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

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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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 CO_2 of 35-40 mmHg, anesthesia was maintained with sevoflurane in O_2/air with FiO_2 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 an 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

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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). For FIBTEM, parameters such as A10, MCF, and MCE, which evaluate clot strength, were only retrieved for analysis, because other parameters, including CT, CFT, α, and LI60, are usually not used for evaluation of fibrin polymerization [4]. 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.

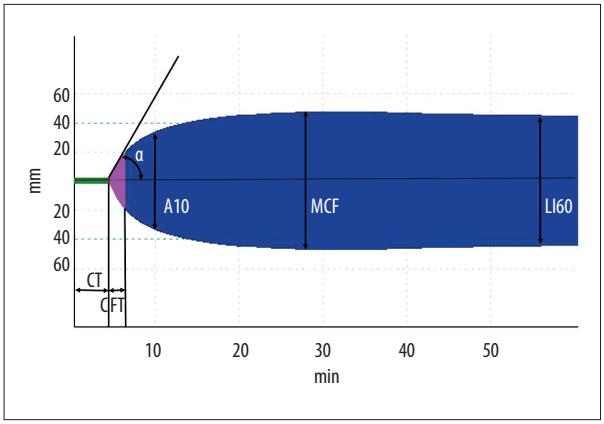

Figure 1. Rotational thromboelastometry (ROTEM®) parameters. CT – clotting time, CFT – clot formation time, A10 – amplitude at 10 min after CT, α – alpha angle, MCF – maximal clot firmness, LI60 – clot lysis index at 60 min.

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 Wilcox 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 Finnegan (2014). referenceIntervals: 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.

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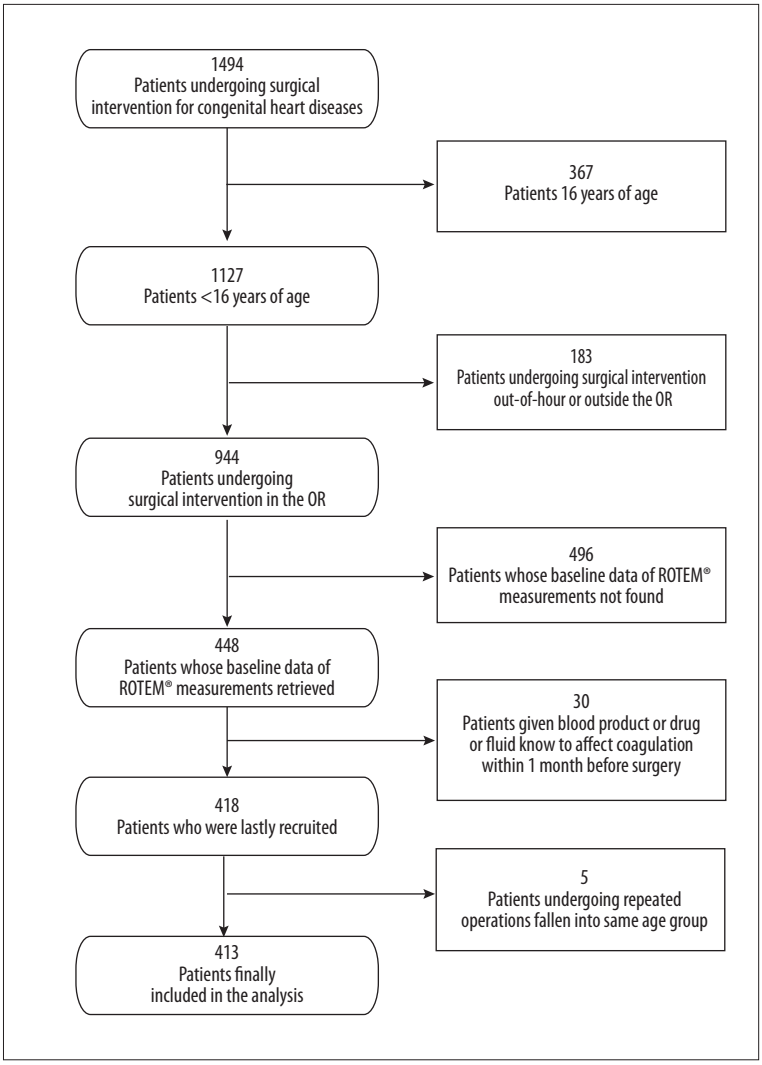

Figure 2. Flow chart representing the process of patient recruitment and selection.

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	15.9±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	
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%)	30 (54.5%)	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	
BSA (m^2)	0.20±0.03	0.24±0.04	0.31±0.05	0.50±0.07	0.64±0.10	0.95±0.17	1.33±0.19	
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.

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Table 2. Hematologic and plasmatic coagulation parameters 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
Hb (g/dL)	12.9 [11.2; 14.4]	11.1* [10.2; 11.8]	11.4* [10.8; 12.6]	12.9†,‡ [11.9; 13.9]	13.7*,†,‡ [12.8; 16.1]	12.9†,‡ [12.2; 13.9]	14.5*,†,‡,§,@ [13.9; 15.3]	<0.001
Hct (%)	38.5 [33.2; 43.0]	32.5* [29.8; 36.0]	34.1* [31.8; 37.5]	37.3†,‡ [35.5; 39.8]	39.8†,‡ [36.5; 49.8]	37.9†,‡ [35.9; 40.4]	42.0*,†,‡,§, @ [41.5; 44.5]	<0.001
Platelet ($\times 10^3/\mu l$)	404.0 [279.5; 481.5]	444.0 [337.5; 534.5]	387.0 [309.5; 451.0]	320.0†,‡ [261.5; 375.5]	307.0*,†,‡ [266.0; 358.0]	255.5*,†, ‡,§,# [232.0; 276.0]	222.0*,†, ‡ [207.0; 305.0]	<0.001
Fibrinogen (mg/dL)	217.0 [175.0; 263.0]	214.0 [182.0; 248.0]	206.0 [170.5; 244.5]	244.0*,†, ‡ [222.5; 284.5]	252.0*,†, ‡ [222.0; 292.0]	247.0‡ [216.0; 286.0]	227.0 [184.0; 256.0]	<0.001
aPTT (sec)	44.5 [39.5; 51.2]	38.1* [32.7; 41.2]	34.8* [31.7; 37.9]	33.6* [31.5; 37.2]	35.0* [33.1; 36.8]	34.9* [33.1; 36.9]	34.7* [34.0; 39.1]	<0.001
PT (sec)	11.9 [10.9; 13.9]	11.5 [10.9; 12.1]	11.7 [11.0; 12.6]	11.5 [10.9; 12.0]	12.1 [11.3; 12.6]	12.4§ [11.6; 13.6]	11.4 [11.0; 11.8]	0.008
ACT initial	143.0 [129.0; 153.5]	144.0 [132.5; 148.5]	132.0*,† [121.0; 143.0]	132.0* [123.0; 136.0]	133.0 [127.0; 142.0]	140.0 [131.0; 150.0]	150.0 [140.5; 150.5]	<0.001

Data are median [interquartile range]. Hb – hemoglobin, Hct – hematocrit, PT – prothrombin time; aPTT – activated partial thromboplastin time. * p<0.05, compared to Neonate; † p<0.05, compared to Early Infant; ‡ p<0.05, compared to Late Infant; § p<0.05, compared to Toddler; @ p<0.05, compared to School; # p<0.05, compared to Preschool.

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 early and late infants compared to that in children of older age groups (all p<0.05). The aPTT was significantly prolonged in neonates in comparison to other age groups (all p<0.05).

INTEM parameters were significantly different among all age group (all p<0.001 except LI60, in which p=0.014). Compared to those in late infant, toddler, preschool, and school age groups, CT was significantly prolonged, CFT was significantly shorter, and α was steeper in neonates and early infants (all p<0.05). Compared to all other age groups, A10, MCF, and MCE were significantly greater in neonates and early infants (all p<0.05) (Table 3). With LI60, no between-group difference was found with post hoc analysis. Apart from INTEM results, CT in EXTEM was significantly shorter in neonates and early infants compared to all other age groups. Other parameters showed a similar pattern to INTEM results: significantly shorter CFT and steeper α, and greater A10, MCF and MCE in neonates and early infants compared to all other age groups (Table 4). For FIBTEM parameters, A10, MCF, and MCE were significantly greater in neonates and infants compared to those in late infant, toddler, and preschool age groups (Table 5).

The results of ROTEM® measurements were classified on the basis of preoperative diagnosis of cyanotic and acyanotic CHD (Table 6). Although prolongation of CT in INTEM in cyanotic patients was not statistically significant compared to acyanotic patients, ROTEM® parameters of INTEM and EXTEM assays in cyanotic patients were shown to have significantly prolonged CT and CFT, and significantly reduced α, A10, MCF, and MCE. 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].

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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 [198.0; 297.0]	238.0 [201.5; 282.0]	221.0*,† [177.0; 301.0]	205.0*,† [170.5; 242.5]	199.0* [169.0; 254.0]	195.0*,† [166.5; 229.0]	173.0*,† [150.0; 202.0]	<0.001
A10	63.0 [59.0; 67.0]	63.0 [58.0; 65.0]	56.0‡,§ [52.0; 62.0]	54.0‡,§ [50.0; 57.5]	53.0‡,§ [50.0; 56.0]	53.0‡,§ [49.0; 57.0]	49.0‡,@ [48.0; 54.0]	<0.001
CFT	50.5 [42.0; 68.0]	52.0 [44.0; 67.0]	69.0‡,§ [53.5; 89.0]	79.0‡,§ [63.0; 94.5]	79.0‡,§ [65.0; 105.0]	86.5‡,§ [71.5; 105.0]	88.0# [66.5; 97.5]	<0.001
MCF	67.0 [64.0; 71.0]	67.0 [63.0; 69.0]	62.0‡,§ [58.0; 67.0]	59.0‡,§ [56.0; 64.0]	59.0‡,§ [57.0; 64.0]	60.0‡,§ [56.0; 65.0]	57.0@,# [54.5; 60.0]	<0.001
α	80.0 [76.0; 82.0]	79.0 [76.0; 81.0]	77.0‡,§ [72.0; 79.0]	74.5‡,§ [71.0; 77.0]	74.0‡,§ [70.0; 77.0]	73.0‡,§ [70.0; 76.0]	73.0* [71.0; 76.5]	<0.001
LI60	94.0 [92.0; 96.0]	93.0 [91.0; 95.0]	93.0 [91.0; 94.5]	91.5 [88.5; 95.0]	92.5 [90.0; 95.5]	94.0 [89.5; 96.0]	92.0 [90.0; 93.0]	0.014
MCE	206.0 [181.0; 245.0]	205.0 [170.0; 224.5]	164.0‡,§ [136.5; 199.5]	144.0‡,§ [128.0; 176.0]	147.0‡,§ [131.0; 176.0]	149.5‡,§ [128.5; 185.0]	134.0†,# [120.0; 148.5]	<0.001

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<0.001, compared to Neonate; § p<0.001, compared to Early Infant; @ p<0.01; compared to Early Infant; # p<0.01, compared to Neonate.

Table 4. EXTEM 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	47.0 [41.5; 54.0]	45.0 [40.5; 52.0]	55.0*,† [49.0; 63.0]	58.0‡,§ [46.0; 65.0]	56.0*,† [52.0; 68.0]	57.0*,† [51.5; 65.0]	62.0@,§ [56.0; 64.0]	<0.001
A10	63.0 [58.5; 67.5]	63.0 [59.0; 66.5]	56.0*,† [52.5; 61.5]	54.0*,† [47.5; 58.5]	56.0*,† [49.0; 59.0]	54.0*,† [48.0; 59.0]	48.0*,† [46.0; 54.0]	<0.001
CFT	59.0 [50.5; 70.0]	58.0 [49.0; 69.5]	72.0*,† [60.0; 87.5]	86.0*,† [69.5; 117.5]	86.0*,† [68.0; 108.0]	86.5*,† [74.0; 113.5]	101.0*,† [75.0; 110.5]	<0.001
MCF	68.0 [63.5; 71.0]	67.0 [63.0; 70.0]	63.0*,† [60.0; 66.0]	60.0*,† [56.0; 64.5]	63.0*,† [60.0; 66.0]	61.0*,§ [56.0; 66.5]	56.0*,† [54.5; 60.0]	<0.001
α	78.0 [76.0; 80.0]	78.0 [76.0; 80.0]	75.0*,† [72.0; 78.0]	73.0*,† [68.0; 76.0]	73.0*,† [69.0; 76.0]	72.0*,† [68.0; 75.0]	70.0*,† [68.5; 74.5]	<0.001
LI60	92.0 [89.0; 95.0]	90.0 [86.0; 92.5]	91.0 [88.0; 93.0]	91.0 [88.5; 95.0]	91.5 [89.0; 94.0]	94.0 [92.0; 96.0]	92.0 [90.0; 92.0]	0.038
MCE	212.0 [174.0; 248.0]	204.5 [171.5; 234.0]	170.0*,† [149.5; 196.5]	153.0*,† [126.5; 181.5]	168.0*,† [147.0; 194.0]	154.5*,† [128.5; 196.5]	128.0*,† [118.0; 150.0]	<0.001

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<0.001, compared to Neonate; † p<0.001, compared to Early Infant; ‡ p<0.01, compared to Neonate; § p<0.01, compared to Early Infant; @ p<0.05, compared to Neonate.

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Table 5. FIBTEM 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
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.

		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.

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Table 7. 95% reference intervals and 90% confidence intervals of lower and upper reference limits of INTEM 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)
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	91–304
	90% CI	Lower	76–113	80–123	14–80	85–124	39–100	66–113	66–113
		Upper	358 – 409	343–402	364–437	286–329	283–369	277–339	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	43–71
	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	36–191
	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	46–72
	90% CI	Lower	56–59	57–60	48–53	44–49	40–53	44–51	41–49
		Upper	77–80	75–78	71–76	70–76	68–81	70–77	65–83
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	63.1–85.1
	90% CI	Lower	63.7–71.4	63.0–74.0	59.5–65.9	52.4–64.3	54.4–64.4	60.8–66.3	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	77.6–95.5
LI60	95% Ref Interval		89–100	87–99	87–100	83–100	84–101	84–104	84–99
	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	76–234
	90% CI	Lower	97–121	108–132	77–100	50–81	63–103	47–90	50–89
		Upper	295–326	267–292	232–257	203–238	204–245	218–263	182–363

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.

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Table 8. 95% reference intervals and 90% confidence intervals of lower and upper reference limits of EXTEM 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)
CT	Ref intervals [5]		38–65		37–77	37–73	46–97	43–74	44–91
	95% Ref Interval		30–65	28–64	31–78	28–83	35–84	29–84	40–80
	90% CI	Lower	28–32	25–31	27–36	24–34	29–39	20–37	16–47
		Upper	62–67	60–68	74–83	77–89	79–90	75–94	74–105
A10	Ref intervals [5]		51-72		46–68	41–68	49–68	49–65	49–67
	95% Ref Interval		58–80	57–74	52–72	48–73	52–75	48–74	45–69
	90% CI	Lower	50–56	55–58	51–54	45–50	50–54	45–51	29–48
		Upper	77–83	73–76	71–73	70–76	69–74	70–77	65–84
CFT	Ref intervals [5]		30–105		44–146	46–139	41–109	49–114	53–115
	95% Ref Interval		21–96	35–96	26–121	35–224	35–139	30–157	38–229
	90% CI	Lower	13–28	32–37	13–40	25–45	25–52	14–43	
		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		55–81	58–77	53–74	48–74	53–73	48–74	45–69
	90% CI	Lower	52–58	56–60	51–54	45–51	50–55	45–51	
		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		71.5–85.0	67.0–89.3	63.1–87.4	56.2–88.5	62.8–82.6	61.8–81.7	55.5–88.0
	90% CI	Lower	70.5–72.6	61.4–72.7	57.8–68.9	50.2–60.5	59.6–65.2	59.1–64.3	34.9–64.8
		Upper	84.0–86.1	83.5–94.6	81.6–92.2	84.0–94.6	79.5–84.3	79.4–83.9	84.6–106.0
LI60	Ref intervals [5]		71–94		71–95	77–94	74–93	70–97	76–94
	95% Ref Interval		78–106	79–100	83–100	84–99	83–100	86–103	85–98
	90% CI	Lower	73–83	77–82	81–85	82–85		82–89	
		Upper	102–111	99–102	98–101	98–101		100–106	
MCE	95% Ref Interval		114–312	121–292	94–246	77–232	96–240	73–252	68–198
	90% CI	Lower	101–126	108–133	83–105	62–93	80–112	53–94	
		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.

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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	Ref intervals [5]		8–22		6–24	5–24	7–22	7–21	3–22
	95% Ref Interval		10–35	9–30	6–30	7–28	6–28	4–36	3–42
	90% CI	Lower	9–11	8–10	5–7	6–8	5–7	3–6	0.7–5
		Upper	32–39	28–33	27–35	25–32	23–33	27–48	25–214
MCF	Ref intervals [5]		8–23		7–25	6–24	7–23	7–22	8–24
	95% Ref Interval		10–36	9–32	6–32	6–28	6–27	5–39	3–41
	90% CI	Lower	9–11	8–10	5–7	5–7	5–7	4–6	1–6
		Upper	33–40	29–36	28–36	24–33	21–32	30–50	25–209
MCE	95% Ref Interval		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
		Upper	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.

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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 level 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 level was 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.

References:

1. Spiess BD, Gillies BS, Chandler W, Verrier E: Changes in transfusion therapy and reexploration rate after institution of a blood management program in cardiac surgical patients. J Cardiothorac Vasc Anesth, 1995; 9: 168–73
2. Shore-Lesserson L, Manspeizer HE, DePerio M et al: Thromboelastography-guided transfusion algorithm reduces transfusions in complex cardiac surgery. Anesth Analg, 1999; 88: 312–19
3. Nakayama Y, Nakajima Y, Tanaka KA et al: Thromboelastometry-guided intraoperative haemostatic management reduces bleeding and red cell transfusion after paediatric cardiac surgery Br J Anaesth, 2015; 114: 91–102
4. Lang T, Bauters A, Braun SL et al: Multi-centre investigation on reference ranges for ROTEM thromboelastometry. Blood Coagul Fibrinolysis, 2005; 16: 301–10
5. Oswald E, Stalzer B, Heitz E et al: Thromboelastometry (ROTEM) in children: Age-related reference ranges and correlations with standard coagulation tests. Br J Anaesth, 2010; 105: 827–35
6. Haizinger B, Gombotz H, Rehak P et al: Activated thrombelastogram in neonates and infants with complex congenital heart disease in comparison with healthy children. Br J Anaesth, 2006; 97: 545–52
7. Osthaus WA, Boethig D, Johanning K et al: Whole blood coagulation measured by modified thrombelastography (ROTEM) is impaired in infants with congenital heart diseases. Blood Coagul Fibrinolysis, 2008; 19: 220–25
8. Strauss T, Levy-Shraga Y, Ravid B et al: Clot formation of neonates tested by thromboelastography correlates with gestational age. Thromb Haemost, 2010; 103: 344–50

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9. Miller BE, Bailey JM, Mancuso TJ et al: Functional maturity of the coagulation system in children: an evaluation using thrombelastography. Anesth Analg, 1997; 84: 745–48
10. Gary LH: EP28-A3C (formerly C28-A3c): Defining, establishing, and verifying reference intervals in the clinical laboratory; Approved guideline. 3rd ed. Wayne (PA): Clinical and Laboratory Standards Institute, 2010
11. Horn PS, Pesce AJ: Reference intervals. A user's guide. Washington DC: AAPC Press, 2005
12. Konietschke F, Placzek M, Schaarschmidt F, Hothorn LA: Nparcomp. An R software package for nonparametric multiple comparisons and simultaneous confidence intervals. J Stat Software, 2015; 64: 1–17
13. Scarpelini S, Rhind SG, Nascimento B et al: Normal range values for thromboelastography in healthy adult volunteers. Braz J Med Biol Res, 2009; 42: 1210–17
14. Subramanian A, Albert V, Saxena R et al: Establishing a normal reference range for thromboelastography in North Indian healthy volunteers. Indian J Pathol Microbiol, 2014; 57: 43–50
15. Orkin SH, Nathan DG, Ginsburg D et al: Nathan and Oski's hematology of infancy and childhood. 7th ed. Philadelphia: Saunders/Elsevier, 2009; 1771–96
16. Cvirn G, Gallistl S, Rehak T et al: Elevated thrombin-forming capacity of tissue factor-activated cord compared with adult plasma. J Thromb Haemost, 2003; 1: 1785–90
17. Tempe DK, Virmani S: Coagulation abnormalities in patients with cyanotic congenital heart disease. J Cardiothorac Vasc Anesth, 2002; 16: 752–65

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