Reference Intervals of Thromboelastometric Evaluation of Coagulation in Pediatric Patients with Congenital Heart Diseases: A Retrospective Investigation

Background: Rotational thromboelastometry (ROTEM®) is a point-of-care test for coagulation, enabling physicians to make a swift decision. The aim of this investigation was to establish reference intervals of thromboelastometric evaluation for coagulation in pediatric patients with congenital heart diseases (CHD).

Material/Methods: As baseline data, 3 assays of ROTEM® (INTEM, EXTEM, and FIBTEM) were measured after anesthesia induction. ROTEM® parameters were clotting time (CT), amplitude at 10 min (A10), clot formation time (CFT), α angle, maximal clot firmness (MCF), clot lysis index at 60 min (LI60), and maximal clot elasticity (MCE). As age is a well-known factor for maturation, age groups were determined as follows: 1) <1 month, 2) 1–3 months, 3) 4–12 months, 4) 1–3 years, 5) 4–6 years, 6) 7–12 years, and 7) 13–16 years. Reference limits representing 95% of distribution of ROTEM® parameters and 90% confidence intervals of upper and lower reference limits were calculated.

Results: The data of 413 patients were analyzed. Although INTEM CT was prolonged, significantly shorter CT and CFT, steeper α, and greater A10, MCF, and MCE were shown in patients age <3 months compared to older children.

Conclusions: Reference intervals of thromboelastometric evaluation for coagulation from pediatric patients with CHD were shown to have similar pattern to those obtained from healthy pediatric patients. Pediatric patients with CHD, even with cyanosis, were demonstrated to have functionally intact coagulation profile before surgery.

MeSH Keywords: Blood Coagulation • Heart Defects, Congenital • Reference Values • Thrombelastography

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Background

Blood viscoelastic measurements, including thromboelastography (TEG®) and rotational thromboelastometry (ROTEM®), are now known to provide a coagulation profile of critically ill patients with rapid visual evaluation of whole blood coagulation, ranging from clot formation to lysis of the clot [1–3]. A critical care team employs a point-of-care test (POCT) for blood coagulation to make a swift decision at the bedside, and ultimately to improve patient outcomes. Medical decision-making with a test or measurement is a process to compare the result of a patient with reference intervals. During cardiac surgery, massive bleeding leads to transfusion, which makes early detection of coagulopathy a critical step to reduce patient morbidity and mortality.

The ROTEM® machine (ROTEM®, TEM International, Munich, Germany) has been available in the operating room designated for congenital heart disease (CHD) surgery since 2009 in our institution. At first, it was mainly utilized for investigational purposes, and was adopted as a routine measurement of blood coagulation in pediatric patients undergoing surgical intervention for CHD approximately 2 to 3 years later. Test results of a pediatric patient had been evaluated using reference intervals embedded in the ROTEM® machine. Those reference intervals were, however, made from measurements in the adult population [4]. Furthermore, to the best of our knowledge, investigations to establish reference intervals of ROTEM® measurement in pediatric patients with CHD are rare. Previous investigations were done in healthy pediatric populations [5] and in a small number of pediatric patients with complex CHD or extreme age [6–8]. The aim of this retrospective investigation was to establish reference intervals of ROTEM® evaluation for coagulation in pediatric patients with CHD.

Material and Methods

The local ethics committee agreed to waive the requirement of informed consent from patients or the next of kin for this retrospective investigation because the ROTEM® measurements were done as a routine procedure. Patients who had undergone surgical interventions for CHD under general anesthesia between October 2010 and May 2016 in a tertiary university hospital were recruited for analysis. Exclusion criteria were: age older than 16 years, inability to find baseline data of ROTEM® measurements, operations done out-of-hours or outside the operating room, and administration of blood products or any drug or fluid known to affect blood coagulation, such as colloid solution, given within a month before surgery. However, prostaglandin E1 for ductal patency was an exception because it could be vital for oxygenation for patients with ductal dependency. As age is a well-known factor for functional maturation in pediatric patients, age groups were determined as follows in the light of previous investigations [5,9]: <1 month (neonate), 1–3 months (early infant), 4–12 months (late infant), 1–3 years (toddler), 4–6 years (preschool), 7–12 years (school), and 13–16 years (adolescent). Preoperative data to be retrieved from electronic medical record system were demographic details, such as age, sex, height, body weight, and preoperative diagnosis, including cyanotic or acyanotic congenital heart disease. For baseline evaluation of blood cell count and coagulation, the latest preoperative results of complete blood count and plasmatic coagulation tests, including hemoglobin (Hb), hematocrit (Hct), platelet count, functional fibrinogen level determined by Clauss method, prothrombin time (PT), and activated partial thromboplastin time (aPTT), were also retrieved.

Anesthetic management

The patients were lightly sedated with IV ketamine <1.0 mg/kg or midazolam 0.5–1.0 mg as appropriate with patient age and body weight when arrived in the holding area of the operating room. The patient’s trachea was intubated after further sedation with additional bolus dose of IV ketamine or midazolam and muscle relaxation with IV vecuronium 0.4–0.5 mg/kg. Once mechanical ventilation was set up to obtain an end-tidal CO₂ of 35–40 mmHg, anesthesia was maintained with sevoflurane in O₂/air with FiO₂ of 0.6 and continuous infusion of sufentanil 0.1–0.3 μg/kg/hr. Central venous and femoral arterial catheters were inserted. When all the anesthetic procedures were completed, blood sampling for ROTEM® measurements was drawn via a 20-G, single-lumen, femoral arterial catheter.

ROTEM® measurements

ROTEM® was measured as a routine procedure of blood coagulation management for pediatric patients undergoing surgical intervention for CHD. To collect baseline data for subsequent comparison, the first ROTEM® was measured after the induction of anesthesia, but before the surgery was initiated. A total of 1.5–2.0 ml blood was sampled after discarding a sufficient volume of blood to prevent contamination with heparin-containing fluid. Drawn blood was immediately transferred to a citrate-coagulated vacuum tube (Vacutainer®, BD, Franklin Lakes, NJ), and inserted into the designated hole in the ROTEM® machine for warming. Measurement was initiated within 15 min after blood sampling. Three assays of ROTEM®, which evaluate contact-activated, tissue factor-activated and fibrinogen polymerization pathways (INTEM, EXTEM, and FIBTEM, respectively) were done in the same ROTEM® machine. ROTEM® was measured at least for 60 min by anesthesiologist with substantial experience in ROTEM® measurement. Each assay of ROTEM® was measured with 300 μl of whole blood, which was warmed to 37°C before initiation of measurement. Warmed blood and assay-specific reagents were poured into a cup fixed in the cup
holder, using an automatic pipette system. Reagents required to activate each coagulation pathway were: 1) star-TEM with calcium chloride for both INTEM and EXTEM, 2) in-TEM: contact activator comprised of partial thromboplastin phospholipid for INTEM, 3) ex-TEM: tissue factor containing thromboplastin for EXTEM, and 4) fib-TEM: platelet inhibitor (cytochalasin D) and calcium chloride for FIBTEM (TEM International, Munich, Germany). Once measurements were initiated, the results were displayed on the screen, and ultimately recorded and stored in the integrated computer of the ROTEM® machine. The parameters of ROTEM® measurement to be retrieved to establish reference intervals were: clotting time (CT, time to initial appearance of clot, in seconds); amplitude at 10 min after initial appearance of clot (A10); clot formation time (CFT, time from the first appearance of clot to an amplitude of 20 mm, in seconds); alpha angle (α, an angle between horizontal axis of time and tangent to main body of trace from a time point of CT, in degrees); maximal clot firmness (MCF, the widest amplitude of main body of trace, in millimeters), clot lysis index at 60 min (LI60, a ratio of amplitude at 60 min after CT to MCF, as percentage); and maximal clot elasticity (MCE, calculated value from a formula of 100×MCF/(100-MCF)) (Figure 1). Data of ROTEM® measurements were retrieved as a text file (*.txt) and then tabulated into a worksheet using MICROSOFT® EXCEL® 2016 (MICROSOFT, Redmond, WA). Calibration of the ROTEM® machine was performed regularly based on instructions from the manufacturer.

**Statistical methods**

The primary endpoint of this retrospective investigation was to establish reference intervals of previously described parameters of ROTEM® measurement in pediatric patients with CHD. Reference intervals were calculated as lower and upper reference limits with 2.5th and 97.5th percentiles of distribution of ROTEM® measurement results, as recommended by CLSI guidelines [10]. Because the number of patients in each age group was not expected to reach 120, which is a recommended number of reference individuals by CLSI guidelines, the robust method, as suggested by Horn and Pesce [11], was used to calculate reference limits and 90% confidence interval of each reference limit. The secondary endpoint was to compare the ROTEM® measurement results to find a possible difference 1) between age groups and 2) between patients with acyanotic and cyanotic CHD. For comparison between age groups, data are presented as mean (SD) or median (interquartile range) and ANOVA or Kruskal-Wallis test were used, respectively, as indicated by results of the normality test. Multiple comparisons with Tuckey’s method were used as a post hoc test. For comparison between cyanotic and acyanotic patients, the t test or Wilcoxon signed rank sum test was done, as appropriate, for normality testing. For testing normality of distribution, the Shapiro-Wilk test was used. Statistical analysis, including calculations of reference limits and confidence intervals, was performed with R studio (Ver. 0.99.902, RStudio Team [2015]). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, URL. http://www.rstudio.com/) and R version 3.3.0 (R Core Team [2016]). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/ with packages named “referenceIntervals” (Daniel Finnegans [2014]). referencelIntervals: Reference Intervals. R package version 1.1.1. https://CRAN.R-project.org/package=referenceIntervals, and nparcomp [12].

**Results**

The data of 1494 patients undergoing surgical interventions for CHD were retrieved. The data of 413 patients were analyzed after the exclusion of patients older than 16 years of age, those undergoing surgery out-of-hours or outside the operating room, those without available baseline data of ROTEM® measurements, those given blood products or any drug or fluid known to affect coagulation, and/or those undergoing repeated operations in which the latest operation was performed while the patient was still in the same age group. Details are depicted in Figure 2.

Patient characteristics are presented in Table 1. Sex ratio was similar between age groups (P=0.963). With advancing age, height, body weight, and body surface area were elevated (p<0.001). There was a difference in the number of children with cyanosis among age groups (P<0.01). Younger children were more likely to have cyanosis compared to older children.
Figure 2. Flow chart representing the process of patient recruitment and selection.

Table 1. Demographic data.

|          | Neonate (n=119) | Early infant (n=76) | Late infant (n=87) | Toddler (n=55) | Preschool (n=37) | School (n=32) | Adolescent (n=7) | p       |
|----------|----------------|---------------------|-------------------|----------------|-----------------|---------------|-----------------|---------|
| Age      | 15±7.1 day     | 54.8±16.5 day       | 5.7±2.0 month     | 1.8±0.6 year   | 4.3±0.8 year    | 8.4±1.7 year  | 13.6±0.6 year   | <0.01   |
| Sex      |                |                     |                   |                |                 |               |                 | 0.093   |
| Male     | 58 (48.7%)     | 37 (48.7%)          | 39 (44.8%)        | 25 (45.5%)     | 17 (45.9%)      | 16 (50.0%)    | 2 (28.6%)       |         |
| Female   | 61 (51.3%)     | 39 (51.3%)          | 48 (55.2%)        | 20 (54.1%)     | 20 (54.1%)      | 16 (50.0%)    | 5 (71.4%)       |         |
| Height (cm) | 49.0±4.5       | 54.0±5.1            | 62.3±6.7          | 82.4±7.8       | 100.5±8.4       | 127.5±10.8    | 156.5±8.9      |         |
| Bodyweight (kg) | 3.3±0.6         | 4.3±0.9             | 6.1±1.4           | 10.1±1.9       | 15.0±3.4        | 25.3±7.3      | 39.4±9.8       |         |
| Cyanosis | 15 (12.6%)     | 11 (14.7%)          | 15 (17.2%)        | 10 (18.2%)     | 15 (40.5%)      | 2 (6.2%)      | 1 (14.3%)      | <0.01   |

Data are number (%) or mean ±SD, as appropriate. BSA – body surface area.
The preoperative results of complete blood count and plasmatic coagulation tests are presented in Table 2. All hematologic and plasmatic coagulation profiles except PT differed among age groups (all P<0.001). The Hb and Hct were significantly lower in early and late infants compared to other age groups (all P<0.05). Platelet count was significantly higher in those in late infant, toddler, preschool, and school age groups (all P<0.001). The Hb and Hct were significantly lower in cyanotic patients compared to that in acyanotic patients. ROTEM® parameters of INTEM and EXTEM assays in cyanotic patients were shown to have significantly prolonged CT and CFT, and significantly reduced CL and CLT. Clot lysis index was significantly different between cyanotic and acyanotic patients only in INTEM assay. FIBTEM parameters of A10, MCF, and MCE were significantly lower in cyanotic patients compared to that in acyanotic patients.

Reference limits representing 95% of distribution of ROTEM® parameters and 90% confidence intervals of upper and lower reference limits are presented in Tables 7–9, in which reference intervals obtained from otherwise healthy pediatric patients are also presented for comparison [5].

### Table 2. Hematologic and plasmatic coagulation parameters classified with age groups.

| Parameter | Neonate (N=119) | Early infant (N=76) | Late infant (N=87) | Toddler (N=55) | Preschool (N=37) | School (N=32) | Adolescent (N=7) |
|-----------|-----------------|---------------------|-------------------|----------------|-----------------|---------------|-----------------|
| Hb (g/dL) | 12.9            | 11.1*               | 11.4*             | 12.9†          | 13.7*,†         | 12.9*         | 14.5*,†         |
|           | [11.2; 14.4]    | [10.2; 11.8]        | [10.8; 12.6]      | [11.9; 13.9]   | [12.8; 16.1]    | [12.2; 13.9]  | [13.9; 15.3]    |
| Hct (%)   | 38.5            | 32.5*               | 34.1*             | 37.3†          | 39.8†           | 37.9†         | 42.0*,†         |
|           | [33.2; 43.0]    | [29.8; 36.0]        | [31.8; 37.5]      | [35.5; 39.8]   | [36.5; 49.8]    | [35.9; 40.4]  | [41.5; 44.5]    |
| Platelet (*10^3/µl) | 404.0          | 387.0               | 320.0*,†         | 307.0*,†       | 255.5*,†       | 222.0*,†       |
|           | [279.5; 481.5]  | [309.5; 451.0]      | [261.5; 375.5]    | [266.0; 358.0] | [232.0; 276.0]  | [207.0; 305.0] |
| Fibrinogen (mg/dL) | 217.0          | 206.0               | 244.0*†          | 252.0*,†       | 247.0*         | 227.0         |
|           | [175.0; 263.0]  | [170.5; 244.5]      | [222.5; 284.5]    | [222.0; 292.0] | [216.0; 286.0]  | [184.0; 256.0] |
| aPTT (sec) | 44.5           | 38.1*               | 34.8*             | 33.6*          | 35.0*           | 34.9*         | 34.7*          |
|           | [39.5; 51.2]    | [32.7; 41.2]        | [31.7; 37.9]      | [31.5; 37.2]   | [33.1; 36.8]    | [33.1; 36.9]  | [34.0; 39.1]    |
| PT (sec)  | 11.9            | 11.5                | 11.7              | 11.5           | 12.1            | 12.4†         | 11.4           |
|           | [10.9; 13.9]    | [10.9; 12.1]        | [11.0; 12.6]      | [10.9; 12.0]   | [11.3; 12.6]    | [11.6; 13.6]  | [11.0; 11.8]    |
| ACT initial | 143.0          | 144.0               | 132.0*,§         | 132.0*         | 133.0          | 140.0         | 150.0          |
|           | [129.0; 153.5]  | [121.0; 143.0]      | [123.0; 136.0]    | [127.0; 142.0] | [131.0; 150.0]  | [145.0; 150.5] |
### Table 3. INTEM parameters of ROTEM measurement classified with age groups.

|                | Neonate (N=119) | Early infant (N=76) | Late infant (N=87) | Toddler (N=55) | Preschool (N=37) | School (N=32) | Adolescent (N=7) | p     |
|----------------|-----------------|---------------------|-------------------|----------------|-----------------|--------------|-----------------|-------|
| **CT**         |                 |                     |                   |                |                 |              |                 |       |
|                | 236.5           | 238.0               | 221.0<sup>*</sup> | 205.0<sup>*</sup> | 199.0<sup>*</sup> | 195.0<sup>*</sup> | 173.0<sup>*</sup> | <0.001 |
|                | [198.0; 297.0]  | [201.5; 282.0]      | [177.0; 301.0]    | [170.5; 242.5] | [169.0; 254.0]  | [166.5; 229.0] | [150.0; 202.0]  |       |
| **A10**        | 63.0            | 63.0                | 56.0<sup>*</sup>  | 54.0<sup>*</sup> | 53.0<sup>*</sup> | 53.0<sup>*</sup> | 49.0<sup>*</sup>  | <0.001 |
|                | [59.0; 67.0]    | [58.0; 65.0]        | [52.0; 62.0]      | [50.0; 57.5]   | [50.0; 56.0]    | [49.0; 57.0]   | [48.0; 54.0]    |       |
| **CFT**        | 50.5            | 52.0                | 69.0<sup>*</sup>  | 79.0<sup>*</sup> | 79.0<sup>*</sup> | 86.5<sup>*</sup> | 88.0<sup>*</sup>  | <0.001 |
|                | [42.0; 68.0]    | [44.0; 67.0]        | [53.5; 89.0]      | [63.0; 94.5]   | [65.0; 105.0]   | [71.5; 105.0]  | [66.5; 97.5]    |       |
| **MCF**        | 67.0            | 67.0                | 62.0<sup>*</sup>  | 59.0<sup>*</sup> | 59.0<sup>*</sup> | 60.0<sup>*</sup> | 57.0<sup>*</sup>  | <0.001 |
|                | [64.0; 71.0]    | [63.0; 69.0]        | [58.0; 67.0]      | [56.0; 64.0]   | [57.0; 64.0]    | [56.0; 65.0]   | [54.0; 60.0]    |       |
| **α**          | 80.0            | 79.0                | 77.0<sup>*</sup>  | 74.5<sup>*</sup> | 74.0<sup>*</sup> | 73.0<sup>*</sup> | 73.0<sup>*</sup>  | <0.001 |
|                | [76.0; 82.0]    | [76.0; 81.0]        | [72.0; 79.0]      | [71.0; 77.0]   | [70.0; 77.0]    | [70.0; 76.0]   | [71.0; 76.5]    |       |
| **LI60**       | 94.0            | 93.0                | 91.5              | 92.5            | 94.0            | 92.0          | 92.0            | 0.14  |
|                | [92.0; 96.0]    | [91.0; 95.0]        | [88.5; 95.0]      | [90.0; 95.5]   | [89.5; 96.0]    | [90.0; 93.0]   |                |       |
| **MCE**        | 206.0           | 205.0               | 164.0<sup>*</sup> | 144.0<sup>*</sup> | 147.0<sup>*</sup> | 149.5<sup>*</sup> | 134.0<sup>*</sup> | <0.001 |
|                | [181.0; 245.0]  | [170.0; 224.5]      | [136.5; 199.5]    | [128.0; 176.0] | [131.0; 176.0]  | [128.5; 185.0] | [120.0; 148.5]  |       |

Data are median [interquartile range]. CT – clotting time; A10 – amplitude at 10 minutes after CT; CFT – clot formation time; MCF – maximal clot firmness; α – alpha angle; LI60 – clot lysis index at 60 minute; MCE – maximal clot elasticity. * p<0.05, compared to Neonate; † p<0.05, compared to Early Infant; ‡ p<0.01, compared to Neonate; † p<0.001, compared to Early Infant; † p<0.01, compared to Toddler; † p<0.001, compared to Toddler.
Table 5. FIBTEM parameters of ROTEM measurement classified with age groups.

| Age Group          | Neonate (N=119) | Early Infant (N=76) | Late Infant (N=87) | Toddler (N=55) | Preschool (N=37) | School (N=32) | Adolescent (N=7) | p     |
|--------------------|-----------------|---------------------|--------------------|---------------|------------------|---------------|----------------|-------|
| A10                | 18.0 [14.0; 21.0] | 16.0 [13.0; 19.0]   | 13.0* [10.0; 17.0] | 12.0** [9.0; 15.5] | 12.0** [9.0; 14.0] | 11.5* [9.0; 17.0] | 11.0 [7.0; 14.0] | <0.001 |
| MCF                | 19.0 [15.0; 23.5] | 17.0 [14.0; 21.0]   | 14.0* [11.0; 19.0] | 13.0* [10.0; 17.5] | 13.0* [11.0; 16.0] | 13.5* [10.0; 18.0] | 12.0 [8.0; 16.0] | <0.001 |
| MCE                | 24.0 [18.0; 30.5] | 20.5 [16.0; 26.0]   | 16.0* [12.0; 23.0] | 15.0* [11.0; 21.0] | 15.0* [12.0; 19.0] | 15.5* [11.5; 21.5] | 13.0 [8.0; 19.5] | <0.001 |

Data are median [interquartile range]. A10 – amplitude at 10 minutes after CT; MCF – maximal clot firmness; MCE – maximal clot elasticity. * p<0.001; compared to Neonate; † p<0.05, compared to Early Infant; ‡ p<0.01, compared to Early Infant; § p<0.05, compared to Neonate.

Table 6. INTEM, EXTEM and FIBTEM parameters of ROTEM measurement classified with acyanotic and cyanotic diagnosis.

| Diagnosis          | Acyanosis (N=344) | Cyanosis (N=69) | p     |
|--------------------|-------------------|-----------------|-------|
| INTEM              |                   |                 |       |
| CT                 | 216.0 [181.0; 272.0] | 227.0 [187.0; 295.0] | 0.353 |
| A10                | 59.0 [54.0; 64.0]  | 55.0 [47.0; 59.0] | <0.001 |
| CFT                | 62.0 [47.0; 81.0]  | 85.0 [55.0; 113.0] | <0.001 |
| MCF                | 65.0 [60.0; 68.0]  | 62.0 [55.0; 66.0] | 0.001 |
| α                  | 77.0 [74.0; 80.0]  | 73.0 [69.0; 79.0] | <0.001 |
| LI60               | 93.0 [91.0; 95.0]  | 94.0 [92.0; 97.0] | 0.008 |
| MCE                | 182.0 [148.0; 216.0] | 164.0 [124.0; 195.0] | 0.001 |
| EXTEM              |                   |                 |       |
| CT                 | 51.0 [45.0; 59.0]  | 56.0 [48.0; 65.0] | 0.014 |
| A10                | 60.0 [54.0; 64.0]  | 56.0 [48.0; 60.0] | <0.001 |
| CFT                | 67.0 [56.0; 86.0]  | 77.0 [64.0; 111.0] | <0.001 |
| MCF                | 65.0 [61.0; 69.0]  | 62.0 [56.0; 67.0] | <0.001 |
| α                  | 77.0 [73.0; 79.0]  | 74.0 [68.0; 77.0] | <0.001 |
| LI60               | 91.0 [88.0; 94.0]  | 92.0 [88.0; 96.0] | 0.155 |
| MCE                | 187.0 [158.0; 222.5] | 160.0 [128.0; 200.0] | <0.001 |
| FIBTEM             |                   |                 |       |
| A10                | 15.0 [11.0; 19.0]  | 12.0 [10.0; 16.0] | <0.001 |
| MCF                | 16.0 [12.0; 21.0]  | 14.0 [11.0; 17.0] | 0.007 |
| MCE                | 19.0 [14.0; 27.0]  | 17.0 [12.0; 21.0] | 0.009 |

Data are median [interquartile range]. CT – clotting time; A10 – amplitude at 10 minutes after CT; CFT – clot formation time; MCF – maximal clot firmness; α – alpha angle; LI60 – clot lysis index at 60 minute; MCE – maximal clot elasticity.
### Table 7. 95% reference intervals and 90% confidence intervals of lower and upper reference limits of INTEM parameters of ROTEM measurement.

| Parameter | Neonate (n=119) | Early infant (n=76) | Late infant (n=87) | Toddler (n=55) | Preschool (n=37) | School (n=32) | Adolescent (n=7) |
|-----------|------------------|---------------------|-------------------|---------------|-----------------|--------------|----------------|
| CT        | Ref intervals [5] | 105–285             | 76–239            | 99–207        | 99–239          | 97–212       | 128–206        |
|           | 95% Ref Interval  | 94–383              | 102–374           | 44–402        | 104–307         | 73–327       | 91–304         |
|           | 90% CI            | Lower 76–113         | 80–123            | 14–80         | 85–124          | 39–100       | 66–113         |
|           |                   | Upper 358–409        | 343–402           | 364–437       | 286–329         | 283–369      | 277–339        |
| A10       | Ref intervals [5] | 50–72               | 47–70             | 45–67         | 45–68           | 45–66        | 48–67          |
|           | 95% Ref Interval  | 57–77               | 57–75             | 50–73         | 47–71           | 45–74        | 47–73          |
|           | 90% CI            | Lower 56–58          | 55–59             | 48–52         | 45–49           | 38–51        | 43–50          |
|           |                   | Upper 76–79          | 73–76             | 71–75         | 69–74           | 68–81        | 69–77          |
| CFT       | Ref intervals [5] | 27–88               | 37–100            | 42–112        | 40–94           | 48–93        | 45–106         |
|           | 95% Ref Interval  | 22–120              | 31–91             | 29–158        | 43–145          | 35–195       | 48–162         |
|           | 90% CI            | Lower 19–26          | 28–33             | 25–34         | 37–49           | 26–46        | 41–54          |
|           |                   | Upper 100–145        | 83–100            | 135–186       | 126–166         | 151–269      | 138–191        |
| MCF       | Ref intervals [5] | 54–71               | 52–73             | 56–72         | 53–73           | 53–69        | 54–71          |
|           | 95% Ref Interval  | 58–78               | 58–76             | 51–74         | 47–73           | 46–75        | 47–74          |
|           | 90% CI            | Lower 56–59          | 57–60             | 48–53         | 44–49           | 40–53        | 44–51          |
|           |                   | Upper 77–80          | 75–78             | 71–76         | 70–76           | 68–81        | 70–77          |
| α         | Ref intervals [5] | 74–85               | 73–83             | 70–82         | 72–82           | 72–80        | 70–81          |
|           | 95% Ref Interval  | 67.5–92.3           | 68.1–90.1         | 63.0–90.0     | 58.7–90.4       | 59.7–87.4    | 63.8–81.9      |
|           | 90% CI            | Lower 63.7–71.4      | 63.0–74.0         | 59.5–65.9     | 52.4–64.3       | 54.4–64.4    | 57.5–67.6      |
|           |                   | Upper 89.0–96.3      | 84.5–95.3         | 87.1–93.2     | 84.8–96.9       | 82.8–92.5    | 79.6–83.9      |
| LI60      | 95% Ref Interval  | 89–100              | 87–99             | 87–100        | 83–100          | 84–101       | 84–104         |
|           | 90% CI            | Lower 88–90          | 86–88             | 86–89         | 82–85           | 81–86        | 79–88          |
|           |                   | Upper 99–101         | 97–100            | 99–101        | 98–102          | 99–104       | 101–108        |
| MCE       | 95% Ref Interval  | 110–310             | 122–280           | 89–244        | 67–221          | 83–224       | 69–239         |
|           | 90% CI            | Lower 97–121         | 108–132           | 77–100        | 50–81           | 63–103       | 47–90          |
|           |                   | Upper 295–326        | 267–292           | 232–257       | 203–238         | 204–245      | 218–263        |

CI – confidence interval; CT – clotting time; A10 – amplitude at 10 minutes after CT; CFT – clot formation time; MCF – maximal clot firmness; α – alpha angle; LI60 – clot lysis index at 60 minute; MCE – maximal clot elasticity.
Table 8. 95% reference intervals and 90% confidence intervals of lower and upper reference limits of EXTEM parameters of ROTEM measurement.

| Parameter | Neonate (n=119) | Early Infant (n=76) | Late Infant (n=87) | Toddler (n=55) | Preschool (n=37) | School (n=32) | Adolescent (n=7) |
|-----------|-----------------|---------------------|-------------------|--------------|-----------------|-------------|-----------------|
| CT Ref intervals [5] | 38–65 | 37–77 | 37–73 | 46–97 | 43–74 | 44–91 |
| 95% Ref Interval Lower | 28–32 | 25–31 | 27–36 | 24–34 | 29–39 | 20–37 |
| 90% CI Upper | 62–67 | 60–68 | 74–83 | 77–89 | 79–90 | 75–94 |
| A10 Ref intervals [5] | 51–72 | 46–68 | 41–68 | 49–68 | 49–65 | 49–67 |
| 95% Ref Interval Lower | 50–56 | 55–58 | 51–54 | 45–50 | 50–54 | 45–51 |
| 90% CI Upper | 77–83 | 73–76 | 71–73 | 70–76 | 69–74 | 70–77 |
| CFT Ref intervals [5] | 30–105 | 44–146 | 46–139 | 41–109 | 49–114 | 53–115 |
| 95% Ref Interval Lower | 13–28 | 32–37 | 13–40 | 25–45 | 25–52 | 14–43 |
| 90% CI Upper | 88–104 | 87–107 | 106–135 | 174–309 | 126–155 | 142–178 |
| MCF Ref intervals [5] | 54–74 | 46–71 | 46–72 | 52–70 | 53–68 | 53–72 |
| 95% Ref Interval Lower | 52–58 | 56–60 | 51–54 | 45–51 | 50–55 | 45–51 |
| 90% CI Upper | 78–84 | 75–78 | 72–75 | 71–77 | 70–75 | 70–77 |
| α Ref intervals [5] | 69–84 | 68–82 | 64–81 | 69–82 | 67–80 | 67–80 |
| 95% Ref Interval Lower | 59–72 | 52–75 | 50–73 | 52–75 | 50–75 | 45–69 |
| 90% CI Upper | 84–86 | 83–94 | 81–92 | 79–83 | 79–83 | 84–106 |
| LI60 Ref intervals [5] | 71–94 | 71–95 | 77–94 | 74–93 | 70–97 | 76–94 |
| 95% Ref Interval Lower | 73–83 | 77–82 | 81–85 | 82–85 | 82–89 | 76–94 |
| 90% CI Upper | 109–111 | 109–111 | 98–101 | 100–106 | 100–106 | 88–101 |
| MCE 95% Ref Interval Lower | 114–312 | 121–292 | 94–246 | 77–232 | 96–240 | 73–252 |
| 90% CI Upper | 299–325 | 277–306 | 234–259 | 216–248 | 222–257 | 231–277 |

CI – confidence interval; CT – clotting time; A10 – amplitude at 10 minutes after CT; CFT – clot formation time; MCF – maximal clot firmness; α – alpha angle; LI60 – clot lysis index at 60 minute; MCE – maximal clot elasticity.
Table 9. 95% reference intervals and 90% confidence intervals of lower and upper reference limits of FIBTEM parameters of ROTEM measurement.

|                  | Neonate (n=119) | Early infant (n=76) | Late infant (n=87) | Toddler (n=55) | Preschool (n=37) | School (n=32) | Adolescent (n=7) |
|------------------|-----------------|---------------------|--------------------|----------------|------------------|---------------|-----------------|
| A10 95% Ref Interval | 10–35           | 9–30                | 6–30               | 7–28           | 6–28             | 4–36          | 3–42            |
| 95% CI Lower      | 9–11            | 8–10                | 5–7                | 6–8            | 5–7              | 3–6           | 0.7–5           |
|                  | 32–39           | 28–33               | 27–35              | 25–32          | 23–33            | 27–48         | 25–214          |
| A10 MCF Reference [5] | 8–23            | 7–25                | 6–24               | 7–23           | 7–22             | 8–24          |                 |
| 95% CI Lower      | 9–11            | 8–10                | 5–7                | 5–7            | 5–7              | 4–6           | 1–6             |
|                  | 33–40           | 29–36               | 28–36              | 24–33          | 21–32            | 30–50         | 25–209          |
| A10 MCE Reference [5] | 10–52           | 10–44               | 6–44               | 6–37           | 6–37             | 5–48          | 3–62            |
| 90% CI Lower      | 1–12            | 9–11                | 5–7                | 5–8            | 4–7              | 4–6           | 1–6             |
|                  | 47–59           | 39–50               | 36–54              | 31–46          | 28–47            | 35–65         | 34–479          |

CI – confidence interval; CT – clotting time; A10 – amplitude at 10 minutes after CT; CFT – clot formation time; MCF – maximal clot firmness; α – alpha angle; Li60 – clot lysis index at 60 minute; MCE – maximal clot elasticity.

Discussion

Patients undergoing surgical interventions for CHD before 3 months of age had a coagulation profile of faster initiation and propagation of clot formation and more enhanced clot strength when compared to older patients. On the other hand, patients with cyanotic CHD showed slower initiation and propagation of clot formation and weaker clot strength compared to patients with acyanotic diseases. Reference intervals obtained from pediatric patients undergoing surgical interventions for CHD had similar results and pattern to those obtained from otherwise healthy pediatric patients [5].

For a specific test or measurement, it is commonly recommended that a laboratory or institute determine their own reference intervals from their own patient population [5,13,14]. Test results depend not only on pre-analytic factors, such as sampling technique and handling or storage of samples, but also on characteristics of the particular patient population. Therefore, this retrospective investigation was planned to obtain reference intervals of thromboelastometric evaluation for coagulation from pediatric patients with CHD in our institution. ROTEM® measurement does have its own reference intervals embedded in the ROTEM® machine and depicted as a “normal guideline”, which enables quick and intuitive interpretation of ongoing measurement result. However, for pediatric patients with CHD, such as in this study, this convenient, real-time interpretation could be inaccurate or misleading because the reference intervals were obtained from an otherwise healthy adult population.

There have been few investigations that compared ROTEM® parameters of pediatric patients with CHD, mostly cyanotic, to those of otherwise healthy pediatric patients or pediatric patients with acyanotic CHD [6,7]. These investigations were, however, done in a limited number of patients and were designed for comparison, not to obtain the reference intervals. The CLSI task force recommended that if the reference population did not reach 120 individuals, which is the minimal number of reference individuals they recommend, a specific formula has to be utilized to calculate reference intervals. One investigation calculated reference intervals of ROTEM® parameters from a large number of otherwise healthy pediatric patients undergoing non-cardiac/minor surgical procedures following CLSI guidelines on reference intervals [5]; when the reference intervals calculated in were compared to those calculated from otherwise healthy pediatric patients, both reference intervals overlapped, but the reference intervals had a tendency to have broader ranges compared to that of otherwise healthy pediatric patients. Furthermore, both reference intervals had a similar age-related pattern of longer INTEM CT but shorter EXTEM CT, shorter CFT and steeper α in INTEM and EXTEM, and greater A10 and MCF in INTEM, EXTEM, and FIBTEM in neonates and infants, compared to those in older children.
For prolongation of INTEM CT, our data (>200 s) was longer than that of otherwise healthy pediatric patients (>170) [5], but shorter than that of pediatric patients with complex CHD, mostly cyanotic CHD (>250) [6]. The prolongation of INTEM CT could be attributed to cyanosis, because, although statistically not significant, patients with cyanotic CHD in this investigation were also shown to have prolonged INTEM CT compared to patients with acyanotic CHD. The EXTEM CT was shown to have a reversed direction against the INTEM CT, shorter than that in older children. This age-related discrepancy in rate of clot initiation between the contact-activated and tissue factor-activated coagulation pathways was not clearly defined because coagulation factor assays are not included in routine preoperative evaluation, but some of the difference in maturation of respective coagulation activators could be attributed to such a discrepancy [5].

Blood cell count and plasmatic coagulation test parameters showed a similar age-related pattern to the results of an investigation by Oswald et al. [5]; red blood mass and fibrinogen levels were elevated with advancing age, but platelets were higher in neonates and infants. The aPTT was prolonged in neonates and infants, but PT was not significantly different among the age groups. These findings are, however, within age-specific reference ranges of blood cell count and plasmatic coagulation tests in healthy pediatric patient population [15].

The age-related pattern of “hypercoagulability” in neonates and infants younger than 3 months of age have been previously demonstrated [5,6,16]. Although the plasmatic coagulation test results of aPTT were prolonged and fibrinogen levels were reduced in neonates and infants compared to older children, functional profile of coagulation was not compromised. Most importantly, reference intervals calculated from pediatric patients with CHD overlapped with the reference intervals calculated from adults [4]. Even the data from patients with cyanotic CHD, a group known to have a certain defect in coagulation [17], were within the reference intervals calculated from adults. Additionally, hyperfibrinolysis, which was reported to be a primary mechanism for coagulation abnormality, especially in patients with cyanotic CHD [17], was not observed preoperatively in this investigation, even in patients with cyanotic CHD. Therefore, patients with CHD, even those with cyanotic CHD, are not to be regarded as having “impaired” coagulation, but rather as having an exaggerated coagulation profile with greater variability.

There are several limitations in the present investigation. First, the number of patients was limited, especially for adolescents, so confidence intervals for several ROTEM parameters could not be calculated. To the best of our knowledge, our investigation is the first to recruit more than 100 pediatric patients with CHD to obtain reference intervals of ROTEM parameters, but further study is needed for refined calculation, with more than 120 patients in each age group. Second, assays of coagulation factors were not included in the routine preoperative evaluation. With such information, further data to explain results of this investigation, for example, the discrepancy between INTEM and EXTEM CTs, would have been possible. Lastly, although the outliers were intrinsically removed within a process of calculation of reference intervals, some moderate outliers would be included for final calculation, and it could be attributed to the broader ranges of reference intervals used in this investigation compared to those calculated prospectively.

**Conclusions**

The reference intervals of preoperative results of thromboelastometric evaluation for blood coagulation from pediatric patients with CHD calculated in this investigation were shown to have a similar pattern to reference intervals obtained from otherwise healthy pediatric patients. Pediatric patients with CHD, even those with cyanotic CHD, were demonstrated to have functionally intact coagulation profile before surgery for CHD.

**Statement**

All sources were departmental source. All authors declare no competing interests.

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