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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13 21 MEA predicted adverse outcome in adult patients with coronary stents.22 Small sample volumes, point-of-care analysis within 6 min, and availability of standardized agonists are features that make use of MEA intriguing in children undergoing heart surgery. We used MEA to explore the peri-operative evolution of platelet aggregation in this high-risk population.

Methods

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 malformations 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 significant right-to-left shunt resulting in an arterial oxygen saturation 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 ventricular 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 adolescents.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 anaesthesia. A bolus of 400 units kg\(^{-1}\) was the trigger for platelet transfusion in the case of clinical bleeding. Fresh-frozen plasma (FFP) was transfused in the case of persisting bleeding 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. Acyanotic 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 anaesthesia 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 available 1.4 ml Monovette\textsuperscript{TM} collection tubes (Sarstedt, Numbrecht, Germany) containing 1.2 mg ml\(^{-1}\) EDTA for whole-blood count (Sysmex XE-2100, Roche Diagnostics, Mannheim, Germany) and 2.6 ml Monovette\textsuperscript{TM} 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 impedance 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 cartridges containing a magnetic stirrer and two independent dual-sensor units of silver-covered electrodes. After incubating blood samples for 3 min at 37°C, the following agonists were added (test name and final concentration in parenthesis): 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 impedance 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 \textit{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 χ² 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 ≤12 months, 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.23

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 postoperatively 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 ≤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 7 Despite normal platelet counts, platelet aggregation in response to all tested agonists was below the age-adjusted reference range in acyanotic 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

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject characteristics. Values are expressed as absolute number, mean (SD), or †mean (minimum, maximum). *P&lt;0.05 vs cyanotic group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cyanotic group</td>
</tr>
<tr>
<td>Number (n)</td>
<td>25</td>
</tr>
<tr>
<td>Age (months)†</td>
<td>2.2 (0.1, 10.4)</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>4496 (2184)</td>
</tr>
<tr>
<td>Size (cm)</td>
<td>55 (8)</td>
</tr>
<tr>
<td>Duration of cardiopulmonary bypass (h)</td>
<td>166 (47)</td>
</tr>
<tr>
<td>Duration of surgery (h)</td>
<td>293 (64)</td>
</tr>
<tr>
<td>Preoperative haemoglobin (g dl⁻¹)</td>
<td>13.8 (2.3)</td>
</tr>
</tbody>
</table>

Point-of-care assessment of platelet aggregation
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.

<table>
<thead>
<tr>
<th></th>
<th>Cyanotic group</th>
<th>Acyanotic group</th>
<th>Subgroup: acyanotic and age &lt;12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>25</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>Blood loss per body weight (ml·kg⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraoperative</td>
<td>215 (111, 305)</td>
<td>131 (38, 689)*</td>
<td>165 (32, 689)</td>
</tr>
<tr>
<td>Until 1st POD</td>
<td>24 (0, 138)</td>
<td>23 (0, 159)</td>
<td>30 (0, 159)</td>
</tr>
<tr>
<td>Until 5th POD</td>
<td>23 (0, 107)</td>
<td>19 (0, 180)</td>
<td>26 (0, 181)</td>
</tr>
<tr>
<td>RBC transfusion (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraoperative</td>
<td>25</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>Until 1st POD</td>
<td>9</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Until 5th POD</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Platelet concentrate transfusion (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraoperative</td>
<td>16</td>
<td>10</td>
<td>5*</td>
</tr>
<tr>
<td>Until 1st POD</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Until 5th POD</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fresh-frozen plasma transfusion (n)</td>
<td></td>
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</tr>
<tr>
<td>Intraoperative</td>
<td>25</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>Until 1st POD</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Until 5th POD</td>
<td>1</td>
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</table>
The mechanism of platelet inhibition in cyanotic patients remains unclear. Medication such as antibiotics, prostaglandins, 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. Horigome and colleagues reported a negative correlation between haematocrit, platelet count, and aggregability. However, in our patients, both haemoglobin levels and platelet count were comparable in children with cyanotic and acyanotic malformations.

Cardiac surgery with CPB leads to impaired haemostasis. Suggested mechanisms include platelet exhaustion, hyperfibrinolysis, decreased clotting factor activities due to haemodilution, 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. Preoperative platelet inhibition in acyanotic children did not aggravate 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 agonists 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 baseline 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 corrects thrombocytopenia and thrombopathia, and desmopressin improves platelet adhesion. Further interventional studies are warranted to test the effect of these haemostatic options on perioperative bleeding tendency and transfusion needs in the case of positive pre-test probability (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 differences 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, it is noteworthy that despite better overall platelet responsiveness platelet concentrate transfusions occurred more frequently and cumulative weight-adjusted blood loss was higher in the cyanotic population (Table 2). Haemostasis involves a balance of plasmatic and cellular elements and it is likely that the overall balance is shifted towards hypercoagulability in cyanosis, despite adequate platelet aggregability. Furthermore, young age and complexity of the surgical procedure could be major risk factors for perioperative bleeding in the cyanotic population. Neither platelet count nor platelet aggregation parameters correlated to blood loss and platelet transfusion requirements. Although statistically significant, the correlation between ADP test before surgery and intraoperative RBC transfusion requirements was weak \( p = -0.41 \). While MEA was found to be predictive 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 transfusion requirements in the paediatric population.

**Conflict of interest**

S.K.-L. received funding for an educational e-learning platform (www.perioperativebleeding.org) from Dynabyte.

**Funding**

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