Perioperative changes in platelet count and function in patients undergoing cardiac surgery

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Abstract
Background: Patients undergoing cardiac surgery are at increased risk of bleeding due to multifactorial coagulopathies. In the present study, we aimed at investigating the changes in platelet count and function during and after surgery as well as determining the association of the platelet dysfunction with bleeding and transfusion requirements in these patients.

Methods: A total of 40 adult patients scheduled for elective valve coronary cardiac surgery were included in this prospective observational study. Changes in platelet count and function with ADP, acid arachidonic, and collagen (light transmission aggregometry) were analyzed at three time points: before CPB, after CPB, and 24 hours after end of surgery. Postoperative bleeding and intraoperative transfusion requirements were recorded.

Results: There were a significant reverse correlation between CPB time and ADP-induced aggregation, particularly after CPB and postoperative AA-induced aggregation. There was not any significant correlation between platelet count and function at all-time points. Both platelet count and platelet aggregation significantly reduced during CPB. While platelet aggregation increased on postoperative Day 1, platelet count reduced by about 40% after CPB, and remained at this level postoperatively. Patients with abnormal ADP-induced aggregation had significant increased postoperative bleeding and transfusion requirements.

Conclusion: The results of this study demonstrate that platelet count and platelet aggregation are reduced during CPB. Our results emphasized the effect of platelet dysfunction on increased postoperative bleeding and transfusion requirements. Perioperative monitoring of platelet function can be considered as a bleeding management strategy for implantation of PBM programs.

Keywords: Platelet Function Tests, Cardiovascular Surgical Procedures, Hemorrhage, Blood Transfusion

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Introduction
Multifactorial coagulopathies including defects in both primary and secondary hemostasis increase the risk of bleeding and transfusion requirements in cardiac surgery with cardiopulmonary bypass (CPB). Platelets, as the main component of the primary hemostatic system, play a key role in the development of blood clotting process. Several factors such as preoperative treatment with antiplatelet agents, the use of CPB, hemodilution, hypothermia, etc. can influence platelet count and function in patients undergoing cardiac surgery (1-4). Dual antiplatelet therapy, containing mostly aspirin and clopidogrel, has traditionally been used for patients with coronary artery disease (CAD) to reduce the risk of thrombotic events. According to the recommendations, antiplatelet drugs should be discontinued between 5 to 10 days prior to the surgery to ensure the recovery of platelet function (5-9). However, some studies have reported the relationship of aspirin withdrawal with myocardial infarction (10, 11). Various studies have evaluated platelet function in monitoring antiplatelet agents or CPB effects to guide hemostatic therapy (12-14). However, most of these studies include cardiac surgeries with low or medium risk of bleeding. Patients undergoing valve- coronary surgery are at increased risk of bleeding due to extensive tissue injury, considerable CPB and operation time, and use of anticoagulant agents. Nonetheless, there is limited information.

What is “already known” in this topic:
Defects in hemostasis system increase the risk of bleeding and transfusion requirements in cardiac surgery with cardiopulmonary bypass (CPB).

→What this article adds:
Platelet count and platelet aggregation are reduced during CPB. Perioperative monitoring of platelet function can be considered as a bleeding management strategy.
Platelet function in cardiac surgery

about perioperative platelet function in these patients.

Patient blood management (PBM) is a multidisciplinary,
evidence-based approach to reduce the need for transfu-
sion and improve patients’ clinical outcomes. The use of
perioperative preventative strategies to minimize bleeding
in surgery is one of the three pillars of the PBM (15-17).
Perioperative monitoring of platelet count and function
can be helpful in better managing bleeding and transfusion
in cardiac surgery. In the present study, we aimed at in-
vestigating perioperative changes in platelet count and
function and determining the association of platelet dys-
function with postoperative bleeding and transfusion re-
quirements in valve-coronary surgery.

Methods
Patients

After obtaining an institutional review board approval,
we enrolled 40 adult patients (20 male (50%), and 20 fe-
male (50%) patients) scheduled for elective valve coro-
nary cardiac surgery at Rajaei cardiovascular medical and
research center in this prospective observational study
between September 2015 and March 2016. Preoperatively,
considering the severity of disease, all patients were into
class II or class III based on ASA (American Society of
Anesthesiologists) and NyHA (New York Heart Associa-
tion) classification (18). Patients continued the routine
prescription with angiotensin converting enzyme, lipid
lowering drugs, beta blocker agents, diuretics, and Ca
channel blocker until the night before operation. All pa-
tients were on aspirin therapy until the operation day,
while clopidogrel was discontinued 5 days prior to the
operation. Exclusion criteria were age younger than 18
yrs., known bleeding disorder, emergency operation, se-
vere liver or renal dysfunction, and intake of antplatelet
drugs other than aspirin or clopidogrel.

Data Collection

According to the surgery routine, blood samples were
obtained before surgery for hemoglobin (Hb) and routine
coaagulation testing. To assess changes in platelet, count,
and function, three time points were selected: after induc-
tion of anesthesia (before CPB), 15 minutes after prota-
mime administration after cessation of CPB (after CPB),
and 24 hours after end of surgery (post operation). Those
values, which were less than the reference value of plate-
let count and function, were considered abnormal. Chest
tube drainage at 24 hours following admission to the in-
tensive care unit (ICU) was recorded as postoperative
bleeding volume. Target Hb for RBC transfusion was
8g/dL. Decision to transfuse with FFP and platelet con-
centrate in patients with ongoing bleeding was made joint-
ly by the anesthetists and the surgeon.

Anaesthesia and Anticoagulation

Patients were kept NPO from the night before operation
for 8 hours. All patients were medicated by intramuscular
promethazine (50 mg) and morphine sulfate (15 mg) be-
fore being transferred to the operating room. Midazolam,
sufentanil, and cisatracurium were used for induction of
anaesthesia. Maintenance of anaesthesia was done by

sevofloran 0.2%, sufentanil, midazolam, and cisatracuri-
um. During anesthesia, the depth of anesthesia was moni-
tored by the routine usage of bispectotial index (BIS) and
nerve stimulator. An initial full dose of heparin (300
IU/kg) was administrated before institution of CPB to
achieve a target ACT>480. Additional doses of heparin
were administrated in cases of ACT<480. After CPB,
heparin was reversed with protamine sulfate to achieve a
target ACT<130. After the surgery, we provided all pa-
tients with patient controlled analgesia (PCA) with mor-
phine (20 mg/24h) to manage the postoperative pain.

Platelet Function Assay

Blood samples were collected in 5-mL tubes containing
0.5 mL 3.2% buffered sodium citrate (0.105M mol/L)
(Becton, Dickinson). Assessment of platelet function was
performed by Light transmission aggregometry (LTA).
The sample tubes were mixed by gentle inversion before
analysis. At first, samples were analyzed for platelet
count. Then, PRP was obtained by the centrifugation for
10 minutes. Speed of centrifugation was determined ac-
cording to the baseline platelet count for PRP containing
200- 400 x 10 9/L. Resultant PRP was separated and trans-
ferred into polypropylene tubes. Platelet-poor plasma was
obtained by the centrifugation of the remaining plasma for
10 minutes at 2499 RCF. Aggregation was measured with
a ChronoLog Aggregometer (ChronoLog 700 model,
USA) after stimulation with 5 µM of adenosine diphos-
phate (ADP), 5 µM arachidonic acid (AA), and 0.5 µg/mL
collagen (COL). All agonists were provided from Hy-
phen-biomed (Neuville sur Oise, France). Aggregation
curves were recorded after 10 minutes and the percentage
(%) of maximal aggregation was reported. According to
the recommendation of Hyphen biomed company, we
calculated and established the reference value with normal
samples for our laboratory. The reference values for
healthy adults were 64-96 % for ADP- induced aggrega-
tion, 73-100% for AA-induced aggregation, and 78-100%
for COL-induced aggregation.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Sta-
tistic 22 for Windows (IBM Inc, Armonk, NY). Kolmogo-
rov–Smirnov test was applied to investigate the normality
assumption of the variables. Nominal data were expressed
as the percentage, and Pearson Chi square test was used to
find the differences between the 2 groups. The results are
presented as mean (±SD). Differences in the scale data
between the 2 groups were assessed using the student’s t
test. Repeated measures ANOVA was used to assess peri-
operative changes in platelet count and function. Correla-
tion between the scales variables was assessed with Pear-
son’s correlation coefficient (r). P-values less than 0.05
were considered statistically significant.

Results

Patient Characteristic

Patient characteristics are presented in Table 1. Baseline
platelet count in all patients was within the reference
value (150-450x10 9/L). There was a significant reverse
The results of perioperative platelet count and function testing are demonstrated in Table 1. Of the total of 160 analyses, platelet count less than 100x10⁹/L were observed in only 2 (1.6%) and 5 (4.2%) patients after CPB and on the first day after surgery, respectively. Platelet count reduced significantly during surgery, especially after CPB (p< 0.001). Overall, platelet count was reduced by 37 (39%) post operation compared with the baseline platelet count.

Time course of platelet aggregation in the ADP test, AA test, and COL test is shown in Fig. 1. Platelet aggregation in response to all agonists was significantly reduced after CPB compared with before CPB (p< .005). Postoperative ADP-induced aggregation significantly increased but did not reach baseline level (p< 0.05). No significant increase was observed in AA- and COL-induced aggregation after operation (p< 0.05).

The relationship between platelet count and function

Platelet count showed a poor correlation to the platelet function for ADP-induced aggregation before CPB (r=−0.31, p= 0.047). No significant correlation was found between platelet count and function for AA- and COL-induced aggregation at all-time points. Preoperative platelet count did not correlate with platelet aggregation at any time points.

Postoperative Blood Loss and Transfusion Requirements

Table 2 demonstrates the percent of patients with abnormal platelet function in response to each agonist at all-time points. Platelet aggregation was below the reference range in more than half of the patients before CPB. The number of patients with abnormal aggregation increased after CPB in all three platelet function tests. We compared the mean of postoperative bleeding volume between patients with normal and abnormal platelet aggregation. The results revealed that patients with abnormal function in ADP-induced aggregation testing had a more significant postoperative bleeding compared to patients with normal function in this test (646±539 mL vs. 158±147 mL, p<0.05). Although the mean of postoperative bleeding volume was higher in patients with abnormal function in AA- and COL- induced aggregation testing compared with patients with normal function in these measurements (584±561 mL vs. 368±400 mL, and 608±528 mL vs. 316±429 mL, respectively), these increases were not statistically significant (p>0.05) (Fig. 2).

Transfusion frequencies are summarized in Table 3. Perioperatively, 23 (57.5%) patients received blood products, 21 (52.5%) received RBC, 12 (30%) FPP, and 8 (20%) platelet concentrate. Figure 3 displays transfusion

**Table 1.** Patients Characteristics and the Results of Perioperative Platelet Count and Function Testing

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(year)</td>
<td>48 (14.1)</td>
</tr>
<tr>
<td>Weight(Kg)</td>
<td>75 (17)</td>
</tr>
<tr>
<td>Hb(g/dl)</td>
<td>12.9 (1.7)</td>
</tr>
<tr>
<td>INR</td>
<td>1.5 (0.5)</td>
</tr>
<tr>
<td>Duration of CPB (min)</td>
<td>106 (33)</td>
</tr>
<tr>
<td>Platelet count (x 10⁹/L)</td>
<td>224 (37)</td>
</tr>
<tr>
<td>Before CPB</td>
<td>196 (36)</td>
</tr>
<tr>
<td>After CPB</td>
<td>134 (29)</td>
</tr>
<tr>
<td>Postoperative</td>
<td>137 (31)</td>
</tr>
</tbody>
</table>

ADP-induced aggregation (%)

| Before CPB | 56 (23) |
| After CPB  | 44 (22) |
| Postoperative | 47 (22) |

AA-induced aggregation (%)

| Before CPB | 54 (37) |
| After CPB  | 38 (32) |
| Postoperative | 39 (27)|

COL-induced aggregation (%)

| Before CPB | 59 (28) |
| After CPB  | 49 (28) |
| Postoperative | 54 (19)|

**Abbreviation:** Hb, Hemoglobin; INR, International normalized ratio; CPB, cardiopulmonary bypass; ADP, Adenosine diphosphate; AA, Acid arachidonic; Col, Collagen

Correlation between CPB time and ADP-induced aggregation, particularly after CPB (r = -0.71, p< 0.001) and postoperative AA-induced aggregation (r = -0.56, p< 0.001). The results of the analyses did not show any significant correlation between CPB time and Col-induced aggregation at any time points. Weight and age were not associated with any of the platelet function tests.

**Fig. 1.** Perioperative changes in platelet aggregation. Platelet aggregation was analyzed by 3 agonists at 3 time points (before CPB, after CPB, and post operation). In all experiments results were expressed as the mean of maximum percentage of aggregation ± SD. The mean of platelet aggregation was significantly reduced after CPB in response to all agonist versus before CPB (p<0.05). Postoperative platelet aggregation significantly increased in ADP-induced test versus before CPB (p<0.05). Postoperative increase in platelet aggregation was not significant in AA- and Col-induced aggregation tests (p>0.05).

Transfusion frequencies are summarized in Table 3. Perioperatively, 23 (57.5%) patients received blood products, 21 (52.5%) received RBC, 12 (30%) FPP, and 8 (20%) platelet concentrate. Figure 3 displays transfusion
Platelet function in cardiac surgery

Fig. 2. Comparison of postoperative bleeding volume between normal and abnormal patients in ADP-, AA-, and COL-induced aggregation testing. Data were expressed as mean ±SD. *P<0.05 was considered as significant.

Table 3. Transfusion Requirements Values are expressed as absolute number (percentage) or mean±SD.

<table>
<thead>
<tr>
<th>Transfusion with</th>
<th>Total number of transfused patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (n)</td>
<td>23 (57.7%)</td>
</tr>
<tr>
<td>Platelet concen. (n)</td>
<td>21 (52.3%)</td>
</tr>
<tr>
<td>FFP (n)</td>
<td>12 (30%)</td>
</tr>
<tr>
<td>The volume of blood products</td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>260±350 ml</td>
</tr>
<tr>
<td>Platelet concen.</td>
<td>350±602 ml</td>
</tr>
<tr>
<td>FFP</td>
<td>30±65 ml</td>
</tr>
</tbody>
</table>

Abbreviation: RBC, Red Blood Cell; FFP, Fresh frozen plasma

Fig. 3. Prevalence of perioperative transfusion in normal or abnormal patients in ADP-, AA-, and COL-induced aggregation testing. Data were expressed as the percentage of patients transfused. *P<0.05 was considered as significant.

Prevalence for patients with normal or abnormal ADP-, AA-, and COL-induced aggregation testing. In patients with abnormal ADP-induced aggregation, transfusion prevalence was 67.9% compared with 33% in patients with normal aggregation (p<0.05). As the figure shows, the differences between groups were not statistically significant in AA- and COL-induced aggregation (65% vs. 45% and 56% vs. 60%, respectively, p>0.05).

Discussion

A variety of studies have evaluated the platelet function in cardiac surgery (19-22). Here, we focused on the perioperative platelet count and function changes in response to ADP, acid arachidonic, and collagen agonists, as well as their relationship with bleeding and transfusion requirements in valve coronary surgery.

In this study, the baseline platelet count was normal in all patients. In contrast to some previous studies reporting that platelet count and function are associated; our results did not show any significant association between platelet count and aggregation in all-time points (23, 24). We found that both platelet count and aggregation reduced significantly during CPB. Although platelet aggregation increased 24 hours after surgery, we did not observe any significant increase in platelet count. In fact, platelet count reduced by about 40% after CPB compared with before CPB and remained at this level 24 hours after surgery. These results confirm those of the previous studies reporting a 50% reduction in platelet count during CPB (24-26). Furthermore, similar to other studies, our results showed that platelet function recovered rapidly one day post operation, but it did not reach baseline level (24, 25, 27, 28). However, a slow recovery of platelet aggregation was observed in children (12, 28).

Our results revealed that CPB affects the ability of platelets for aggregation. It is likely that contact of platelets with CPB circuit induces some changes in the expression of molecules involved in adhesion and aggregation or signaling pathway. Furthermore, this issue may be due to the inhibiting effects of heparin on thrombin as the main activator of platelets during CPB.

Based on the traditional recommendations, antiplatelet drugs should be discontinued between 5 to 10 days prior to high bleeding risk procedures (5, 7, 29). Price et al. reported the recovery of platelet function in 75% of patients with coronary diseases by 5 days after cessation of clopidogrel (30). Furthermore, Manach et al. reported highly individual variable platelet recovery after clopidogrel withdrawal in patients undergoing major vascular surgery (31). In our study, although clopidogrel was discontinued 5 days before surgery, the platelet aggregation was abnormal in more than half of the patients in response to all agonists, especially ADP before CPB. We found that bleeding and transfusion prevalence were higher in patients with abnormal aggregation compared to patients with normal aggregation. However, in contrast to reports of pediatric cardiac surgery, these differences were statically significant only in cases of abnormal ADP-induced aggregation (24). Interestingly, despite the continuation of aspirin until the day of surgery, bleeding and transfusion were not significantly associated with abnormality in AA-induced aggregation. Light transmission aggregometry using ADP agonist is considered as a reference method to evaluate residual platelet reactivity (RPR) in high-risk patients on clopidogrel treatment (32-34). Our results combined with those of the other studies suggested that a 5- day waiting period for surgery after clopidogrel withdrawal is not enough to reach optimal platelet function and it is also associated with risk of bleeding (21).

In conclusion, many studies have been conducted with the aim of optimizing bleeding to reduce the need for transfusion in various types of surgery (35-38). Our results emphasized perioperative changes in platelet function and considerable association between platelet dysfunction and...
increased bleeding, as well as increased transfusion requirements in patients undergoing valve coronary surgery. Because of the critical role of the platelet in hemostasis, we recommend perioperative evaluation of platelet function to manage bleeding in these patients. Perioperative monitoring of platelet function can be considered as a bleeding management strategy for implantation of PBM programs.

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Conflict of Interests
The authors declare that they have no competing interests.

References
Platelet function in cardiac surgery
