Platelet activation after aortic prosthetic valve surgery

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Abstract

Objective: Platelet activation, which may lead to thrombus formation, is poorly understood after valve surgery when analysed by flow cytometry. It is still unclear whether platelet activation is due to the implanted prosthesis itself or to its aortic or mitral position. The present prospective study was aimed at assessing platelet P-selectin expression, platelet-leukocyte conjugate formation and platelet microparticles in patients undergoing isolated aortic valve replacement, or in patients with either bioprostheses or mechanical valves. Methods: Thirty-three patients were included (15 bioprostheses, 11 bileaflet and 7 tilting disc mechanical valves). Blood samples were analyzed by flow cytometry immediately before surgery, on postoperative day 8 (D8) and 2 months after surgery (M2). Results: At D8, patients with bileaflet valves demonstrated a significant increase in platelet-leukocyte conjugates and in platelet microparticles whereas only platelet-monocyte conjugates were significantly increased in patients with bioprostheses. At M2, platelet activation had returned to the basic level observed prior to surgery in the bileaflet valve group whereas, it was still increased in the bioprosthesis group. Neither at D8 nor at M2 did patients with tilting disc mechanical valves demonstrate any platelet activation. Conclusions: Platelet activation after aortic valve replacement was observed with the use of bioprostheses and bileaflet mechanical valves, but not with tilting disc mechanical valves.

Keywords: Platelets; Flow cytometry; Heart valve; Bioprosthesis; Mechanical valve; Cardiac surgery

1. Introduction

Platelets play a major role in hemostasis and are involved in inflammatory and thromboembolic mechanisms. They can be activated in various circumstances such as blood exposure to biomaterials or by shear stress. Platelet activation involves changes to platelet metabolism, shape, surface receptors, and membrane phospholipid orientation. Activation of platelets results in an increased circulating number of microparticles which are vesicles shed from the platelet membrane. As they contain a high amount of phosphatidylserine (PS) to anchor the prothrombinase complex, both activated platelets and PMPs are procoagulant. A similar increase is observed in the surface expression of platelet CD62P (or P-selectin expression), which is a constituent of the platelet α-granule only expressed upon cell activation. The upregulation of CD62P is followed by the formation of platelet-leukocyte conjugates by binding to leukocytes via the P-selectin Glycoprotein Ligand 1 (PSGL-1) receptor.

Platelet P-selectin expression and platelet-leukocyte conjugates are increased after valve surgery [1–3]. Platelet microparticles are also increased in valvular prosthesis patients presenting a stroke [4]. However, in previous reports, the markers of platelet activation have been generally studied in patients with either aortic or mitral valve replacement, or in patients with either bioprostheses or mechanical valves. In some reports, baseline expression of platelet markers before surgery was not indicated. Hence it is unclear whether platelets are activated in the same way whatever the type of prostheses used and/or to their anatomical location.

The purpose of the present study was to analyse by flow cytometry platelet P-selectin expression, platelet-neutrophil and platelet-monocyte conjugate formation and platelet microparticles in patients undergoing isolated aortic valve replacement using a bioprosthesis or a tilting disc or a bileaflet mechanical valve. The flow cytometry markers were assessed in the three groups, immediately before surgery, before hospital discharge at day 8 and two months after surgery.

2. Materials and methods

Between April and December 2004, one hundred and seventeen patients underwent aortic valve replacement at...
our institution. Among them, 33 patients were recruited because they could be followed in our institution for flow cytometry measurements. Fifteen bioprostheses (5 SJM Epic, 3 Mitroflow and 7 Carpentier-Edwards Perimount) and 18 mechanical valves (7 Omnicarbon and 11 SJM Regent) were implanted, according to the surgeon’s choice. Exclusion criteria were previous cardiac surgery, combined aortic and other valve surgery, infection, diabetes and atrial fibrillation. The protocol was approved by the Hospital Institutional Review Board.

2.1. Blood samples

Venous blood samples were collected using Vacutainer® blood collection tubes with EDTA (Becton Dickinson Biosciences, Le Pont de Claix, France) after discarding the first 3 ml. Blood samples were taken the day before surgery in order to assess the baseline level of platelet markers in all patients (D-1), after surgery and before discharge from the department of surgery (D8) and 2 months after surgery (M2). Patients with either a bioprosthesis or mechanical valve were all on oral anticoagulants at D8 and M2 which were then replaced with an antiplatelet drug in biological valve patients when possible after M2.

2.2. Monoclonal antibodies

The monoclonal antibodies (mAb) were purchased from Becton Dickinson Biosciences (Le Pont de Claix, France). Anti-CD61 conjugated with PerCP and anti-CD42b conjugated with phycoerythrin were used for platelet immunostaining, anti-CD62P conjugated with phycoerythrin identified platelet P-selectin receptor, anti-CD45 conjugated with PE-Cy7* was used for leukocyte immunostaining. Anti-CD11b conjugated with FITC identified αMβ2 CD11b integrin on neutrophils and monocytes (Serotec Inc, Cergy Saint-Christophe, France). Irrelevant isotype-specific mouse control antibodies were used in all panels to identify non-specific fluorescence. All antibodies were used in saturated concentrations.

2.3. Flow cytometry

The protocol for platelet-neutrophil conjugates analysis in whole blood by flow cytometry was modified from Tuttle et al. [5]. Briefly, blood was mixed with PE-Cy7 anti-CD45 mAb, FITC anti-CD11b mAb and PE anti-CD42b mAb. Red blood cells were lysed using ImmunoPrep Reagent System (Beckman Coulter, Villepinte, France). To distinguish leukocytes from platelets, a threshold was set on events that stained positive for PE-Cy7 anti-CD45. Neutrophils or monocytes were then defined and gated by combination of both their Forward Scatter/Side Scatter (FSC/SSC) characteristics as well as the fluorescence intensity of CD11b. The platelet-leukocyte population was then analysed for the co-expression of both markers, i.e. CD11b and CD42b in the population of cells only positive for CD11b. A total number of 11,000 events was acquired per sample.

The protocol for platelet-labeling in whole blood was performed as described by McDonagh et al. [6]. Whole blood was mixed with PerCP anti-CD61 mAb and PE anti-CD62P mAb. Michelson et al. [7] have summarized the advantages of evaluating the platelet function using samples of whole blood, rather than isolated platelets. To distinguish platelets from leukocytes and erythrocytes, a threshold was set to include only the events that stained positive for PerCP anti-CD61. PE fluorescence of the anti-CD62P was used to determine the expression of P-selectin on platelets. The total number of platelets acquired for each blood sample was 50,000.

Table 1: Patient characteristics at the time of surgery between biological and mechanical valve groups

<table>
<thead>
<tr>
<th></th>
<th>Biological valves</th>
<th>Bileaflet valves</th>
<th>Tilting disc valves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years old)</td>
<td>76 ± 2</td>
<td>64 ± 2*</td>
<td>62 ± 2*</td>
</tr>
<tr>
<td>Sex ratio (M/F)</td>
<td>7/8</td>
<td>8/3</td>
<td>5/2</td>
</tr>
<tr>
<td>Preop LVEF (%)</td>
<td>64 ± 4</td>
<td>67 ± 5</td>
<td>66 ± 9</td>
</tr>
<tr>
<td>Indications for operation (S/R/D)</td>
<td>10/2/3</td>
<td>5/2/4</td>
<td>4/-3</td>
</tr>
<tr>
<td>Other surgical procedures (nb)</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>CABG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic root</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>ECC time (min)</td>
<td>113.5 ± 8.5</td>
<td>107.7 ± 15.2</td>
<td>104.8 ± 13.7</td>
</tr>
<tr>
<td>Aortic cross clamp time (min)</td>
<td>90.0 ± 7.5</td>
<td>85.7 ± 11.3</td>
<td>81.3 ± 9.4</td>
</tr>
</tbody>
</table>

Preop LVEF: preoperative left ventricular ejection fraction; S: stenosis; R: regurgitation; D: disease; CABG: coronary artery bypass grafting; ECC: extracorporeal circulation.

* P < 0.02 between biological and mechanical valves.

Table 2: Platelet count (x10¹²/mm³) before and after surgery between biological and mechanical valve groups

<table>
<thead>
<tr>
<th></th>
<th>Biological v.</th>
<th>Bileaflet v.</th>
<th>Tilting disc v.</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-1</td>
<td>233.5 ± 9.7</td>
<td>186.6 ± 25.4</td>
<td>215.2 ± 27.0</td>
</tr>
<tr>
<td>D8</td>
<td>261.5 ± 23.6</td>
<td>309.3 ± 42.5*</td>
<td>250.7 ± 54.6</td>
</tr>
<tr>
<td>M2</td>
<td>284.6 ± 16.0</td>
<td>205.4 ± 30.6</td>
<td>197.6 ± 28.3</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M. D-1: day before surgery; D8: post-operative day 8; M2: 2 months after surgery; v. for valve.
* P < 0.05.

Table 3: Percentage of platelet-neutrophil conjugates before and after surgery between biological and mechanical valve groups

<table>
<thead>
<tr>
<th></th>
<th>Biological v.</th>
<th>Bileaflet v.</th>
<th>Tilting disc v.</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-1</td>
<td>7.3 ± 1.6%</td>
<td>8.8 ± 1.5%</td>
<td>14.6 ± 8.1%</td>
</tr>
<tr>
<td>D8</td>
<td>7.8 ± 1.9%</td>
<td>16.1 ± 2.8%*</td>
<td>9.9 ± 3.0%</td>
</tr>
<tr>
<td>M2</td>
<td>11.4 ± 2.8%</td>
<td>11.1 ± 3.4%</td>
<td>3.9 ± 1.6%</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M. D-1: day before surgery; D8: post-operative day 8; M2: 2 months after surgery; v. for valve.
* P < 0.02 between biological and mechanical valves.
The protocol for platelet microparticles (PMPs) was evaluated in whole blood labeled with PE anti-CD61. Platelet events were determined from other cells by thresholding on positive events for PerCP anti-CD61. Platelet microparticles were then distinguished from platelets by their smaller FSC/SSC specificities. Before acquisition flow count beads (Flow-Set, Beckman Coulter) were added to the samples. A total of 20,000 total events were acquired and the results were expressed as a number of PMPs/100 platelets.

Data acquisition was performed using a Cytomics FC 500 flow cytometer (Beckman Coulter) after performing an instrument quality control procedure. Experimental panels and data analyses were performed using the CXP Software for the Cytomics FC500. From the FACS analysis, the percent positive cells (%Pos) and the mean channel of fluorescence (MCF) were obtained. The results were expressed as total fluorescence intensity (TFI), where TFI = (%Pos/100) × MCF [6].

2.4. Statistical analysis

All results are expressed as mean ± standard error of the mean (S.E.M.). A Mann–Whitney Rank Sum Test was used to compare two different prosthesis or patient characteristics. A Student t-test was used to compare D8 and M2 with D-1. P < 0.05 was considered as statistically significant (SigmaStat 3.0).

3. Results

3.1. Population

Table 1 summarizes the patients' characteristics. No statistical differences, except for patient age, were observed between biological and mechanical valves. Although it was not significant, platelet count was increased throughout the study in patients with bioprostheses. The increase in platelet count at D8 was almost significant in patients with bileaflet valves but not in patients with tilting disc valves (Table 2). Patients with either mechanical valve nearly returned to their baseline count at M2.

3.2. Platelet P-selectin expression

P-selectin expression on platelets increased at D8 and returned to its baseline level at M2 in the bioprosthetic valve group. Platelet P-selectin expression also increased at D8 and significantly decreased at M2 in the bileaflet valve group (TFI 8.8 ± 2.5% vs. 4.3 ± 2.6%, P < 0.05). Conversely, it was almost unchanging after surgery as compared to the baseline level when a tilting disc valve was implanted (Fig. 1).

3.3. Platelet-neutrophil conjugates

In the bioprosthesis group, the percentage of platelet-neutrophil conjugates was comparable to the baseline level at D8 and was increased by 1.5 at M2. In the bileaflet valve group, platelet-neutrophil conjugates significantly increased at D8 as compared to the baseline level (16.1 ± 2.8% vs. 8.8 ± 1.5%, P < 0.02) and then returned to the baseline level. In the tilting disc valve group, platelet-neutrophil conjugates decreased to reach a quarter of the baseline level at M2 (3.9 ± 1.6% vs. 14.6 ± 8.1%, P = NS) (Fig. 2).

At D8, the difference between the bioprosthesis and the bileaflet valve groups was statistically significant (7.8 ± 1.9% vs. 16.1 ± 2.8%, P < 0.02) (Table 3).

3.4. Platelet-monocyte conjugates

In the bioprosthesis group, platelet-monocyte conjugates doubled at D8 as compared to their baseline level (respectively 21.0 ± 6.7% vs. 9.4 ± 1.3%, P < 0.02) and remained increased at M2. In the bileaflet valve group, the percent-

![Fig. 1. Platelet-selectin expression before and after surgery between biological and mechanical valve groups. Results are expressed as a percentage of their baseline level. D-1: day before surgery; D8: postoperative day 8; M2: 2 months after surgery; v. for valve. *P < 0.05 intragroup comparison with D-1.](image1)

![Fig. 2. Percentage of platelet-neutrophil conjugates before and after surgery between biological and mechanical valve groups. Results are expressed as a percentage of their baseline level. D-1: day before surgery; D8: postoperative day 8; M2: 2 months after surgery; v. for valve. **P < 0.02 intragroup comparison with D-1.](image2)
age of platelet-monocyte conjugates was also significantly increased at D8 as compared to its baseline level (23.6 ± 3.9% vs. 12.1 ± 1.9%, P < 0.05). It then decreased near its baseline level at M2. In the tilting disc valve group, platelet-monocyte conjugates remained at their baseline level throughout the period of study (Fig. 3).

At M2, bioprostheses and bileaflet valve groups were significantly different (16.5 ± 1.2% vs. 9.5 ± 4.5%, P < 0.05) (Table 4).

3.5. Platelet microparticles

Platelet microparticles increased throughout the time course in the bioprosthesis group to reach a 1.5-fold increase at M2. In the bileaflet valve group, PMPs increased significantly at D8 (19.5 ± 2.1% vs. 13.0 ± 1.1%, P < 0.02) and returned to the baseline level at M2. Again, platelet microparticle percentage did not vary throughout the time course in the tilting disc valve group (Fig. 4).

4. Discussion

Our results demonstrate that inflammation occurs in patients undergoing aortic valve replacement with bioprostheses and bileaflet mechanical valves. At D8, bioprostheses and mechanical valves are responsible for platelet activation which appears to be more important in the mechanical group. Conversely, no platelet activation was observed throughout the study in patients with tilting disc valves.

Platelet count was increased at D8 with all types of valvular substitutes although it was significant only in the bileaflet valve group. These results, suggesting platelet activation, are consistent with those observed by Wahba et al. [2].

The inflammatory process related to surgery with the aid of cardiopulmonary bypass may not provide an explanation of our results since platelet function studied by flow cytometry returns to its baseline level on the day following surgery [8]. Heparin administration is known to modify platelet P-selectin expression [9]. However, in the present experience, all patients were on oral anticoagulants at D8. At D8, an increase in platelet P-selectin expression was observed, associated with an increase in platelet-leukocyte conjugate and microparticle formation, evidencing platelet activation after aortic valve replacement. Our findings are consistent with the results reported by Wahba et al. [2] demonstrating an increase in platelet-monocyte conjugates after aortic valve surgery. We have demonstrated a significant increase in platelet-monocyte conjugates in homogeneous groups of patients with either bioprostheses or bileaflet mechanical valves whereas Wahba et al. [2] reported on a group in which various types of prostheses were mixed. In the same study, the rate of platelet-neutrophil conjugates was not increased after surgery. Conversely, we did observe an increase in the bileaflet valve group. The present study also demonstrates an increase in platelet microparticle formation in patients with a bileaflet valve.

As concerns the results at M2, a persistent platelet activation process was observed only in patients with a bioprosthesis as shown by the increase in platelet microparticles and platelet-leukocyte conjugates. A significant decrease in platelet P-selectin expression was observed in the bileaflet valve group. Using both types of mechanical valves, the percentage of platelet-neutrophil and platelet-monocyte conjugates was also reduced at M2, although not significantly. In a recent study of a population of 55 patients with mechanical valves and, therefore, receiving oral anticoagulant therapy, platelet P-selectin expression and platelet-neutrophil conjugates were increased when compared to patients receiving oral anticoagulant therapy only [1]. This study was done 60 months after valve surgery and included either aortic or mitral valve replacements, suggesting a persisting inflammatory process late after surgery. Although in our study the last blood sample was taken earlier after surgery, we also observed an increase in the percentage of platelet-neutrophil conjugates in the bioprosthesis group. However, by contrast with the study of Mauger et al. [1], we did not observe a persistent increase in platelet P-selectin expression. As already mentioned, our study was carried out in patients undergoing aortic valve surgery only and the blood samples were not collected at similar intervals after surgery. Furthermore, in Mauger’s study [1] the levels of the different flow cytometry panels were not analysed before surgery, which could not allow to conclude that platelet activation may be observed after valve surgery.
In our study, neither platelet activation, as measured by four different platelet markers nor platelet count, were observed after implantation of a tilting disc valve. Because of the small number of tilting disc valves studied (seven patients) any statistical comparison between tilting disc and bileaflet mechanical valves proved to be irrelevant. During surgery, the mechanical valves were implanted in their optimal orientation, i.e. the large orifice of the tilting disc directed toward the noncoronary cusp and one leaflet of the bileaflet valve facing the right coronary cusp[10]. Laas et al. [11] demonstrated that, in such positions, HITS, reflecting the prosthesis turbulences, were almost physiologic for tilting disc valves and less numerous than those observed with bileaflet valves. Since platelets are in part a component of HITS, a physiological number of HITS in patients with tilting disc mechanical valve suggests strongly that no platelet activation occurs with such valves, as demonstrated by our results. This finding requires confirmation during a longer follow-up with an increased number of patients.

Platelet activation was observed at D8 and M2 in patients with biological valve whereas they were still on oral anticoagulants. These drugs are not known to be responsible for platelet activation. One explanation could be the biological tissue itself. Lehner et al. [12] compared the bio-compatibility of commercially available cardiac bioprostheses either pre-treated or not with autologous endothelial cells in a model of adult baboons. All the valves were explanted for analysis 40 days after implantation. No endothelial cells were detectable on the leaflets surface of the non-endothelialized prostheses. Fibrin deposits and platelet aggregates were observed on the bioprosthesis surface, but not on the pre-treated valves. Those results may explain the platelet activation observed at M2 in our study since platelet adhesion to biomaterials is an important and early step for further platelet activation and aggregation. This finding may argue in favour of an earlier start of antipla-telet drugs such as aspirin after surgery and it needs to be confirmed by a longer follow-up when oral anticoagulants are replaced with aspirin. The lack of platelet activation throughout the study in patients with tilting disc valves suggests a better design when compared with bileaft valves, resulting in less downstream turbulence and a lower thromboembolic rate. Tilting disk valves might be preferred for mechanical aortic valve replacement.

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References


