Point-of-care assessment of platelet aggregation in paediatric open heart surgery

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Editor’s key points

- Perioperative platelet dysfunction is common in paediatric open heart surgery.
- Changes in platelet aggregation were followed in 50 children undergoing cardiopulmonary bypass.
- Platelet aggregation was reduced before operation in acyanotic but not cyanotic subjects, and decreased during surgery with slow recovery in both.
- Platelet aggregation did not correlate with blood loss, and so does not appear to be a good predictor.

Background. Congenital heart disease (CHD) is associated with complex coagulation abnormalities. Platelet aggregability has not been investigated in detail in children with acyanotic and cyanotic malformations undergoing open heart surgery. The method of whole-blood multiple electrode aggregometry (MEA) appears suitable for rapid platelet analysis in children, for example, because of small sample volumes. We investigated perioperative evolution of platelet aggregation by means of MEA in children with CHD.

Methods. Fifty children with acyanotic or cyanotic malformations were included in a prospective observational study. Laboratory testing was assessed before anaesthesia, and during and after surgery until the fifth postoperative day. MEA was performed in hirudin-anticoagulated blood using adenosine diphosphate (ADP), arachidonic acid, and thrombin receptor-activating peptide for platelet activation. Surgical variables, bleeding volumes, and transfusion requirements were documented during hospital stay.

Results. Mean platelet count was within the normal range in all patients with no intergroup differences. Before surgery, aggregation to all agonists was within the age-adjusted normal range in cyanotic children and was statistically significantly higher compared with acyanotic children. Platelet aggregation decreased significantly during surgery in both groups followed by a slow recovery not reaching baseline levels. Bleeding and platelet transfusions were higher in the cyanotic group. Transfusion requirements correlated with ADP-induced platelet aggregation.

Conclusions. These results indicate higher blood loss, despite better platelet aggregation in cyanotic patients compared with acyanotic patients. MEA alone might not be suitable for predicting increased perioperative blood loss.

Keywords: anaesthesia; blood coagulation; cardiac surgical procedures; heart defects, congenital; infant; platelet function tests

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Congenital heart disease (CHD) is associated with complex coagulation abnormalities, including platelet dysfunction. While some studies indicate a reduction in platelet count and function, others report platelet hyperactivity in CHD. Platelet aggregability has not yet been systematically investigated in children with cyanotic and acyanotic CHD.

Cardiac surgery with cardiopulmonary bypass (CPB) affects platelets, and the resulting platelet exhaustion is considered to be an important risk factor for early postoperative blood loss and transfusion requirements. Bleeding, in turn, increases morbidity and mortality. Since allogeic blood products and specifically platelet concentrate transfusions have a well-recognized infection risk, prediction and prevention of transfusions by perioperative monitoring of platelet-dependent parameters could prove useful for management of haemostasis. Accordingly, the aim of our study was to compare preoperative platelet aggregation in paediatric patients with cyanotic or acyanotic CHD, and to describe the intra- and postoperative course of platelet function.

Light transmission aggregometry in platelet-rich plasma has several limitations in paediatric surgical patients, such as the need for large sample volumes, long turn-around times, and poor standardization. Multiple electrode aggregometry (MEA) is a relatively new method which measures electrical impedance changes in whole blood induced by platelet adhesion and aggregation onto metal wires. MEA has been proven sensitive for platelet-inhibiting agents and the effects of extracorporeal circulation during CPB.
i.v. continuous infusion of 10 ml kg⁻¹ given when the haematocrit was 
the anaesthetist. Red blood cell (RBC) transfusions were 
ing to the haemodynamic requirements at the discretion of 
Ringer’s solution and 6% hydroxyethyl starch 130/0.4 accord-
coronary stents. 22 Small sample volumes, point-of-care 
anaesthesia. A bolus of 400 units kg⁻¹ and sevoflurane and sufentanil were for maintenance of 
procedures. Midazolam, cisatracurium and thiopental, or 
all patients according to the hospital’s standard operating 

Subjects
After obtaining institutional review board approval and 
written informed consent from the parents, 50 consecutive 
children < 7 yr of age undergoing repair of cardiac malfor-
mations with CPB at the General Hospital Linz were included 
in a prospective non-interventional observational study. 
Exclusion criteria were hereditary bleeding disorders. 

Patients were divided according to the direction of the 
shunt into a cyanotic group with a haemodynamically signifi-
cant right-to-left shunt resulting in an arterial oxygen satur-
ation under 90% (tetralogy of Fallot, transposition of the 
great arteries, hypoplastic left heart syndrome, double 
outlet right ventricle, pulmonary atresia) and an acyanotic 
group with a left-to-right shunt (ventricular septal defect, 
atrial septal defect, truncus arteriosus communis, atrial ven-
tricular septal defect, or after correction of right-to-left shunting as first repair of malformation).

After performance of this study, reference values have 
been established for healthy infants, children, and adoles-
cents. 23 Accordingly, subjects were respectively divided into 
three age groups: ≤ 12 months, 1.1–4, and 4.1–7 yr. 

Anaesthesia and surgery
The operative and anaesthetic management was similar in 
all patients according to the hospital’s standard operating 
procedures. Midazolam, cisatracurium and thiopental, or 
S-ketamine (in patients with Fallot) was used for induction, and sevoflurane and sufentanil were for maintenance of 
anasthesia. A bolus of 400 units kg⁻¹ unfractionated heparin was administered before institution of CPB. 
Additionl doses of heparin were given on CPB to maintain 
an activated clotting time above 480 s. After CPB, heparin 
was neutralized with protamine sulphate, 1 mg protamine 
per 100 units of total heparin dose. The standard non-
pulsatile CPB circuit with a membrane oxygenator and a 
roller pump was primed with human albumin 20% and crys-
talloids and applied with moderate hypothermia of 34–35°C. 
Weaning off CPB was performed after rewarming to 36°C 
using dobutamine, nitroglycerin, and milrinone (in single 
ventricle lesions). Patients received an i.v. bolus injection of 
10 mg kg⁻¹ tranexamic acid before surgery followed by an 
i.v. continuous infusion of 10 ml kg⁻¹ h⁻¹ until the end of 
surgery. Infusion therapy was performed with lactated 
Ringer’s solution and 6% hydroxyethyl starch 130/0.4 accord-
ing to the haemodynamic requirements at the discretion of 
the anaesthetist. Red blood cell (RBC) transfusions were 
given when the haematocrit was < 25% while on CPB and 
< 30% after CPB (35% in biventricular lesions). In the case 
of diffuse bleeding after weaning from CPB, despite neutral-
ization of heparin and completion of surgical haemostasis, 
platelets were transfused as a first-line therapy, especially 
in neonates with low platelet counts before CPB. The ration-
ale for transfusing platelets is the proposed platelet con-
sumption during extracorporeal circulation. 24 After surgery, 
platelet count below 100 g litre⁻¹ was the trigger for platelet transfusion in the case of clinical bleeding. Fresh-frozen 
plasma (FFP) was transfused in the case of persisting bleed-
ning and prothrombin time > 40% above control. Frequency 
and volume of transfused RBC, FFP, and platelet concentrates 
and also blood loss during the hospital stay were recorded.

After surgery, activated partial thromboplastin time was 
maintained at 50–60 s using unfractionated heparin. Aca-
notic patients more than 14 yr old received enoxaparin for 
thrombosis prophylaxis once daily starting at the first post-
operative day.

Sample collection
Measurement time points were after induction of anaesthe-
sia but before surgery, 15 min after start of CPB, at the end of 
surgery, at arrival to the intensive care unit, on the first post-
operative day, and on the fifth postoperative day. Blood was 
drawn from an indwelling arterial catheter after discarding 
the first 2.6 ml. Blood was collected into commercially avail-
able 1.4 ml Monovette™ collection tubes (Sarstedt, Numb-
rech, Germany) containing 1.2 mg ml⁻¹ EDTA for 
whole-blood count (Sysmex XE-2100, Roche Diagnostics, 
Mannheim, Germany) and 2.6 ml Monovette™ collection 
tubes containing 25 µg ml⁻¹ hirudin for MEA analyses. All 
tests were run at the hospital central laboratory 0.5–2 h 
after blood sampling. The results of the platelet function 
analyses were not provided to the anaesthetists, surgeons, 
or intensivists in charge.

Platelet aggregometry was performed using the impe-
dance aggregometer Multiplate® (Dynabyte, Munich, 
Germany), an instrument with five channels for parallel 
determinations. Three hundred microlitres of saline and 
300 µl of hirudinized blood were pipetted into disposable car-
tridges containing a magnetic stirrer and two independent 
dual-sensor units of silver-covered electrodes. After incubat-
ing blood samples for 3 min at 37°C, the following agonists 
were added (test name and final concentration in paren-
thesis): adenosine diphosphate (ADP test, 6.4 µM), arachidonic 
acid (ASPI test, 0.5 mM), or thrombin receptor-activating 
peptide (TRAP test, 32 µM) using commercially available 
test reagents for MEA analysis. The change in electrical impe-
dance as a result of platelet aggregation and adhesion to the 
metal sensors was recorded and plotted against time.

Statistical analysis
The study was considered exploratory; therefore, sample size 
was not calculated a priori. Data of all parameters were 
examined by the Kolmogorov–Smirnov test for suitability 
for parametric testing. Differences in laboratory data
between cyanotic and acyanotic patients were assessed using Student’s t-test, Wilcoxon’s test, or \( \chi^2 \) test as appropriate. Analysis of variance for repeated measures with post hoc comparisons by two-sided paired t-test was used to assess changes during the observation period. A P-value of < 0.05 was considered statistically significant. Linear regression was used to analyse the relationship between platelet parameters, bleeding, and transfusion requirements. Data are presented as mean (standard deviation) or median (minimum, maximum).

**Results**

Subject characteristics are presented in Table 1. In the cyanotic group, all 25 subjects were younger than 1 yr, whereas in the acyanotic group, 17 of 25 were \( \leq 12 \) months of age. Five subjects were 1.1–4 yr, and three subjects were 4.1–7 yr. This explains differences in patient characteristic parameters between the cyanotic and acyanotic groups (Table 1), and consideration of age-adjusted reference values for platelet aggregation in the following text.

**Laboratory data**

Mean platelet count was within the normal range in cyanotic and acyanotic patients with no intergroup differences. Time course of platelet count before and after cardiac surgery is shown in Figure 1a.

Time course of platelet aggregation in the ADP test, TRAP test, and ASPI test before and after cardiac surgery is shown in Figure 1a–d. Before surgery, aggregation responses to all agonists were in the age-adjusted reference range in cyanotic children. Platelet aggregation was below the age-adjusted lower range at baseline in acyanotic children and significantly lower compared with the cyanotic group. In the small subgroup of acyanotic children older than 1 yr (\( n=8 \)), aggregation values were slightly lower compared with the younger acyanotic population.

Platelet aggregation in response to all tested agonists decreased significantly during surgery in both groups below the reference range. Platelet function recovered in both groups but did not reach baseline levels until the fifth postoperative day.

**Intra- and postoperative bleeding and transfusions**

Bleeding and transfusion frequencies are summarized in Table 2. Children with cyanotic malformations had higher intraoperative blood loss per body weight compared with acyanotic subjects.

The number of subjects receiving RBC concentrates and FFP was comparable between the groups. Platelet concentrate transfusion occurred more often in cyanotic subjects compared with acyanotic subjects \( \leq 12 \) months of age. Platelet aggregation in the ADP test before surgery correlated inversely with intraoperative RBC transfusion requirements \((r = -0.41, P=0.0054)\). Neither platelet count nor platelet aggregation parameters correlated with blood loss and platelet transfusion requirements. Platelet counts were \(< 100 \) g litre\(^{-1} \) before transfusion in 88% of subjects receiving platelet concentrates, while platelet aggregation was below the age-adjusted reference range in 100%. Fifty-four per cent of subjects received no platelet transfusion but had abnormal platelet counts and abnormal platelet aggregation.

No subject underwent re-exploration due to diffuse bleeding and all children had an uncomplicated postoperative period.

**Discussion**

The present investigation partially confirms previous findings that children with CHD have compromised platelet function.\(^2\) Despite normal platelet counts, platelet aggregation in response to all tested agonists was below the age-adjusted reference range in cyanotic children before surgery. In cyanotic children, however, the aggregation response was within the reference range of healthy neonates and infants. This finding indicates that cyanosis per se does not inhibit platelet aggregation. Shear stress-induced overproduction of procoagulant platelet microparticles in cyanotic CHD has been reported,\(^8\) and might explain the increased platelet aggregation response in the cyanotic group (Fig. 1a–d). Platelet activation and endothelial dysfunction causing an enhanced procoagulant potential have further been suggested to play an important role in the increased rate of thromboembolic events in patients with cyanotic CHD.\(^7\)
Fig 1. Platelet aggregation and platelet count before and after cardiac surgery. Data are given as maximum aggregation (arbitrary units, AU) determined at 37°C. Values are expressed as mean (SD). *P < 0.05 between groups. ADP, adenosine diphosphate; ASPI, arachidonic acid; POD, postoperative day; R1, lower 10th percentile of reference range in subjects ≤12 months; R2, lower 10th percentile of reference range in subjects ≥12 months; TRAP, thrombin receptor-activating peptide.

Table 2. Blood loss and transfusion requirements. POD, postoperative day; RBC, red blood cell. Values are expressed as absolute number of patients or median (minimum, maximum). *P < 0.05 vs cyanotic group
The mechanism of platelet inhibition in cyanotic patients remains unclear. Medication such as antibiotics, prostaglan-
dins, analgesic drugs, or direct anti-platelet drugs can have side-effects on platelet aggregation but cyanotic paediatric
patients are not routinely exposed to such substances. Hor-
gome and colleagues reported a negative correlation
between haematocrit, platelet count, and aggregability. How-
ever, in our patients, both haemoglobin levels and plate-
let count were comparable in children with cyanotic and
acyanotic malformations.

Cardiac surgery with CPB leads to impaired haemostasis.
Suggested mechanisms include platelet exhaustion, hyperfibri-
nolysis, decreased clotting factor activities due to haemodilu-
tion, activation, and increased consumption. MEA is
dependent upon platelet counts; for counts <80–100 g litre⁻¹, MEA generates only a qualitative estimation of platelet
aggregation during CPB. In our study, platelet aggregability
decreased during surgery to a similar level in both groups. Pre-
operative platelet inhibition in acyanotic children did not aggra-
vate CPB-related platelet impairment. This finding suggests
that preoperative platelet aggregability does not determine
the course of platelet dysfunction during cardiac surgery.

Interestingly, platelet response to the strong agonist TRAP
remained within the reference range in cyanotic patients
even after CPB while the response to the weak platelet ago-
nists ADP and ASPI was below normal. The reason for the low
dual response remains to be determined at the molecular
level, for example, by flow cytometry.

Slow recovery of platelet aggregability did not reach base-
line levels until the fifth postoperative day, despite adequate
clinical haemostasis. In adults, a rapid increase in platelet
reactivity has been observed. Therapeutic interventions
might enhance platelet recovery. Platelet transfusion cor-
rects thrombocytopenia and thrombopathia, and des-
mopressin improves platelet adhesion. Further interventional
studies are warranted to test the effect of these haemostatic options on periparative bleeding tendency and transfusion needs in the case of positive pre-test prob-
ability (decreased aggregation in MEA).

A limitation of this study with respect to the clinical
outcome parameters is the small sample size of 50 subjects.
Accordingly, the study was not powered to analyse differ-
ces in bleeding tendency and transfusion requirements.
Furthermore, the clinical management was not controlled
in the observational study design and platelet concentrate
transfusions were at the clinician’s discretion. Nevertheless,

blood loss and platelet transfusion requirements. Although
statistically significant, the correlation between ADP test
before surgery and intraoperative RBC transfusion require-
ments was weak (p = −0.41). While MEA was found to be pre-
dictive for platelet concentrate transfusion in adult patients
undergoing cardiac surgery, monitoring of platelet aggregation alone might not be suitable for identifying a
perioperative disposition for increased blood loss and trans-
fusion requirements in the paediatric population.

**Conflict of interest**

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form (www.perioperativebleeding.org) from Dynabyte.

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