Perioperative evaluation of primary hemostasis in patients undergoing mitral valve repair

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ABSTRACT

Introduction: No data exist on the prevalence of primary hemostatic defects and acquired von Willebrand disease in mitral valve prolapse with severe regurgitation.
Methods: Primary hemostasis was evaluated by PFA-100, von Willebrand Factor Antigen (vWF:Ag) and Ristocetin cofactor (vWF:RiCof) assays in a prospective observational trial. Sixty-five consecutive patients with mitral regurgitation (study group) or aortic stenosis (control group) who were operated for mitral valve repair or aortic valve replacement were enrolled in the study.
Results: There were no differences in Closure Time in the two groups at all time points. The concentration of plasma vWF: Ag was within normal limits in all patients preoperatively; after surgery, a significant increase was observed in both groups from baseline (199 +/- 144 mcg/dL vs. 295 +/-141 mcg/dL in the study group, p = 0.002; 243 +/- 141 mcg/dLl vs 338 +/- 154 mcg/dL in the control group, p = 0.009).
The ratio of vWF:RiCof to vWF:Ag was slightly decreased preoperatively in both groups (ratio = 0.91) and showed a marked increase in the postoperative period (ratio = 0.22) as, probably, new hemostatically effective large multimeric forms of vWF were released.
Conclusions: Patients who present for surgery with a valvular pathology with high shear stress have some degree of primary hemostasis defect; nevertheless, the potent stimulus of surgery and the correction of the underlying disease allow quick restoration of vWF activity and normalization of PFA-100.

Keywords: *von Willebrand, mitral surgery, aortic surgery, platelect function, cardiac surgery.*

INTRODUCTION

Acquired von Willebrand syndrome in high intracardiac shear stress conditions, such as aortic stenosis (1-8), ventricular septal defect (9) and patent ductus arteriosus (10), has been clearly defined in its epidemiology, pathophysiology and clinical correlations. Similarly, patients with other cardiac defects might occasionally exhibit such abnormality.
Platelet and blood clotting activation has been shown in patients with mitral valve prolapse but no data exist on the prevalence of primary hemostatic defects and acquired von Willebrand disease in mitral valve prolapse with severe regurgitation (11).
The fluid dynamics of a regurgitant valve are, in many ways, similar to the fluid dynamics of a stenotic valve; however, the anatomy of the regurgitant orifice and the

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dynamics of the regurgitant flow are complex and not fully understood. The shape and the direction of the regurgitant jet depends on multiple factors, including the anatomy and orientation of the regurgitant orifice, the driving force across the valve, and the size and compliance of the receiving chamber.
Given these facts, we hypothesized that primary hemostasis defects could be a feature in patients with mitral valve prolapse.
Aim of this study was to evaluate the prevalence of primary hemostasis disorders in patients with severe mitral regurgitation due to prolapse without an history of pathological mucocutaneous bleeding, by means of PFA-100 and to define their pathophysiology and clinical implications.

METHODS

65 consecutive patients undergoing open heart surgery for valve operations were prospectively evaluated; 41 patients underwent mitral valve repair for severe mitral regurgitation (study group), 24 of them underwent aortic valve replacement for severe aortic stenosis (control group). 5 patients were excluded from the analysis for postoperative re-exploration with evident source of surgical bleeding.
Patients were not included if operated on urgency-emergency priority or with a minimally invasive approach or had chronic atrial fibrillation. No patient received warfarin or antiplatelet drugs within 7 days before surgery; patients on intravenous heparin had it stopped at least 6 hours preoperatively. Patients were not considered eligible if the preoperative platelet count was $< 80{,}000 \times 10^9$/l, if they had preoperative PT and/or APTT ratios > 1.2 or if they had a history of hematological or hepatic disease (e.g., active hepatitis or cirrhosis), or chronic renal failure requiring dialysis.
All patients signed an informed consent, according to the Declaration of Helsinki, after approval of the protocol by the Ethics Committee of our Institution.

PFA-100

The PFA-100 provides an ex vivo quantitative measure of platelet function under high shear conditions simulating primary haemostatic events following vascular injury.
The platelet function analyzer (PFA-100, Dade Behring, Inc, Deerfield, Ill.) performs analyses on citrated whole blood samples (Vacutainer tubes, Becton Dickinson; citrate 129 mmol/L; 3.8 %) and measures the time needed for a platelet plug to form after activation of platelets by collagen and adenosin diphosphate (CADP, 50 µg) or collagen and epinephrine (CEPI, 10 µg). The sample is aspirated under constant negative pressure through a 200 µm capillary into a small reservoir, by passing through an 150 µm opening coated with collagen/epinephrine (CEPI) or collagen/ADP (CADP). As blood contacts the collagen-coated membrane, the platelet agonist (epinephrine or ADP) is solubilized, resulting in platelet stimulation. The test terminates when the developed primary hemostatic plug obstructs the opening. The instrument provides the Closure Time (CT) parameter, indicating the time duration of the test: results are expressed in seconds, with a maximum test time of 300 seconds. Any value greater than that is reported as non closure. Our assays were performed with CADP cartridges in order to remove the effect of antiplatelets acting on ADP receptors.
All PFA-100 measurements were performed within 30 minutes of sample collection.

Patients' management

Prior to institution of cardiopulmonary bypass (CPB), patients received intravenous porcine heparin (300 IU/kg of body weight)

and, during CPB, additional doses (5000 IU), if required, to maintain the activated clotting time (ACT) >480 sec. (ACT II, Medtronic, Minneapolis, MN, USA). ACT measurements were carried out 2 minutes after heparin administration and every 20 minutes thereafter. After termination of CPB and surgical hemostasis, heparin was neutralized with protamine sulphate (1:1 ratio). All patients received an intraoperative infusion of tranexamic acid (1g in 20 minutes before skin incision, followed by a continuous infusion of 400 mg/h until completion of surgery) according to our institutional protocol.

At the end of the surgical procedure, patients were transferred to the Intensive care unit (ICU). Postoperative blood loss was collected in a graduated reservoir connected to a closed evacuation system (Argyle, Aqua-seal, Sherwood Medical, Tullamore, Ireland).

Cardiopulmonary bypass circuit

Extracorporeal circulation was instituted in all patients by draining blood by gravity into an open venous reservoir (Avant Reservoir, Dideco, Mirandola, Italy), and by driving it by means of a roller peristaltic pump (Caps, Stöckert Instruments, Munich, Germany) through a heat exchanger integrated with a hollow fiber membrane oxygenator (Avant D903, or Avant D903 Ph.i.s.i.o., Dideco, Mirandola, Italy) and through an arterial filter (D734 MICRO 40, Dideco, Mirandola, Italy) back into the patient at a mean flowrate of 2.4 l/min/m^2. The same custom pack of PVC/silicone tubing (Dideco, Mirandola, Italy) was used for all patients. The circuit was primed with 1500-ml Ringer lactate solution, 100 mL Mannitol 18%, and 5000 IU porcine heparin. Intermittent cold blood cardioplegia (4°C) was infused by means of a heat exchanger (D720 Helios C, Dideco, Mirandola, Italy) and two roller pumps, according to Buckberg's protocol. Mild-to-moderate hypothermia-was employed (mean internal temperature: 31.4°C).

Shed mediastinal blood suction and left heart venting were actively performed with two separated roller pumps. Additionally, blood was aspirated from the operative field with a vacuum suction device (D745, Dideco, Mirandola, Italy), processed in a cell saver (Compact-A, Dideco, Mirandola, Italy), and then reinfused after closure of the chest.

Laboratory assays

Blood samples were collected from a radial arterial catheter by means of a two-syringe technique. The first syringe was used to withdraw 10 ml of blood and then the sample was obtained in the second syringe. Sample time points were as follows:

A) preoperatively (Baseline);
B) before heparin administration (Pre-hep);
C) after removal of the aortic crossclamp (Unclamp);
D) 4 minutes after the administration of protamine (Post-prota);
E) at ICU arrival (ICU-arrival);
F) 4 hours after ICU arrival (ICU-4h);
G) the morning following surgery (ICU-18h).

Blood samples were collected in siliconized Vacutainer tubes (Becton Dickinson, Plymouth, UK) containing tri-sodium citrate (0.19 M); within 1 hour from blood collection, platelet poor plasma was obtained by centrifugation for 20 minutes at 2000 RPM at room temperature.

Plasma aliquots of platelet-poor plasma (0.4 mL) were snap-frozen with methanol and dry ice and finally stored at –80 °C until assayed.

Assay methods

Von Willebrand Factor Antigen (vWF:Ag) was determinated by commercially available ELISA assays (Asserachrom vWF, Di-

agnostica Stago, Asnier sur Seine, France). Ristocetin cofactor activity (vWF:RiCof) was measured by commercially available lyophilized platelet reagents (Dade-Behring, Marburg, Germany) using an automated coagulometer (ACL 9000; Instrumentation Laboratory, Lexington, Massachusset, USA) (12).
All results are the mean of the two determinations and expressed as IU/dL of plasma.
The authors had full access to the data and take responsibility for its integrity. All authors have read and agree with the manuscript as written.

Statistical analyses

Descriptive data are expressed as mean standard deviation or median with interquartile range (IQR) as appropriate according to the skewedness of the observed distribution. Statistical significance of the difference between continuous variables was assessed by using a Student t-test or a Mann-Whitney U-test as appropriate. Comparison of proportions was performed by Chi-Square or Fisher Exact tests as appropriate. All reported p values are two tailed. Differences were considered statistically significant in case p-values were lower than 0.05.
Statistical analyses were performed using the SPSS Statistical Package version 11.5.1 (SPSS Inc, Chicago, USA).

RESULTS

Patients' demographics were similar in the two groups (mitral valve repair/study group vs aortic valve replacement/control group). Platelet count and hematocrit did not differ between the two groups at all time points (*Figure 1 and 2*)
There were no differences in Closure Time in the two groups at all time points (*Figure 3*): the median closure time at baseline was above the normality range (less than 118 sec.) in both groups (146 sec. [106-299, 25th-75th percentile] in the study group vs 183 sec. [128-299, 25th-75th percentile] in the control group, p = 0.22); only three patients in the control group exhibited a "normal" CT preoperatively (12 %) vs 14 patients (38 %) in the study group. Five pa-

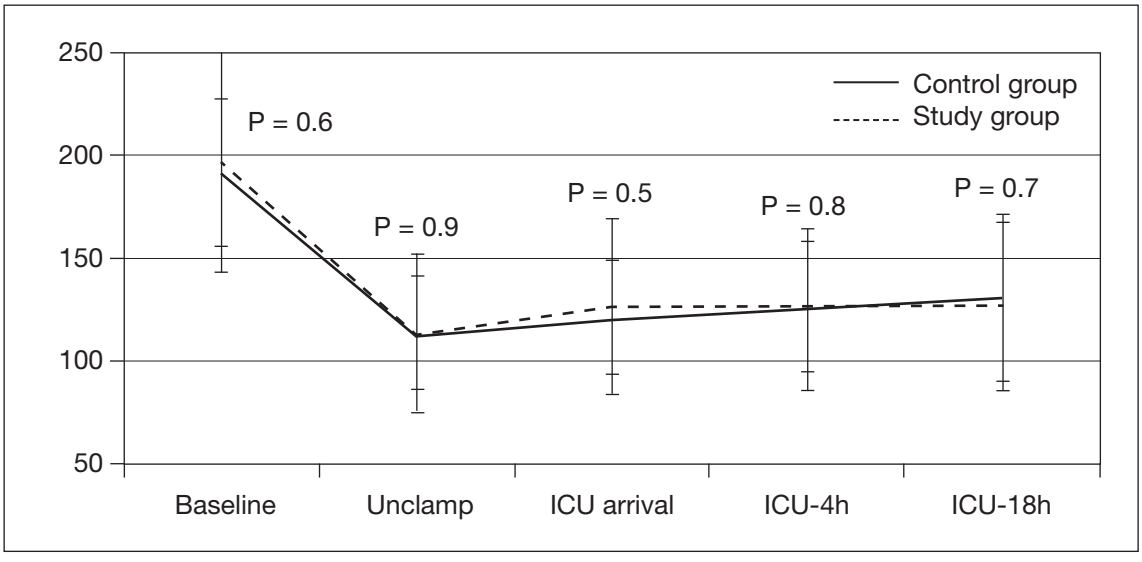

Figure 1 - *Time course of platelet count (t-test) mean ± SD.*

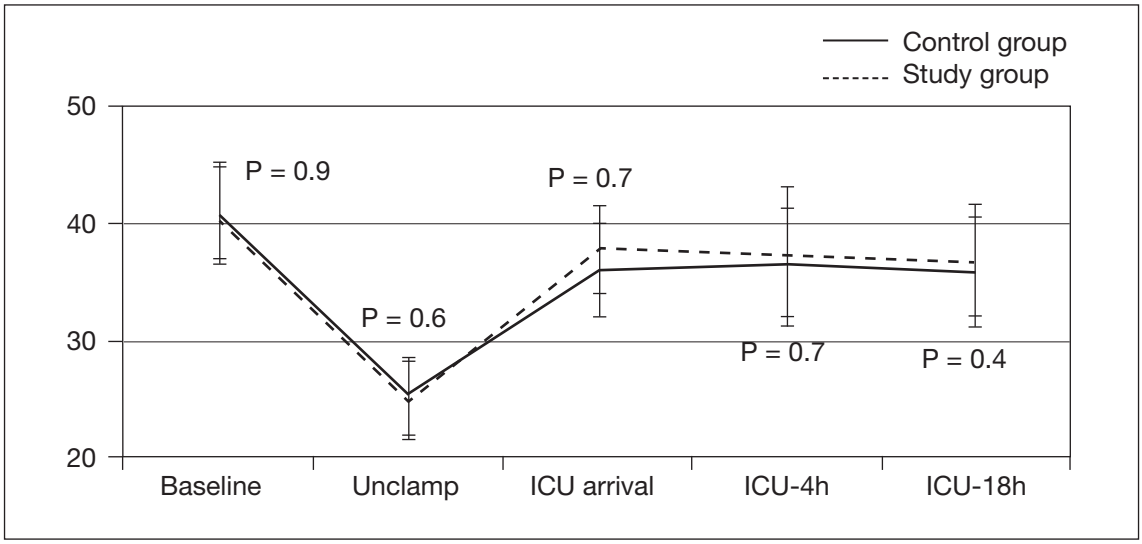

Figure 2 - *Time course of hemostasis: (%) (t-test) mean ± SD.*

tients in the control group had non closure (CT > 300 sec.) preoperatively.
Preoperative CT increased following heparin administration and again with initiation of CPB. Fifteen minutes after protamine sulphate administration, CT returned to near baseline values.
Median (CT) of all patients significantly decreased from baseline at ICU arrival and 18 hours postoperatively (163sec. [109.5-299, 25th-75th percentile] vs 83sec. [66-112, 25th-75th percentile], $p < 0.001$) (*Table 1*); similarly the haematocrit and platelet count decreased. These variables were inversely related to CT at all time points (PLT $p < 0.001$, $r = -0.424$; Hct $p < 0.003$, $r = -0.353$ at ICU arrival (Spearman test).
The concentration of plasma vWF:Ag was

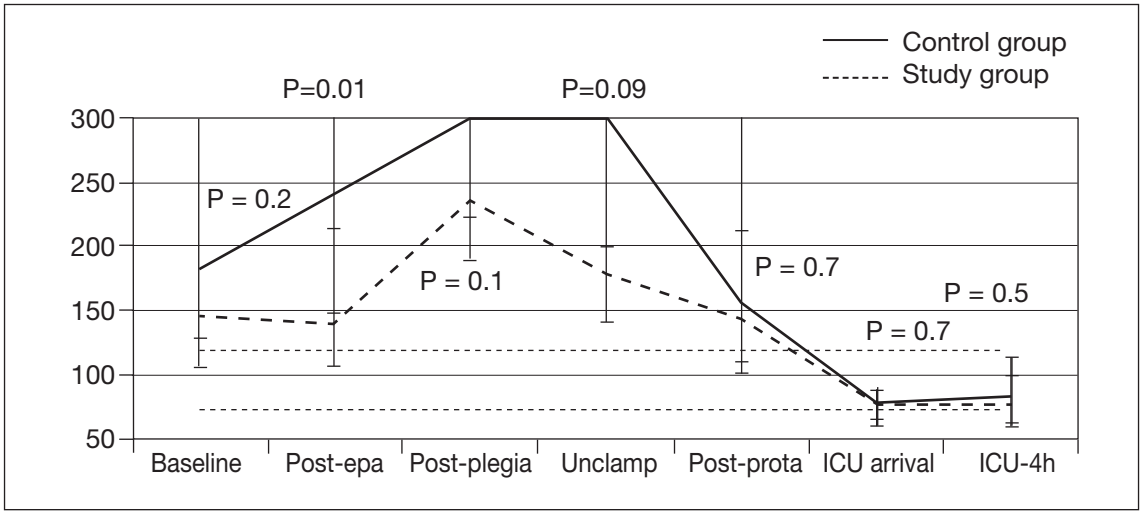

Figure 3 - *Closure Time (Mann-Whitney) median (25th-75th percentile).*

Table 1 - *Time course of Platelet Function Analyzer Closure Time [median (25th – 75th percentile)] in the overall population.*

	25th percentile	median	75th percentile	p
Baseline	109.5	163	299	
Pre-hep	132.75	185	299.75	0.389
Post-hep	120	163	299	0.112
Post-CPL	205.5	293	299	< 0.001
Unclamp	151.5	256.5	300	0.009
Post-prota	106	151	247	0.109
ICU arrival	63.5	78	90	< 0.001
4h after ICU arrival	62	79	102	< 0.001
18h after ICU arrival	66	83	112	< 0.001

within normal limits in all patients preoperatively. (VWF:Ag) levels did not differ at all time points in the two groups; after surgery, a significant increase was observed in both groups from baseline (199 + /-144 mcg/dL vs 295 + /-141 mcg/dL in the study group, p = 0.002; 243 + /-141 mcg/dL vs 338 + /-154 mcg/dL in the control group, p = 0.009) (*Figures 4, 5 and 6*).
The ratio of vWF:RiCof to vWF:Ag was slightly decreased preoperatively in both groups (ratio = 0.91; normal range greater than 0.7) and showed a marked increase in the postoperative period (ratio = 0.22) as, probably, new hemostatically effective large multimeric forms of vWF were released.
The correlations between CT and vWF:Ag and vWF:RiCof could not be calculated because of a number of patients had an infinite CT (> 300 sec.). However, our results indicate that, on the whole, the prolongation of the CT was inversely proportional

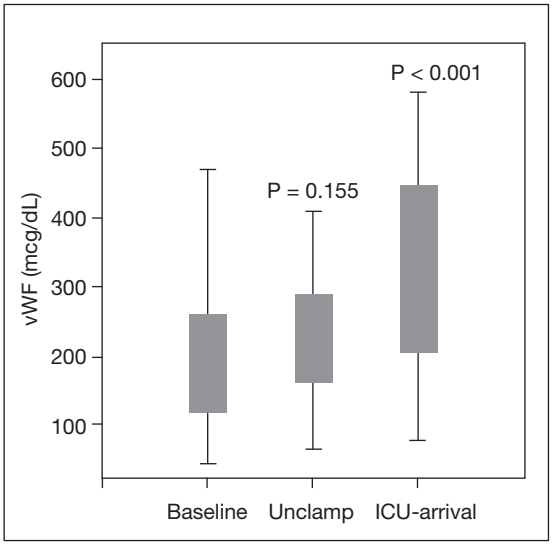

Figure 4 - *von Willebrand Factor Antigen concentration: time course in the overall study population.*

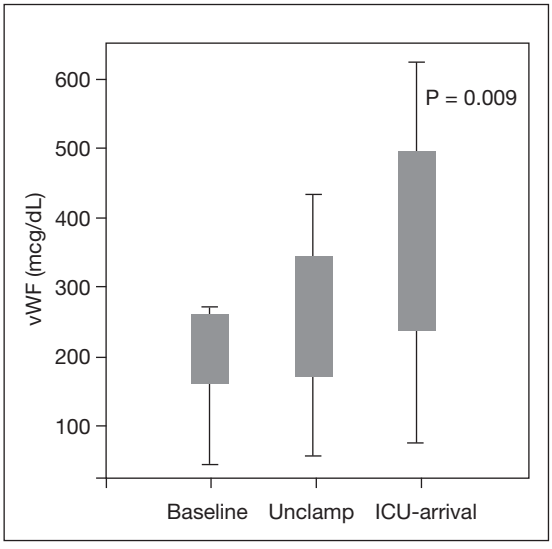

Figure 5 - *von Willebrand Factor Antigen concentration: time course in the control group.*

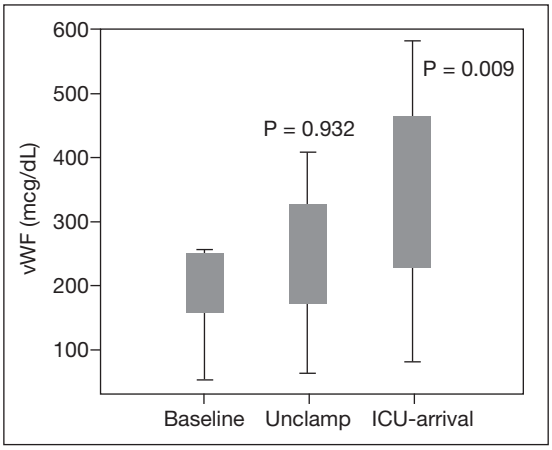

Figure 6 - *von Willebrand Factor Antigen concentration: time course in the study group.*

to the level of vWF:Ag and of vWF:RiCof (*Figure 3*).

Postoperative bleeding did not differ in the two groups (280 ml [220-400, 25th-75th percentile] vs 300 ml [180-400, 25th-75th percentile]) and no significant correlation was detected between nor preoperative or post-protamine CT and postoperative mediastinal drainage. However, among the patients population, five patients had no formation of the platelet plug at baseline (CT > 300 or non-closure): this subgroup showed a statistically significant higher postoperative bleeding as compared to the rest of other patients (350 ml [240-400, 25th-75th percentile] vs 220 ml [180-290, 25th-75th percentile]), $p = 0.05$) which was related to CT at baseline ($r = 0.441$, $p = 0.035$).

No patients suffered of any thromboembolic complication.

DISCUSSION

Patients with high intracardiac shear stress have a certain degree of proteolysis of high molecular weight vWF and such a condition may contribute to the increased risk of postoperative bleeding. PFA-100 has been found to be both sensitive (90-100 %) and specific (88-95 %) in the detection of von Willebrand disease (13-16).

Our study shows that patients with mitral valve regurgitation due to prolapse have high intracardiac shear stress which is responsible for the development of defects of primary hemostasis, which can be detected by means of PFA-100.

Despite these biological alterations, however, these patients do not show any pathological mucocutaneous or surgical bleeding, confirming the discrepancy between the low frequency of bleeding symptoms and the high prevalence of hemostatic abnormalities in this setting. This subclinical defect of primary hemostasis is quickly corrected by valve replacement due to the quick restoration of vWF levels; however it is responsible for a higher postoperative bleeding in those patients with the most severe deficiencies.

The PFA-100, however, is a sensitive, specific and reproducible test which might be useful to screen patients preoperatively to identify those who could benefit from correct diagnosis and appropriate therapy to reduce their complications.

High shear rates prevail in stenotic valves: under these conditions, large molecular weight multimers of vWF are changed in shape and exposed to the action of, which results in their proteolysis. High molecular weight multimers of vWF are the most effective in platelet mediated hemostasis. Platelet adhesion to the subendothelial matrix of injured arterial vessels wall requires adhesion of platelets to subendothelially located vWF and of plasma vWF to subendothelial collagen. In addition, vWF enhances the adhesion of platelets to fibrin clots and stabilizes coagulation factor VIII (17-20).

Although aortic stenosis is a relevant in vivo model of shear stress induced cleavage of vWF with resultant loss of its largest multimers, fluid dynamics of mitral re-

gurgitation are also characterized by high shear stress.

These anomalies measured with PFA-100 were reversed by surgical correction of the regurgitant valve on the first postoperative day, a finding which is consistent with the 12-to-20-hour half-life of vWF in vivo.

The correction of PFA-100 abnormalities after surgical repair or replacement of the diseased valve indicates that the passage of blood through the diseased valve causes a disturbance of flow, thereby generating high shear stress and turbulence, may be responsible, at least in part, for a heightened vWF proteolysis. However, the overall imbalance of primary hemostasis is usually mild and the PFA-100 abnormalities are not an accurate predictor of a bleeding tendency, unless non closure is present (21).

Froom et al. (22) reported their evaluation of vWF in mitral valve prolapse: they conclude that there is a relationship between mitral valve prolapse and low levels of vWF:Ag. However, vWF:Ag levels are normal in those patients with mitral valve prolapse and heart failure, as it may account for an increased release of vWF.

Slaughter et al. (23) have investigated the PFA-100 in CABG patients: they showed that an impaired ADP mediated platelet aggregation is not corrected after surgery in 17 of 19 patients. Moreover, they reported that 80% of patients having CABG with abnormal chest tube output had prolonged PFA-100 measurements 15 minutes after administration of protamine sulphate.

Raman et al. (24) identified 16 bleeders after CABG: in 15 of those whose bleeding was controlled by platelet transfusion, PFA-100 after protamine sulphate was prolonged. They suggested a potential role for PFA-100 measurements to help identify which patient could benefit from platelet transfusion after CABG.

Several limitations of our study should be acknowledged. First, plasma concentration of vWF has large variations, as it is influenced by ABO blood type, Lewis blood type race and age (25).

Moreover, the plasma level of vWF transiently increases in response to a variety of physiological conditions including trauma, surgery, atherosclerosis, inflammatory states and β-adrenergic stimulation.

CONCLUSIONS

In conclusion, mitral regurgitation secondary to prolapse is responsible for a certain degree of proteolysis of high molecular weight vWF as shown by abnormal shear induced platelet aggregation. This defect of primary hemostasis is quickly corrected by valve repair due to the prompt restoration of vWF levels and this condition is not associated with an increased risk of bleeding, except for those patients with the most severe deficiencies.

On the basis of these data, postoperative bleeding after mitral valve repair should prompt a search for other causes than von Willebrand disease.

In addition, our observation confirms the discrepancy between the low frequency of bleeding symptoms and the high prevalence of hemostatic abnormalities in the setting heart valve disease. Although aortic stenosis and mitral regurgitation are a relevant in vivo model of shear-stress induced cleavage of vWF with resultant loss of its largest multimers, the diagnosis of von Willebrand syndrome should be restricted to those patients who have bleeding.

Shear induced platelet aggregation is significantly increased after cardiac surgery, but the role that this phenomenon might play in the development of postoperative thromboembolic complications remains to be addressed.

No conflict of interest acknowledged by the authors.

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