 3.3 (SD 0.8); $p = 0.003$] at baseline than matched healthy controls. There was a nonsignificant trend toward lower median sP-sel levels in patients with mitral stenosis [64 (inter quartile range 47–91) vs. 109 (46–128); Mann-Whitney test, $p = 0.08$]. Following PBMVP, there was a significant increase in venous sP-sel levels immediately post procedure (paired Wilcoxon test, $p = 0.03$) and at 24 h afterward ($p = 0.01$). Arterial s-Psel levels correspondingly increased immediately post procedure ($p = 0.008$). There was a significant increase in mean venous (at 24 h) but not arterial vWf levels post PBMVP. There were no significant changes in mean venous or arterial plasma fibrinogen levels following PBMVP.

Conclusion: Patients with mitral stenosis and chronic atrial fibrillation have increased plasma levels of vWf and fibrinogen levels compared with healthy controls, suggesting increased endothelial dysfunction and coagulation at baseline in these patients. The increased levels of sP-sel immediately post procedure and at 24 h, in association with increased vWf levels at 24 h after PBMVP, are in keeping with an increase in platelet activation and endothelial dysfunction following PBMVP. These changes may contribute to the increased risk of thromboembolism following PBMVP and suggest the need for adequate antithrombotic therapy following PBMVP.

Key words: mitral valve, valvuloplasty, atrial fibrillation, endothelial dysfunction, fibrinogen, von Willebrand factor

Introduction

Percutaneous balloon mitral valvuloplasty (PBMVP) is associated with systemic thromboembolism, with a reported incidence of around 2%.[1] Nevertheless, this rate has not been reported consistently.[2] During PBMVP, one or both of the fused commissures of the mitral valve are physically split,[3] leading to possible "trauma" to both endocardium and cellular blood elements. Although blood stagnation is usually resolved, spontaneous echocontrast on transesophageal echocardiography either persists or develops anew in the left atrial cavity,[4,5] indicating continued atrial stasis. Thus, exposure of blood to a disrupted endocardium and cardiovascular devices (including the transeptal procedure and balloon dilatation during the PBMVP procedure) may initiate complex clotting

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Received: August 27, 1999
Accepted: November 15, 1999

mechanisms, whereby platelets and plasma coagulation factors interact with the endocardium, leading to an increased propensity to thrombogenesis (thrombus formation).

We hypothesized that the enhanced risk of thromboembolism in patients with mitral stenosis in atrial fibrillation (AF) undergoing PBMVP may be related to measurable changes in hypercoagulability, perhaps explaining some of the differences in thromboembolic rate between different series.[1,2] We focused on three such markers that are representative of different aspects of the process of thrombogenesis, which were measured prior to, immediately post procedure, and (for venous samples) at 24 h following PBMVP. We measured levels of soluble P-selectin (a marker of platelet activation), von Willebrand factor (vWf, as a marker for endothelial cell dysfunction) and plasma fibrinogen (as a hemorheological and clotting factor).

Methods

Patients

Consecutive patients with mitral stenosis who were in chronic AF, admitted to our unit for elective primary PBMVP over a 12-month period (between May 1997 and April 1998), were studied. Patients with associated medical conditions known to influence the three parameters under investigation, namely, coronary artery disease, infections, diabetes mellitus, hypertension, chronic liver failure, chronic renal failure, acute/chronic inflammatory conditions, connective tissue disease, or cancer, were excluded. Informed consent was obtained and the study was conducted in accordance with the declaration of Helsinki and approved by the ethics committees of both Coventry and West Birmingham districts.

All patients were anticoagulated prior to the procedure [target International Normalized Ratio (INR) of 2–3]. In keeping with our routine clinical practice, warfarin was stopped in all patients 2 days prior to the procedure and was recommenced 24 h afterward, after the last venous blood sample for this study had been taken. The PBMVP procedure was performed only if the INR was < 2.0. All patients underwent transesophageal echocardiography to exclude atrial thrombus and the suitability of mitral valve morphology for PBMVP. The actual procedure and choice of balloon were at the discretion of the cardiologist. Baseline blood samples (peripheral vein) in patients with mitral stenosis were compared with those from healthy controls in sinus rhythm, who were matched for age and gender, recruited from healthy hospital staff members, and from patients admitted for minor surgical procedures, for example, hernia repair.

Blood Sample Collection

Blood samples were obtained from a peripheral vein prior to, immediately after, and at 24 h following PBMVP. Arterial samples were taken from the femoral artery prior to and immediately post procedure. As the femoral arterial line was removed immediately after the procedure, no 24-h arterial sample was taken. All samples were collected in 0.105 M sodium citrate vacutainer tubes. After the initial (pre-PBMVP) venous and arterial blood samples were obtained, 10,000 IU of heparin was given for the valvuloplasty procedure. No further anticoagulation was administered until oral warfarin was restarted 24 h later. The blood samples were immediately centrifuged at 3,000 rpm for 20 min at 4°C, and the plasma separated and stored at –70°C until analyzed for the three plasma markers under investigation.

Assay Procedures

Plasma samples obtained were analyzed for soluble P-selectin (sP-sel, ELISA, R&D Systems, Albington, U.K.), von Willebrand factor (vWf, ELISA, Dako, Denmark), and fibrinogen [g/l, modified Clauss assay using thrombin (Pacific Haemostasis, Hunterville, N.C., USA]. Intra- and interassay coefficients of variation for all ELISA assays were < 5 and < 10%, respectively.

Data Analysis and Statistics

Data were entered onto a computerized data base and statistical calculations were performed on a microcomputer using a commercially available statistical package (STATISTICA for windows v 4.3, StatSoft Inc., USA). Von Willebrand factor and fibrinogen were expressed as mean [standard deviation (SD)] and, as sP-sel levels are non-parametrically distributed, they were expressed as median [inter quartile range (IQR)]. Continuous variables were compared between patients and controls using the Student's *t*-test, while nonparametric comparisons were made using the Mann-Whitney test. The paired Wilcoxon sign rank test was used to analyze the difference between results at baseline (pre-PBMVP) and immediately post procedure, and between results at baseline and 24 h post procedure. A value of $p < 0.05$ was considered significant in all statistical analyses.

Results

We studied 16 patients [mean age 59 years (SD 10); 1 male, 15 female] (Table I). All patients were in atrial fibrillation, and had severe mitral stenosis. Two patients had previously suffered a thromboembolic event, while two patients had been smoking regularly prior to their hospital admission. Pre-procedure echocardiography (transthoracic and transoesophageal) showed no left atrial thrombus. All patients had received warfarin therapy which was routinely stopped 2 days prior to the procedure. There were no episodes of thromboembolism following PBMVP.

Mitral Stenosis versus Healthy Controls

Patients with mitral stenosis demonstrated significantly higher mean levels of vWf [148 (SD 24) vs. 102 (SD 37); *t*-

test, $p < 0.001$] and plasma fibrinogen [4.2 (SD 0.8) vs. 3.3 (SD 0.8); $p = 0.003$] at baseline compared with matched healthy controls. There was a nonsignificant trend toward lower median sP-sel levels in patients compared with controls [64 (IQR 47–91) vs. 109 (IQR 46–128) ng/ml; $p = 0.08$]. There was no difference in mean age between patients and controls ($p = 0.34$) (Table I).

Changes in Indices with Valvuloplasty

Venous samples: Following PBMVP, there was a significant increase in median venous sP-sel levels immediately post procedure [pre, 64 (IQR 47–91) vs. post, 87 (IQR 65–102) ng/ml; paired Wilcoxon test, $p = 0.03$] and at 24 h post procedure [pre, 64 (IQR 47–91) vs. 24 h post, 120 (IQR 73–129) ng/ml; $p = 0.011$]. There was also a significant increase in mean plasma vWf levels at 24 h post procedure (pre, 148 ± 24 vs. 24 h post, 161 ± 15 IU/dl; $p = 0.02$), but not immediately after the procedure (149 ± 24 vs. 151 ± 35 IU/dl; $p = 0.6$).

There were no significant changes in plasma fibrinogen levels immediately post procedure (pre, 4.2 ± 0.8 vs. post, 3.9 ± 0.9 g/l; $p = 0.1$) or at 24 h (4.2 ± 0.8 vs. 4.1 ± 1.0 g/l; $p = 0.6$).

Arterial samples: When pre-PBMVP levels were compared with those obtained immediately post procedure, there was a significant increase in arterial sP-sel levels [pre, 60 (IQR 50–76) vs. post, 83 (IQR 60–101) ng/ml; $p = 0.008$], but not vWf levels (pre, 148 ± 28 vs. post, 146 ± 35 IU/dl; $p = 0.9$).

There was no significant change in mean plasma fibrinogen levels post procedure [pre, 4.1 (SD 0.6) vs. post, 4.0 (SD 0.9) g/l; $p = 0.2$].

TABLE I Demography of patients with mitral stenosis and healthy controls[a]

	Mitral stenosis n = 16	Healthy controls n = 16	
Mean age (SD)	58.8 ± 10[a]	59.6 ± 10[a]	p = 0.34
Males:females	1 : 15	1 : 15	
Rhythm			
Sinus rhythm	0	(100) 16	
Atrial fibrillation	(100) 16	0	
Smoking status			
Currently smoking	13 (2)	—	
Nonsmokers	87 (14)	(100) 16	
Warfarin therapy			
Yes (stopped 2 days prior to PBMVP)	(100) 16	0	
Echocardiography data			
Mean mitral valve area [cm (SD)]	1.06 ± 0.4	—	
Left atrial size [cm (SD)]	5.05 ± 0.8	—	

[a] Values are percentage (numbers) unless otherwise specified.
Abbreviations: SD = standard deviation, PBMVP = percutaneous balloon mitral valvuloplasty.

Discussion

In patients with mitral stenosis, systemic thromboembolism can occur in 9–20% without antithrombotic therapy, and these are mostly cerebrovascular (60–75%).[6–8] Indeed, mitral stenosis is a well recognized risk factor for stroke, especially if an enlarged left atrial cavity, AF, and impaired left ventricular function are present.[9] A hypercoagulable state has also been reported in patients with mitral stenosis, even in sinus rhythm.[10] Our observations of increased plasma levels of vWf and fibrinogen levels in patients with mitral stenosis compared with healthy controls, suggesting abnormal endothelial dysfunction and hemorheology/clotting, respectively, are consistent with these observations.[11–14] Nevertheless, levels of sP-sel, an index of platelet activation,[15–17] were not significantly different between patients and controls, but this may be a reflection of chronic antithrombotic therapy usage prior to the procedure.

Thromboembolism, despite adequate anticoagulation, remains a serious complication in patients immediately following PBMVP.[1] In the present study, we have observed a significant increase in sP-sel and vWf levels following PBMVP. The rise in sP-sel level was noted in both venous and arterial samples, supporting the hypothesis that platelet activation occurs after intracardiac and intravascular invasive procedures. The further increase in plasma levels of sP-sel at 24 h post procedure suggests that platelet activation, once initiated, continues to be present during the first 24 h after the procedure. Although we found no significant change in venous or arterial plasma vWf levels immediately post procedure, levels were significantly elevated at 24 h following PBMVP. Indeed, vWf has previously been shown to be elevated in patients in mitral stenosis, as in the present study.[18] The (adverse) changes in sPsel and vWf levels with PBMVP would be consistent with the risk of thromboembolic events in the early period following PBMVP, necessitating continued anticoagulation. In particular, our observations would be consistent with our hypothesis that the PBMVP procedure results in trauma to the endocardium (leading to increased vWf), resulting in interactions with blood constituents (hence, platelet activation and increased sPsel), in the context of flow abnormalities (AF). Thus, the three components of Virchow's triad are fulfilled, increasing the prothrombotic or hypercoagulable state following PBMVP. The changes in the hypercoagulable state may complement clinical variables that have been associated with thromboembolism related to PBMVP, such as a history of previous embolism and atrial thrombi.[1] Nevertheless, careful transesophageal echocardiography would rule out the latter.

In the present study, the elevations in s-Psel and vWf levels were noted despite the standard administration of intravenous heparin immediately prior to the valvuloplasty procedure, but after taking the first baseline samples. These findings are in contrast to the report by Yamamoto *et al.*[19] who found a reduction in some hemostatic markers at 1 week following PBMVP. However, they studied patients who were in both AF and sinus rhythm, and postprocedure blood samples were tak-

en after the patient had been reestablished on antithrombotic therapy.[19] As chronic antithrombotic therapy may alter levels of some hemostatic markers, we have tried to avoid this by being consistent with defining our patient population, stopping anticoagulation in all patients 2 days prior to the procedure, and by taking our last blood sample prior to re-initiation of warfarin therapy.

The present study is limited by its cross-sectional nature when comparing baseline levels with healthy controls, and by the small numbers. The possibility remains that some of the changes seen may be related to the actual procedure and hospitalization, although our patients were elective admissions for PBMVP. We have not attempted to relate indices of hypercoagulability at baseline to underlying clinical variables, also in view of the small numbers, and because the prime objective of the present study was the investigation of the effects of PBMVP on these indices. In addition, we have not related indices of hypercoagulability to hemodynamic data. Indeed, Penny *et al.*[18] have reported that increased vWf in patients with mitral stenosis (as in the present study) can be correlated with pulmonary vascular resistance and pulmonary artery pressure. In our study, levels post-PBMVP would simply be compared with paired samples pre procedure, with patients acting as their own control for the purposes of studying the effects of PBMVP on the various indices of hypercoagulability. In addition, there is limited information on the effects of the various types of contrast media used in left ventriculography on levels of various indices of hypercoagulability, but certain contrast agents may damage endothelial cells and perhaps activate thrombogenesis.[20, 21] The possibility remains that this may have contributed to the rise in sPsel and vWf levels post procedure in our patients. Nevertheless, this limitation would be present in studies similar to ours.[19]

Conclusion

Patients with mitral stenosis were observed to have increased plasma levels of vWf and fibrinogen levels compared with healthy controls, consistent with abnormal endothelial dysfunction and hemorheology/clotting, respectively, in these patients at baseline. Furthermore, the increased levels of sP-sel immediately post procedure and at 24 h, in association with increased venous vWf levels at 24 h after PBMVP, are in keeping with an increase in platelet activation and endothelial dysfunction following PBMVP. These changes may contribute to the increased risk of thromboembolism following PBMVP and suggest the need for adequate antithrombotic therapy following PBMVP.

Acknowledgments

The authors thank Dr. M.F. Shiu and David Antrum for their help in data collection. IG is supported by a nonpromotional research fellowship from Sorin Biomedica Cardio SpA, Saluggia, Italy. The support of the City Hospital NHS Trust Research and Development programme for the Haemostasis, Thrombosis and Vascular Biology Unit is gratefully acknowledged.

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