Platelet count and function in paediatric cardiac surgery: a prospective observational study

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Background. Platelet deficiency, impaired platelet function, or both increase the risk of bleeding complications. We assessed platelet count and function during and after paediatric cardiac surgery. Secondary aims included the effect of modified ultrafiltration, identification of factors associated with platelet dysfunction, and to assess associations between platelet function and transfusion requirements.

Methods. Fifty-seven patients were included in a prospective observational study. Platelet count and platelet function (multiple-electrode impedance aggregometry) were analysed before and during cardiopulmonary bypass (CPB), after modified ultrafiltration, on arrival at the intensive care unit, and on the first postoperative day. Intraoperative transfusions of blood products were registered.

Results. Both platelet count and platelet aggregation were markedly reduced during surgery with the greatest reduction at the end of CPB. On postoperative day 1, platelet count was still reduced by 50%, while platelet aggregation had returned to—or above—preoperative levels. There were only moderate correlations between platelet count and platelet aggregation. Modified ultrafiltration had no significant influence on platelet count or aggregation. Young age, low weight, and long operation time were associated with poor platelet aggregation during surgery, while young age, low weight, high preoperative haemoglobin levels, and low preoperative platelet count were associated with poor aggregation after operation. Patients with impaired platelet function during CPB had markedly increased intraoperative transfusion requirements.

Conclusions. Platelet count and platelet aggregation are markedly reduced during and immediately after paediatric cardiac surgery, especially in neonates. The recovery in aggregation is faster than that in platelet count. Intraoperative platelet dysfunction is associated with increased transfusion requirements.

Keywords: blood, platelets; complications, haemorrhage; heart, congenital defects; surgery, cardiovascular; transfusion

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Platelet deficiency, impaired platelet function, or both increase the risk of bleeding complications and transfusion requirements in cardiac surgery.1–3 During surgery, a number of different factors influence platelet count and function, including the use of cardiopulmonary bypass (CPB), haemodilution, hypothermia, and preoperative treatment with platelet-inhibiting agents.4–7

Monitoring of perioperative platelet function increases the possibility of identifying clinically important platelet disturbances during and after cardiac surgery and can also help to guide haemostatic therapy.8–10 However, classical methods of assessing platelet function, such as light-transmission aggregometry, are time-consuming and require large sample volumes,9–11 making them less suitable in paediatric patients. New whole blood-based point-of-care instruments evaluate platelet function considerably more rapidly. One of these instruments is multiple-electrode aggregometry (MEA), which measures adhesion and aggregation of platelets to metal wires. The method allows analysis within 10 min after sampling.12 The method has been used previously in adult and paediatric cardiac surgery patients for monitoring platelet-inhibiting agents11 13 and to follow the effects of CPB on platelet function.10 15 16
In the present study, we investigated platelet count and function in paediatric cardiac surgery. Five prospectively defined aims were established: (i) to determine changes in platelet count and platelet function during and after paediatric cardiac surgery; (ii) to determine possible associations between platelet count and platelet function; (iii) to determine how modified ultrafiltration influences platelet count and platelet function; (iv) to identify factors associated with impaired platelet function during and after surgery; and (v) to determine the relation between intraoperative platelet function and transfusion requirements.

Methods

Patients

Fifty-seven patients undergoing paediatric cardiac surgery with CPB were included in a prospective observational study between September 2008 and November 2012. During this period, a total of 839 children underwent open cardiac surgery at our institution. Patients were included when the platelet test device and research assistants were available. Twenty-seven patients were included during 2008–2010 and 30 during 2011–2. The study was approved by the Regional Medical Research Ethics Committee and conducted in accordance with the Declaration of Helsinki. Informed written consent was given by all parents. All patients were operated on and anaesthetized by the same group of surgeons and anaesthetists. Patients with a known bleeding disorder, or severe renal or hepatic disorder, were excluded. Two patients were on acetylsalicylic acid treatment and seven on prostaglandin treatment. The patient characteristics and the types of congenital heart defects are given in Table 1.

Anaesthesia

Midazolam and ketamine were used for induction of anaesthesia. Maintenance of anaesthesia included isoflurane, fentanyl (25–75 \( \mu \text{g} \text{kg}^{-1} \)), midazolam (0.1–0.3 mg kg\(^{-1} \)), and atracurium (0.5–0.7 mg kg\(^{-1} \)), supplemented with propofol if indicated during CPB. The anaesthesia procedure was unaltered during the study period.

Anti-coagulation and reversal

An initial i.v. bolus of unfractionated heparin (Leo Pharma A/S, Ballerup, Denmark), 350 U kg\(^{-1} \) body weight, was administered before CPB cannulation. The level of anti-coagulation was repeatedly controlled during bypass with activated clotting time (ACT) (Hemocron Jr II; ITC, Edison, NY, USA) with kaolin as initiator. Reversal of heparin was achieved with protamine (Leo Pharma A/S), 1 mg per 100 U of the total heparin dose. Additional doses of protamine were administered on clinical indication in combination with excessive post-bypass ACT.

Bypass technique

CPB was conducted with a hard-shell reservoir and a patient size-adapted membrane oxygenator (Terumo, Tokyo, Japan). Target rectal temperature (28–36°C) was decided by the surgeon depending on the type of surgery. The total pump prime volume ranged from 350 to 700 ml, depending on the tubing and the oxygenator. The priming solution consisted of crystalloid fluid and allogenic blood, mannitol (5 ml kg\(^{-1} \)), and 100 ml Tribonat\textsuperscript{20} (Fresenius Kabi AB, Uppsala, Sweden) and heparin. Packed red blood cells (RBC) were added to the prime aiming at a target haematocrit (Hct) of 27–30% during CPB. Forty-nine out of 57 patients (86%) received RBC in the prime. During bypass, heparin was administered whenever ACT was <480 s. Myocardial protection was achieved with cold intermittent blood cardioplegia. Modified ultrafiltration was performed after weaning from CPB with cannulae in place, aiming at an Hct of 35–40%. In children <3 kg, or children planned for complex surgical procedures or elective reoperations, tranexamic acid was administered before initiation of CPB (50 mg kg\(^{-1} \)) and after CPB (30 mg kg\(^{-1} \)); aprotinin was not used in any of the patients.

Transfusions

The decision to transfuse patients with blood products intraoperatively was made jointly by the anaesthetist and surgeon responsible. According to the institutional protocol, RBC should be transfused during CPB when Hct is <25%. After CPB, RBC should be transfused when haemoglobin (Hb) levels are <110 g litre\(^{-1} \) except in children with cyanotic lesions and shunts where the limit is 130 g litre\(^{-1} \). Plasma, platelets, and fibrinogen concentrate were transfused in patients with ongoing bleeding, haemodynamic derangement, or both.

Table 1: Patient characteristics, operative variables, and preoperative laboratory analyses. Mean (so), median (range), or number (%). AS, aortic stenosis; ASD, atrial septal defect; AVSD, atrio-ventricular septal defect; CPB, cardiopulmonary bypass, DORV, double outlet right ventricle; HLHS, hypoplastic left heart syndrome; INR, international normalized ratio; SD, standard deviation; TGA, transposition of the great arteries; VSD, ventricular septal defect.

| Age (months) | 5 (0.1–90) |
| Weight (kg) | 5.8 (2.4–23) |
| Girls [n (%)] | 24 (42%) |
| Diagnosis | |
| ASD | 1 |
| VSD | 13 |
| AVSD | 11 |
| Tetralogy of Fallot | 8 |
| TGA | 3 |
| AS | 3 |
| HLHS, DORV, hypoplastic aortic arc | 7 |
| Truncus arteriosus | 2 |
| Others | 9 |
| CPB time (min) | 124 (69) |
| Aortic clamp time (min) | 67 (48) |
| Hb (g litre\(^{-1} \)) | 130 (22) |
| Prothrombin time (INR) | 1.2 (0.2) |
and signs of impaired haemostasis analysed with thromboplastometry and activated coagulation times as previously described. Transfusions were not guided by thromboplastometry in the intensive care unit (ICU). Data from the platelet aggregometry analyses were not available to the physicians responsible for transfusions.

Study protocol
Platelet count, platelet aggregometry, and Hct were analysed in all patients at five pre-set time points: after induction of anaesthesia, at the end of CPB (after rewarming), after modified ultrafiltration (after weaning from bypass but before protamine administration), on arrival at the ICU after surgery, and on the first postoperative day. Impaired platelet function during CPB and on arrival at the ICU was defined as adenosine diphosphate (ADP)-initiated aggregation ≤ 30 U. This level has been used previously to identify patients with an increased risk of bleeding in adult cardiac surgery.

Analyses
Hb was analysed with routine methods before surgery, on arrival at the ICU after surgery, and on the first postoperative morning. Prothrombin time was analysed before surgery with standard clinical methods. All samples were collected from an arterial line, except the sample for prothrombin time, which was collected from a venous line. An accredited university hospital laboratory performed all the analyses except platelet aggregometry.

For aggregometry, whole blood samples were collected in heparinized tubes (Vaccuette LH Lithium Heparin; Greiner Bio-One, Kremsmünster, Austria). Platelet aggregation was analysed by MEA (Multiplate®; Roche Diagnostics, Basel, Switzerland) as described previously. Briefly, the device has five test cells for parallel testing, and each test cell has two independent sensor wires to reduce systemic errors. Each unit consists of two silver-coated, highly conductive copper wires. The analysis is based on platelet adhesion, which results in platelet aggregation onto the metal sensor wires in the test cell, resulting in increased electrical impedance between the wires. All results were included in the analysis independently on platelet count. According to the manufacturer, a platelet count above 100 × 10^9 litre^-1 gives valid results. The analysis is performed in the test cell with 300 μl saline pre-heated to 37°C and 300 μl of heparin-anticoagulated whole blood. The test kits used were ADP test kit (ADP final concentration 6.5 μmol litre^-1) for the detection of P2Y_{12}-dependent aggregation, ASPI test kit (final concentration of arachidonic acid (AA): 0.5 mmol litre^-1) to assess cyclooxygenase-dependent platelet aggregation, and TRAP test kit (final concentration of thrombin receptor-activating peptide (TRAP)-6: 32 μmol litre^-1), which detects PAR-1 receptor-dependent platelet aggregation. The change in impedance in the test cell is measured over 6 min and expressed as a graph where the area under the curve is a quantification of platelet aggregation, reported in arbitrary aggregation units (U). The manufacturer’s reference values for healthy adults using heparin tubes are 55–117 U for ADP-induced aggregation, 79–141 U for AA-induced aggregation, and 87–147 U for TRAP-induced aggregation. No reference values have been established for heparin tubes in neonates and children.

Statistical analysis
The results are presented as mean and standard deviation (sd), or median and range. Any P-value of < 0.05 was considered statistically significant. The paired t-test was used to compare continuous variables before and after ultrafiltration. In group comparisons, Student’s t-test was used to compare normally distributed continuous variables, the Mann–Whitney test to compare non-normally distributed continuous variables, and categorical variables were compared with the χ² test. Normality of data was tested with the Kolmogorov–Smirnov test. Correlation was assessed with Pearson’s test. Because of the exploratory nature of the study, no power calculation was performed. Statistical analysis was carried out using SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results
Clinical course
All children completed the study protocol. There was no perioperative mortality.

Baseline variables
The patient characteristics are presented in Table 1. Twenty children weighed <5 kg and 27 had CPB time of more than 120 min. Baseline platelet count, Hct, and platelet aggregometry variables are given in Table 2. There were weak inverse correlations between preoperative age and weight, and platelet count (r = −0.35, P = 0.009 and r = −0.31, P = 0.020, respectively) and between preoperative age and weight, and preoperative AA-induced platelet aggregation (r = −0.29, P = 0.031 and r = −0.31, P = 0.021, respectively). Age and weight did not correlate with ADP- and TRAP-induced aggregation.

Platelet count and function
Platelet count and all aggregation tests were significantly reduced during surgery in comparison with preoperative levels, with the greatest reduction at the end of CPB (Table 2 and Fig. 1). The reduction was largest in ADP-induced aggregation (72 (28%) (mean and sd)] followed by platelet count [−56 (17%)]. Immediately after ultrafiltration, platelet count was reduced by 53 (19%), while the reduction in ADP-, AA-, and TRAP-induced aggregation was less pronounced (−38, −21, and −20%, respectively) compared with baseline. On postoperative day 1, platelet count was reduced by 47 (30%), while platelet aggregation had returned to or was above preoperative levels (Table 2 and Fig. 1).

In 20 of the 286 analyses (7.0%), platelet count was <100 × 10^9 litre^-1 (none before surgery, eight during CPB, five after ultrafiltration, three at arrival to ICU, and four on postoperative day 1).
Relationship between platelet count and platelet function

There were moderate statistically significant correlations between platelet count and platelet aggregation at all time points, except for TRAP-induced aggregability before operation (Table 3). The best correlation was achieved during CPB (ADP: $r = 0.54$; AA: $r = 0.65$; TRAP: $r = 0.55$; all $P < 0.001$) (Supplementary Fig. S1).

Influence of modified ultrafiltration on platelet count and platelet function

Ultrafiltration increased Hct from 28% to 36% ($P < 0.001$) but had no significant influence on platelet count or ADP- and TRAP-induced aggregation (Table 2). AA-induced aggregation was marginally improved by ultrafiltration [from 34 (25) to 40 (28) U; $P = 0.038$] (Table 2). There were highly significant correlations between the measurements before and after ultrafiltration for platelet count ($r = 0.67$), and ADP-induced ($r = 0.79$), AA-induced ($r = 0.74$), and TRAP-induced aggregation ($r = 0.85$) (all $P < 0.001$). The mean absolute difference for platelet count was $3 \times 10^9$ litre$^{-1}$ (95% CI $-8$ to 13), for ADP-induced aggregation, it was 2 ($-2$ to 5) U, for AA-induced aggregation, it was 5 ($0$–$11$) U, and for TRAP-induced aggregation, it was 4 ($-1$ to 9) U.

Factors associated with impaired platelet function

The following variables were univariately associated with impaired platelet function during CPB: age ($P < 0.001$), weight ($P = 0.001$), and aortic clamp time ($P = 0.013$) (Supplementary Table S1). On arrival at the ICU, age ($P = 0.023$), weight ($P = 0.032$), preoperative Hb ($P = 0.001$), and preoperative
platelet count ($P=0.035$) were associated with platelet dysfunction (Supplementary Table S2).

**Relationship between platelet function, transfusion requirements, and postoperative blood loss**

Intraoperatively, 27 of 57 patients (47%) received transfusions of blood products, 18 (32%) with RBC concentrate, nine (16%) with platelets, and 17 (30%) with fibrinogen concentrate. None of the patients received plasma transfusion intraoperatively. Twenty-three of 27 patients received intraoperative transfusions because of bleeding. After operation (after arrival to ICU), 23 patients (40%) were transfused, 22 (39%) with RBC, and two (4%) with platelets. No patients received plasma or fibrinogen after operation.

Impaired intraoperative platelet function, as measured with impedance aggregometry during CPB, was highly associated with the prevalence of intraoperative transfusion (before arrival to the ICU). Figure 2A shows the transfusion prevalence for patients with none, one, two, or three of the ADP-, AA-, and TRAP-induced aggregation measurements of ≤30 U. In patients with all three measurements at ≤30 U, the transfusion prevalence was 81%, when compared with 31% in patients with all measurements at >30 U ($P=0.006$) and 33% in patients with one measurement at ≤30 U ($P=0.004$). Figure 2A–D shows the transfusion prevalence in patients with ADP-, AA-, and TRAP-induced aggregation measurements of ≤30 or >30 U. The differences were statistically significant in AA- and TRAP-induced aggregation ($P=0.012$ and 0.002, respectively) but not in ADP-induced aggregation ($P=0.19$).

**Table 3** Correlation coefficients according to Pearson’s test between platelet count and platelet aggregation before, during and after surgery. AA, arachidonic acid; ADP, adenosine diphosphate; CPB, cardio pulmonary bypass; ICU, intensive care unit; TRAP, thrombin receptor-activating peptide.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

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<th>After ultrafiltration</th>
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**Fig 2** (A) Prevalence of intraoperative transfusions for patients with none, one, two, or three of the ADP-, AA-, and TRAP-induced aggregation measurements ≤30 U. (B–D) Prevalence of intraoperative transfusions in patients with ADP-induced (A), AA-induced (C), or TRAP-induced (D) aggregation < or > 30 U. ADP, adenosine diphosphate; AA, arachidonic acid; TRAP, thrombin receptor-activating peptide.
Platelet function during CPB was not significantly associated with postoperative transfusions (Supplementary Fig. S2).

The median postoperative bleeding (chest drain volume) to the first postoperative morning was 13.8 ml kg$^{-1}$ (range 4.1–50). There were no significant correlations between ADP-, AA-, and TRAP-induced aggregation at any time point with postoperative chest drain volume.

**Discussion**

In the present study, significant reductions in platelet count and aggregation were observed intraoperatively, but while platelet aggregation was restored after 24 h, platelet count remained reduced by ~50% compared with the count before operation. The low platelet count during and after surgery confirm previous observations in paediatric cardiac surgery. In contrast, studies of perioperative platelet function have given conflicting results. Guay and colleagues and Ranucci and colleagues reported increased platelet reactivity, whereas Hofer and colleagues reported reduced function during and after paediatric cardiac surgery. The diverging results may be consequences of the multifaceted paediatric cardiac surgery in patients with immature coagulation systems, of complex surgical procedures, and of the range of patients (cyanotic–acyanotic, neonates, older children, etc.) but may also be related to differences in study design and analysis. Increased aggregation despite low platelet count may be caused by the activation of platelets via the systemic inflammatory response, as previously suggested. It has also been suggested that platelet turnover is increased during surgery and that newly produced platelets may have better aggregation capacity. One may speculate that the restored platelet aggregation early after surgery may be followed by a hyper-reactive phase that increases the risk of thrombotic complications, especially in children with intra- and extra-cardiac shunts and implanted mechanical assist devices. It may therefore be important to follow platelet aggregation during the postoperative period and, if necessary, consider treatment with anti-platelet agents. The only moderate correlations between platelet count and aggregation found in the present study (Table 3) lend further support to the idea that measurements of platelet count alone are insufficient for the estimation of platelet function during and after paediatric cardiac surgery.

In the present study, modified ultrafiltration did not influence platelet count and ADP- and TRAP-induced platelet aggregation. Ultrafiltration has previously been shown not to significantly affect viscoelastic tests (thromboelastography and thromboelastometry) for the assessment of intraoperative coagulation. Monitoring of platelet count, platelet function, and coagulation could therefore be performed at the end of CPB instead of waiting until after weaning from bypass. This approach might accelerate the diagnosis of platelet dysfunction and coagulation disturbances and improve tailored treatment.

Low weight, young age, and long aortic clamp time were the most prominent factors associated with impaired platelet function during CPB (Supplementary Table S1). Interestingly, the same factors have previously been identified as predictors of bleeding and transfusion requirement in paediatric cardiac surgery. This underscores the importance of platelet function for perioperative haemostasis. On arrival at the ICU, low weight and young age were still important factors for platelet dysfunction, but low preoperative platelet count and high Hb levels also emerged as being important (Supplementary Table S2). The observation of high Hb is not surprising, as previous studies have found that cyanotic patients are more prone to bleeding and coagulation disturbances than acyanotic patients during and after surgery.

Impaired intraoperative platelet function, as measured with impedance aggregometry during CPB, was significantly associated with the intraoperative transfusion prevalence (Fig. 2). As aggregometry results were not available for the physicians who prescribed transfusions, this would indicate that clinical observations and analysis of platelet count can identify the majority of patients with impaired intraoperative platelet function. It is, however, possible that routine perioperative platelet aggregometry would improve our ability to identify patients with clinically significant platelet dysfunction, and consequently help tailor specific transfusion therapy, but this requires further studies to be fully elucidated.

The present study had important limitations. Platelet function was only measured with MEA, which is still a new method, especially regarding paediatric patients. The restricted number of patients limited subgroup analysis. The observational study design was also a limitation, and the true value of platelet function monitoring in paediatric surgery should be confirmed in randomized studies. There is no commonly accepted definition of perioperative platelet dysfunction. The definition we used in the present study (<30 U in ADP-induced aggregation) originates from a study in adult coronary artery bypass graft patients treated with P2Y$_{12}$-inhibitors. It is possible that other definitions would have yielded different results. The present results indicate that TRAP-induced aggregation <30 U (Fig. 2a) or a combination of ADP-, AA-, and TRAP-induced aggregation (Fig. 2a) might be a better marker of clinically relevant platelet dysfunction in paediatric cardiac surgery. Furthermore, 7% of the analyses were performed when the platelet count was <100 × 10$^9$ litre$^{-1}$. It could be argued that these analyses should have been excluded. However, the results reflect the total aggregability in these samples and we decided thus to include the results. In addition, the statistical analyses did not change significantly when these results were excluded. Another limitation is that the patients were collected over a long period of time. The long inclusion period and the low inclusion rate increase markedly the risk for bias because of selection, recruitment, and change of practice.

The risk would have been greater if different groups of patients or different treatment protocols would have been compared. In the present study, no such comparisons were made. Instead, we performed serial measurements of platelet count and function at different time points during the operation and present the results in relation to the baseline measurement or to a previous measurement (e.g. before and after ultrafiltration). With this study design, every patient serves as its own control which reduces the risk for bias.
In conclusion, there were substantial reductions both in platelet count and in platelet function during and immediately after paediatric cardiac surgery. Intraoperative platelet dysfunction was associated with increased transfusion requirements. Low age, low weight, and long operation time were associated with impaired intraoperative platelet dysfunction. Ultrafiltration did not affect platelet count or function. Platelet function, but not platelet count, recovered during the first 24 h after surgery.

Supplementary material
Supplementary material is available at British Journal of Anaesthesia online.

Authors’ contributions
B.S.R.: study design, recruitment of patients, data analysis, and writing of the paper. F.S.: recruitment of patients and revision of the manuscript. H.W.: study design and revision of the manuscript. B.N.: study design and revision of the manuscript. F.B.: analysis and interpretation of data and revision of the manuscript. A.J.: analysis and interpretation of data and revision of the manuscript. All authors have approved the final version of the paper.

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Declaration of interest
None declared.

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