Multivariate model for predicting postoperative blood loss in children undergoing cardiac surgery: a preliminary study

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Editor’s key points

- Bleeding after cardiac surgery is associated with worse outcomes and increased costs.
- Identification of risk factors can prompt management strategies to prevent bleeding.
- Retrospective analysis of a clinical database was performed to identify risk factors for postoperative bleeding in children.
- Risk factors were found to be low weight, cyanotic heart disease, and prolonged wound closure duration.

Background. Postoperative bleeding and blood product transfusion increase morbidity, mortality, and costs after cardiac surgery. However, factors that could accurately predict bleeding have not been well studied in children undergoing cardiac surgery. This study aims at determining factors that could be used to predict postoperative bleeding in this paediatric population.

Methods. We included 182 children undergoing congenital heart surgery. Significant bleeding was defined as a blood loss that exceeds 10% of total blood volume within the first 6 postoperative hours. Univariate and multivariate logistic regression analyses were performed to determine variables independently associated with bleeding. These variables were used to calculate a probability for each individual child to develop postoperative bleeding.

Results. According to the definition of bleeding, 44 patients were included into the ‘bleeder’ group and 138 into the ‘non-bleeder’ group. Factors independently associated with postoperative bleeding were preoperative body weight, the presence of a cyanotic disease, and the time required for wound closure. Based on these three parameters, we calculated the probability of bleeding and found a significant relationship with postoperative bleeding. Finally, a calculated probability of 0.59 can predict significant postoperative blood loss with a sensitivity of 84% and a specificity of 64%.

Conclusions. This study shows that preoperative body weight, cyanotic disease, and wound closure duration are best predictors of bleeding in the paediatric population after cardiac surgery. The combination of these three factors could be used at the end of the surgery to estimate the probability of postoperative bleeding.

Keywords: children; congenital heart diseases; postoperative blood loss; risk factors

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Cardiac surgery with cardiopulmonary bypass (CPB) is often associated with excessive blood loss and blood product transfusions, which have been associated with increased postoperative morbidity, early and late mortality, prolonged hospitalization stay, and costs.1 Outcome may further be worsened by the presence of pre-existing cofactors (e.g. anaemia in adult), massive transfusion, and re-exploration for bleeding.2 Excessive blood loss often results from the development of a perioperative coagulopathy which can be triggered by several factors such as contact between blood and non-endothelial surfaces, anticoagulation using unfractioned heparin (UFH), protamine overdosage, and hypothermia.3 In the paediatric population, other specific factors have to be considered such as the immaturity of the haemostatic system, the higher degree of bypass haemodilution, and the presence of cyanotic disease.4 Therefore, the coagulopathy observed in children undergoing cardiac surgery with CPB is more complex and has been associated with bad outcomes.5 Rapid detection of this coagulopathy should allow early goal-directed haemostatic therapy, aiming at preventing postoperative bleeding, reducing morbidity, mortality, and costs.6 This goal-directed therapy is best obtained through the development of specific algorithms,7 which are applied after identification of abnormal bleeding.8 9 Insufficient data exist to accurately predict
bleeding complications both in adult and paediatric cardiac surgery populations. There is urgent need for further studies that will identify patients with increased bleeding risk in order to improve their perioperative management.10

We therefore designed a retrospective analysis of prospectively collected data to evaluate parameters that could be used to define and/or predict postoperative bleeding in children undergoing cardiac surgery with CPB. Our goal was to develop a simple model that would allow pre- and intraoperative identification of children at higher risk for bleeding using variables available in routine clinical practice.

Methods

Study design
After approval by the local Ethics Board, we performed a retrospective analysis of data collected in our departmental database, which included children aged between 0 and 16 yr old undergoing a cardiac surgery with CPB between September 2010 and January 2012. Exclusion criteria were: pre-existing acquired and/or congenital coagulopathy (defined as platelet count < 100 000 mm$^{-3}$, activated partial thromboplastin time (aPTT) > 45 s, prothrombin time (PT) < 70%, fibrinogen < 100 mg dl$^{-1}$), liver (aspartate aminotransferase and alanine aminotransferase > two-fold our normal range) and/or kidney diseases (creatinine level > 1.5 mg dl$^{-1}$ and/or haemodialysis), emergency procedures for life-threatening conditions, and Jehovah’s Witnesses. The local Ethic Board waived the requirement for written informed consent due to the retrospective nature of the protocol.

Anaesthesia protocol and intraoperative management
Standard monitoring included five-lead ECG, pulse oximeter, non-invasive arterial pressure, arterial and central venous pressures, urinary output, and rectal temperature. I.V. anaesthesia based on midazolam, sufentanil, and rocuronium was used in all children, except those with univentricular physiology undergoing a cavopulmonary connection. In this particular situation, early extubation was warranted and anaesthesia was based on propofol, remifentanil, and atracurium. All children received 30 mg kg$^{-1}$ methylprednisolone and cefazoline 25 mg kg$^{-1}$ after the induction of anaesthesia. Tranexamic acid was systematically administered according to the following dose scheme: a bolus of 10 mg kg$^{-1}$ was given after anaesthesia induction, followed by a continuous infusion of 10 mg kg$^{-1}$ h$^{-1}$ until the end of wound closure. A second bolus of 10 mg kg$^{-1}$ was added to the CPB prime solution.

Before aortic cannulation, 4 mg kg$^{-1}$ UFH was administered to reach an activated clotting time (ACT, ACTII monitor, Medictronic BV, Kerkrade, The Netherlands) > 480 s. The level of anti-coagulation was repeatedly checked during bypass using ACT testing and additional heparin boluses (1 mg kg$^{-1}$) were administered to maintain ACT > 480 s. At the end of CPB, protamine was administered to antagonize UFH. We used a standardized scheme in which the protamine dose corresponds to half the total amount of UFH administered during the whole CPB duration. Heparin reversal was controlled with ACTII monitor comparing ACT measured in cartridge with and without heparinase (Medtronic BV). An additional 1 mg kg$^{-1}$ of protamine was administered if required to ensure adequate antagonization of heparin, defined as a difference < 10% between ACTs with and without heparinase.

The CPB circuit was primed with 6% hydroxyethyl starch (130/0.4) in 0.9% sodium chloride (Voluven©, Fresenius-Kabi GmbH, Bad Homburg, Germany), 20% mannitol (1.5 ml kg$^{-1}$), 20 mEq litre$^{-1}$ sodium bicarbonate, and 50 mg litre$^{-1}$ UFH. In neonates up to 1 month of age, the colloid was replaced by fresh-frozen plasma (FFP). When preparing the CPB prime, the haemotocrit on bypass was calculated based on the volume of the prime and the estimated blood volume (EBV) of the child. Packed red blood cells (RBCs) were added to the prime when predicted haemotocrit was < 24% after cardioplegia (crystalloid cold balanced electrolyte solution enriched with potassium chloride 30 mmol litre$^{-1}$). All patients were rewarmed to a rectal temperature > 35.5°C before weaning from CPB. After weaning from CPB, modified ultrafiltration was used to increase haemotocrit of the residual blood volume in the CPB circuit.

After separation from CPB, packed RBCs were transfused to maintain a haemotocrit > 24% in the case of haemorrhage or to increase oxygen delivery in the case of persistent significant R- to L-shunt resulting in a low arterial oxygen saturation ($S_{PaO_2}$ < 90%), or in the case of persistent lactic acidosis after optimization of cardiac output with inotropes, vasoactive agents, or both. In these conditions, the volume of blood transfused was adapted to each child according to the clinical situation. Platelet concentrates and FFP were transfused after CPB in the presence of abnormal clinical bleeding (based on surgeon and anaesthesiologist’s judgement), using the algorithm developed by Despotis and colleagues, based on platelet count, PT, and aPTT.11

Data recorded
Several parameters, which we considered as factors that may influence postoperative blood loss in children undergoing cardiac surgery, were recorded. These parameters included: age, weight, height, anaesthetic risk (ASA score) and surgical complexity (RACHS-1),12 cyanotic heart disease (CHD), previous sternotomy, preoperative laboratory data (haemoglobin level, platelets count, aPTT, PT, fibrinogen level, creatinine level), routine coagulation assays performed at the end of CPB (fibrinogen level, haemoglobin level, platelets count, aPTT, and PT), length of surgery, aortic clamping, and CPB, minimal temperature during CPB, intraoperative blood loss, wound closure duration, intraoperative exposition to allogeneic blood products (FFP, RBCs, platelets), and volume of priming solution.

Intraoperative blood loss was determined by weighing sponges and measuring suction volumes after separation from CPB. Postoperative blood loss was assessed by measuring chest tube drainage from admission to the paediatric intensive care unit. Clinically significant postoperative bleeding was defined as > 10% of EBV blood loss in the first 6 postoperative hours, which corresponds to the 75th percentile of our population.
Statistical analysis

Continuous variables were tested for normality with the Kolmogorov–Smirnov test. Data are presented as mean [standard deviation (SD)] or median (percentile 25 to percentile 75). Categorical variables are expressed as number and fraction (%). When appropriate, continuous variables were compared using Student’s t-test or Mann–Whitney U-test. The χ² was used for categorical variables. For all possible determinants of postoperative bleeding, univariate logistic regression analysis was performed. All variables with a P-value of <0.1 (defined ‘a priori’) were considered relevant and included into the multivariate logistic regression analysis. This second analysis was used to define the factors that were independently associated with postoperative bleeding.

Finally, receiver operating characteristic (ROC) curves were constructed for variables that significantly predict bleeding. Areas under the curve with the 95% confidence interval (CI) were calculated. These parameters were also used to calculate the probability of bleeding according to the formula developed by Apfel and colleagues.13

According to this formula:

\[ \text{Probability (P)} = (1 + e^{-z})^{-1} \]

where \( z = b_1 \cdot x_1 + b_2 \cdot x_2 + b_3 \cdot x_3 + \ldots + b_Y \cdot x_Y \), where \( b \) is the coefficient beta obtained from the multivariate logistic regression analysis and \( x \) the parameter.

A P-value of <0.05 was considered as statistically significant for all tests.

Statistical analyses were performed with Prism 6 for Mac OS (version 6.0a; GraphPad Software Inc., San Diego CA, USA, www.graphpad.com), Statistix software for Windows (version 9, Analytical Software, Tallahassee, FL, USA, www.statistix.com), and MedCalc software for Windows (Version 12.3.0.0, MedCalc Software, Ostend, Belgium, http://www.medcalc.org).

Results

Patient characteristic data

From the 191 screened patients, nine were excluded, leaving 182 for the final analysis (Fig. 1). Forty-four patients (24%) were included in the ‘bleeder’ group and 138 patients (76%) in the ‘non-bleeder’ group. Patient characteristic data and surgical characteristics are summarized in Table 1.

Children included in the ‘bleeder’ group were significantly younger, had a lower preoperative body weight, lower arterial saturation, were more severely ill, and were exposed to more complex surgeries and allogeneic blood products.

Determination of the parameters associated with blood loss

Univariate logistic regression analysis defined 19 variables associated with postoperative blood loss (Table 2) that were included in the multivariate regression analysis.

From this analysis, preoperative weight (kg), the presence of a cyanotic disease, and the wound closure duration (min) were significant predictors of postoperative bleeding. A P-value of <0.05 was considered as statistically significant for all tests.

Statistical analyses were performed with Prism 6 for Mac OS (version 6.0a; GraphPad Software Inc., San Diego CA, USA, www.graphpad.com), Statistix software for Windows (version 9, Analytical Software, Tallahassee, FL, USA, www.statistix.com), and MedCalc software for Windows (Version 12.3.0.0, MedCalc Software, Ostend, Belgium, http://www.medcalc.org).

Table 1 Patient characteristic and surgical data. RACHS-1, Risk Adjustment on Congenital Heart Surgery; CPB, cardiopulmonary bypass; BP, allogeneic blood products

<table>
<thead>
<tr>
<th>Group ’bleeder’ (n=44)</th>
<th>Group ’non-bleeder’ (n=138)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (month)</td>
<td>4.3 (1.8–13)</td>
<td>13 (3.2–40)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>24 (54)</td>
<td>76 (55)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>58 (52–68)</td>
<td>71 (57–100)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>4.6 (3.5–6.4)</td>
<td>7.6 (4.8–14)</td>
</tr>
<tr>
<td>( \text{SpO}_2 ) (%)</td>
<td>86 (80–96)</td>
<td>95 (89–97)</td>
</tr>
<tr>
<td>ASA (%)</td>
<td>2 (5)</td>
<td>12 (9)</td>
</tr>
<tr>
<td>II</td>
<td>20 (45)</td>
<td>93 (67)</td>
</tr>
<tr>
<td>III</td>
<td>8 (18)</td>
<td>38 (27)</td>
</tr>
<tr>
<td>IV</td>
<td>22 (50)</td>
<td>33 (24)</td>
</tr>
<tr>
<td>Previous sternotomy (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operative complexity</td>
<td>RACHS-1</td>
<td>3 (2–4)</td>
</tr>
<tr>
<td>Length of surg. (min)</td>
<td>219 (83)</td>
<td>224 (51)</td>
</tr>
<tr>
<td>Aortic clamp (%)</td>
<td>41 (93)</td>
<td>108 (78)</td>
</tr>
<tr>
<td>Aortic clamp duration (min)</td>
<td>58 (21)</td>
<td>52 (22)</td>
</tr>
<tr>
<td>CPB duration (min)</td>
<td>126 (86–167)</td>
<td>103 (77–128)</td>
</tr>
<tr>
<td>Wound closure duration (min)</td>
<td>77 (70–100)</td>
<td>67 (57–81)</td>
</tr>
<tr>
<td>Lowest temperature (ºC)</td>
<td>32 (28–33)</td>
<td>32 (31–35)</td>
</tr>
<tr>
<td>Priming volume (ml kg⁻¹)</td>
<td>52 (14)</td>
<td>41 (17)</td>
</tr>
<tr>
<td>Exposition to BP (%)</td>
<td>10 (23)</td>
<td>9 (6)</td>
</tr>
</tbody>
</table>
remained the only variables independently associated with postoperative blood loss in the studied population (Table 3). Each variable was used separately to build ROC curves (Fig. 2A and s). In addition to cyanotic disease, increased bleeding risk was found in children weighing <6.5 kg (sensitivity: 80%, specificity: 62%) and when wound closure duration exceeded 64 min (sensitivity: 84%, specificity: 47%).

**Bleeding probability calculations**

The three parameters obtained were then used to calculate the probability for bleeding for each child included in the study:

\[
\text{Probability (P)} = \left(1 + e^{-z}\right)^{-1}
\]

where \(z = 1.12\) (cyanogen) – 0.22 (weight) + 0.04 (wound closure duration) – 1.30

We observed that the calculated probability was significantly associated with postoperative blood loss (Fig. 3, AUC: 0.80, 95% CI: 0.73–0.85). Finally, we determined that for each child, a calculated probability of 0.59 can predict postoperative blood loss higher than 10% of the expected blood volume with a sensitivity of 84% and a specificity of 64%.

**Discussion**

Our results indicated that preoperative weight, the presence of a cyanotic disease, and the duration of wound closure were significantly correlated with postoperative blood loss. In addition, we showed that the combination of these three parameters could be used to calculate the probability of postoperative bleeding with a good sensitivity and specificity.

Assessment of the haemostatic system has significantly evolved over the last ten years.14 For these reasons, we wanted to assess the current parameters that could be used to identify children at higher risk for postoperative bleeding and design a multifactorial parameter that could be used to determine the need for specific haemostatic assessment.

Point-of-care (POC) monitoring, which allows direct assessment of coagulation at the bedside, is increasingly used in the operating theatre.15 These monitors led to the development of algorithm-based haemostatic management, especially in adult patients.16 Similarly, there is growing evidence that the use of either thromboelastography (TEG®), Hemostasis system, Haemoscope Corporation, Niles, IL, USA) or rotational thromboelastometry (ROTEM®, TEM® International GmbH, Munich, Germany) might be useful to guide haemostatic therapies in children bleeding after cardiac surgery.6 17 It is now recognized that this monitoring should be used in with specific algorithms in patients with abnormal bleeding. Bleeding disorders and abnormal haemostasis have been known for some time to be associated with cyanotic congenital heart diseases in children.18 19 Coagulation defects, such as thrombocytopenia, and factor deficiencies including low fibrinogen level and fibrinogen polymerization impairment have been also described.20 Osthaus and colleagues21 confirmed that whole blood coagulation measured by ROTEM® is impaired in infants with congenital heart disease. They reported that baseline ROTEM® parameters are impaired in these children, in particular in those with a cyanotic disease. In a recent study, Jensen and colleagues21 reported that children with cyanotic disease are in a hypocoagulable state, mainly related to impaired fibrinogen function assessed by TEG Functional Fibrinogen assay, while thrombocytopenia, if present, was not associated with severe platelet dysfunction.

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>Std error</th>
<th>Coef./SE</th>
<th>P-value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-1.3</td>
<td>1</td>
<td>-1.3</td>
<td>0.1931</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyanotic disease (%)</td>
<td>1.12</td>
<td>0.48</td>
<td>2.33</td>
<td>0.0198</td>
<td>3.07</td>
<td>1.20–7.90</td>
</tr>
<tr>
<td>Wound closure duration (min)</td>
<td>0.04</td>
<td>0.01</td>
<td>3.7</td>
<td>0.0002</td>
<td>1.04</td>
<td>1.02–1.07</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.22</td>
<td>0.07</td>
<td>-3.04</td>
<td>0.0024</td>
<td>0.8</td>
<td>0.69–0.92</td>
</tr>
</tbody>
</table>

**Table 2** Univariate logistic regression analysis. RACHS-1, Risk Adjustment on Congenital Heart Surgery; ACT, activated clotting time

**Table 3** Multivariate logistic regression analysis
It is therefore not surprising that, in our study, the presence of a cyanotic disease was significantly correlated with postoperative blood loss.

The other predictive factor found in our study was body weight. This finding is in agreement with other studies showing that younger children, especially neonates, are at increased risk of perioperative bleeding. In 1998, Williams and colleagues reported that blood loss and transfusion requirement varied inversely with age. When expressed in kilograms per body weight, neonates bled more and received more blood donor products than any other age group. In another study, Miller and colleagues showed that children weighing <8 kg bled more than other children. Finally, in a retrospective study including 73 children, the authors reported that neonates bled significantly more than children weighing >13 kg. In our population, we observed that children weighing <6.5 kg were at increased risk of bleeding.

These two parameters could be used to identify before operation children who are at increased risk of perioperative bleeding. However, not only this risk, but also the presence of abnormal bleeding should be recognized as soon as possible after the weaning of CPB and protamine administration.

According to our results, wound closure was delayed in children who experienced significantly more blood loss. From a clinical point of view, this observation is not surprising although it has not been precisely studied in the literature. We observed that a duration >64 min was significantly correlated with ‘abnormal’ bleeding.

In addition, we first used these three parameters, which are independently associated with postoperative blood loss, to design a multivariable probability calculator.

Interestingly, we did not observe any relationship between post-CPB fibrinogen level and postoperative blood loss in the multivariate analysis. The patient characteristic repartition of the studied population could explain this result. Indeed, we included children <1 month of age in the analysis. These children systematically received FFP in the prime solution, FFP administration led to an increased fibrinogen level, which was not independently correlated with postoperative blood loss in our multivariate analysis.

The main limitation of this study comes from the definition of abnormal bleeding, which remains controversial. Indeed, massive bleeding is defined as one of the following by Martino-witz and Michaelson: loss of entire blood volume within 24 h, loss of 50% of blood volume within 3 h, or blood loss at a rate of 150 ml min \(^{-1}\), 1.5 ml kg \(^{-1}\) min \(^{-1}\) for >20 min. A definition for excessive bleeding has also been published in the recent ESA guidelines. However, all these definitions were essentially
developed to define massive bleeding in the adult population and could not be applied for the paediatric cardiac population. Other authors used more specific criteria in cardiac surgery. Rahe-Meyer and colleagues used sponges’ weight to define the 5 min bleeding mass after the weaning of CPB. The authors used a threshold of 250 g to define abnormal blood loss after protamine administration.27 Even if this approach appears attractive, this threshold was based on a single-centre clinical experience and no data are available for the paediatric population. In 1999, Williams and colleagues28 proposed a definition of ‘significant bleeding’ as a proportion of EBV. They observed that blood loss > 20% EBV within the first 6 postoperative hours was best correlated with transfusion in children. However, this definition could only be applied in their population and we considered that many changes have been made between 1999 and now. For this reason, we decided to adopt a definition according to the distribution of postoperative blood loss volume observed in our population. We observed that the 75th percentile for blood loss corresponded to 10% of the EBV and decided to use this value as the cut-off value between ‘bleeder’ and ‘non-bleeder’ in our population. Using this definition, the likelihood to receive blood products was significantly higher in the ‘bleeder’ group, which is a clinically relevant observation. Another relevant definition of ‘significant bleeding’ should have been the amount of blood loss in children re-operated for haemostasis exploration, but fortunately, such an event did not occur in our studied population. The 6 h delay (compared with 24 h) was also modified from the study published by Williams and colleagues.28 If the child bled significantly, we aimed to treat the bleeding before the 24 h delay. For this reason, we believed that 24 h is a too long waiting period. Also, the amount of blood loss recorded 24 h after surgery could be influenced by several factors, including haemostatic interventions.

Our study presents some other limitations. We performed a retrospective analysis of data collected from a single-centre departmental database and the results could only be applied in our population. A multifactorial probability calculation was performed in order to improve the sensitivity and specificity of the probability obtained compared with single factor determination of bleeding. Nevertheless, the present results should be considered as preliminary and need to be validated in a much larger cohort. Using a larger cohort, we could increase the sensitivity and specificity of our probability calculation model to predict bleeding. An ‘acceptable’ sensitivity and specificity threshold to predict bleeding is difficult to define as such a parameter has never been determined in the literature. Further studies are needed to better define the relationship between sensitivity, specificity, and postoperative outcomes due to abnormal bleeding, and costs related to the use of POC testing.

The retrospective nature of this study could not guarantee the absence of bias. In order to decrease this bias as much as possible, we performed univariate and multivariate regression analyses, which are recommended in the case of retrospective design.

Bleeding tendency and incidence of re-exploration for bleeding is very low in our population. Consequently, some factors would have been missed and our results might not apply to other centres. However, if validated, our probability calculation method would provide a simple scoring system that could be used at the bedside, as the first step of an algorithm-based management of the bleeding paediatric cardiac patients. Indeed, using this multifactor identification of the bleeding children, POC monitoring should be used to guide haemostatic therapies when the probability of bleeding observed by the individual child exceeds 0.59. This approach is in accordance with the published literature showing that POC testing should be used in a bleeding patient to guide haemostatic therapy, and not routinely to predict bleeding.8 9

Indeed, Weber and colleagues8 performed POC testing and followed an algorithm-based management of the bleeding patient, if diffuse bleeding from capillary beds at wound surfaces occurred and this required haemostatic therapy as assessed by the anaesthesiologist and surgeon and/or intraoperative or postoperative (during the first 24 postoperative hours), blood loss exceeding 250 ml h\(^{-1}\) or 50 ml per 10 min was observed. Using this approach, the authors reported that haemostatic therapy based on POC testing in a bleeding patient reduced patient exposure to allogenic blood products and provided significant benefits with respect to clinical outcomes, including mortality and costs. Similarly, Rahe-Meyer and colleagues26 used a 5 min bleeding mass determined by weighing dry surgical cloths and compresses to define inadequate haemostasis. These two trials confirmed that based on the current well-designed randomized, controlled trials, POC assessment is needed in a bleeding situation.

In conclusion, based on the results of this study, we defined a simple probabilistic model including preoperative body weight, the presence of a cyanotic disease, and the duration of wound closure that could be used to predict excessive postoperative blood loss in children undergoing cardiac surgery with CPB. Further large multicentre studies are needed to validate this model and to assess if it could be used as the first step of an algorithm-based management of the bleeding cardiac children.

Authors’ contributions
V.S.: recorded the data, wrote the manuscript, and accepted the final version of the manuscript. A.W.: designed the study, recorded the data, helped write the manuscript, and approved the final version of the manuscript. D.F.: designed the analyses, treated the data, performed the statistical analyses, wrote the manuscript, and approved the final version. P.V.d.L.: helped design the study, helped record the data, helped write the manuscript, and approved the final version of the manuscript.

Declaration of interest
None declared.

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