

PAEDIATRICS

Thromboelastometry (ROTEM®) in children: age-related reference ranges and correlations with standard coagulation tests

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Key points

- Monitoring whole-blood coagulation using viscoelasticity assays is useful in managing perioperative bleeding.
- This observational study compared thromboelastometry [ROTEM®] assays with standard coagulation tests in 359 children in six age groups.
- Age-dependent differences identified in both standard and ROTEM® assays provide important reference ranges for monitoring paediatric surgical patients.

Background. The small sample volume needed and the prompt availability of results make viscoelastic methods like rotational thromboelastometry (ROTEM®) attractive for monitoring coagulation in small children. However, data on reference ranges for ROTEM® parameters in children are scarce.

Methods. Four hundred and seven children (ASA I and II) undergoing elective surgery were recruited for this prospective, two-centre, observational study. Subjects were grouped as follows: 0–3, 4–12, 13–24 months, 2–5, 6–10, and 11–16 yr. Study objectives were to establish age-dependent reference ranges for ROTEM® assays, analyse age dependence of parameters, and compare ROTEM® data with standard coagulation tests.

Results. Data from 359 subjects remained for final analysis. Except for extrinsically activated clot strength and lysis, parameters for ROTEM® assays were significantly different among all age groups. The most striking finding was that subjects aged 0–3 months exhibited accelerated initiation (ExTEM coagulation time: median 48 s, Q1–Q3 38–65 s; $P=0.001$) and propagation of coagulation (α angle: median 78°, Q1–Q3 69–84°; $P<0.001$) and maximum clot firmness (median 62 mm, Q1–Q3 54–74 mm), although standard plasma coagulation test results were prolonged (prothrombin time: median 13.2 s, Q1–Q3 12.6–13.6 s; activated partial thromboplastin time: median 42 s, Q1–Q3 40–46 s). Lysis indices of <85% were observed in nearly one-third of all children without increased bleeding tendency. Platelet count and fibrinogen levels correlated significantly with clot strength, and fibrinogen levels correlated with fibrin polymerization.

Conclusions. Reference ranges for ROTEM® assays were determined for all paediatric age groups. These values will be helpful when monitoring paediatric patients and in studies of perioperative coagulation in children.

Keywords: blood, coagulation; gender; measurement techniques, thromboelastograph; paediatrics; thromboelastometry

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Viscoelastic coagulation tests have gained a renewed interest in the safe management of surgery- or trauma-induced coagulopathy in adult and paediatric patients.^{1–4} Viscoelastic whole-blood analyses, such as thromboelastography (TEG®) and thromboelastometry (ROTEM®), reflect initiation and propagation of coagulation, fibrinogen/fibrin-platelet interaction, and clot lysis. In 1997, the ROTEM® device was introduced into clinical routine at our institution. A multi-centre study published by Lang and colleagues⁵ presented reference ranges for ROTEM® assays for adults aged 17–85 yr. With the exception of some data referring to preterm

neonates, newborns, and children exhibiting cardiac failure, reference data are scarce for paediatric patients.^{6–10}

The main objective of our study was to investigate reference ranges for ROTEM® assays in a representative group of normal children undergoing elective surgery encompassing all paediatric age groups. Because platelet number, fibrinogen concentration, and coagulation factor XIII (FXIII) contribute particularly to the clinically relevant parameter clot strength, these parameters were analysed in addition to conducting standard coagulation tests and ROTEM® assays.

Methods

Study protocol

The study was conducted at two tertiary care hospitals. Innsbruck Medical University Hospital performs ~35 000 anaesthesia procedures per year. After 1 yr of recruitment, a second study site, Zurich University Children's Hospital, which performs about 7000 anaesthesia procedures per year, was included to facilitate recruitment within an acceptable time period.

The study was approved by the institutional Ethics Committee at each hospital. After written informed consent was received from parents for blood sampling, a total of 407 children (ASA physical status I–II) aged 0–16 yr who were undergoing minor surgery were enrolled in this prospective study.

Inclusion criteria were elective surgical procedures requiring placement of an i.v. catheter. Exclusion criteria were technical problems encountered in blood sampling, prematurity, age above 16 yr, known haematological disease, emergency surgery, acute systemic infection, known history of congenital or acquired coagulopathy including renal, hepatic, and bone marrow disease, any medication interfering with haemostasis, and cardiac disease. A standardized bleeding history was obtained based on the recommendation of the task force on perioperative coagulation by OEGARI (www.oegari.at/dateiarchiv/205/guidelines_english_version.pdf).

Age groups were defined according to the studies by Chan and colleagues¹¹ observing children <1 to 16 yr and the study of Andrew and colleagues¹² showing haemostatic maturation, especially during the first 3 months after birth. Six age groups were defined as follows: 0–3, 4–12, 13–24 months, 2–5, 6–10, and 11–16 yr. The scheduled group size was at least 50 children according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines for establishing reference ranges.¹³ Since the drop-out rate was expected to be high because of difficulties in blood sampling or because baseline laboratory test results were outside the reference range, a total of 407 children had to be recruited.

Routine patient management

All children were fasted until 4 h (<1 yr) or 6 h (>1 yr) before surgery and those who were older than 5 months received midazolam 0.5 mg kg⁻¹ orally 30–45 min or rectally 15–20 min before anaesthesia. Anaesthesia was induced by inhalation administration of sevoflurane in oxygen or i.v. by injection of fentanyl or alfentanil and propofol. Anaesthesia was then continued using either propofol or sevoflurane in oxygen/air or oxygen/nitrous oxide mixture, fentanyl or remifentanyl, and rocuronium or atracurium according to the planned surgical procedure.

Blood sampling

Using an 18, 20, 22, or 24 G catheter, blood samples were immediately obtained with a light tourniquet used to prevent blood stasis. If blood samples were drawn from already established i.v. lines, the first 2 ml of blood was

discarded. Blood samples were collected in 1.2 ml tubes containing 1.6 mg EDTA ml⁻¹ blood (Sarstedt, Nuembrecht, Germany) for measuring blood count and two 1.4 ml tubes containing 0.14 ml citrate solution (0.106 mol litre⁻¹ trisodium citrate solution) (Sarstedt) for analysing concentrations of FXIII, performing standard coagulation tests [prothrombin time (PT), activated partial thromboplastin time (aPTT), antithrombin, and fibrinogen concentration] and ROTEM[®] tests.

Laboratory analyses

Standard coagulation parameters were determined with the following assays (all reagents manufactured by Siemens Healthcare AG, Erlangen, Germany): PT (Thromborel S[®]), aPTT (Pathromtin SL[®]), antithrombin (Berichrom[®] Antithrombin III), fibrinogen concentration (Multifibren[®]), and FXIII (Berichrom[®]).

ROTEM[®]

Technical details on the ROTEM[®] device are presented elsewhere.¹

ROTEM[®] analyses were performed within 10 min after blood withdrawal by four anaesthetists highly experienced with ROTEM[®] analyses as bedside tests (Innsbruck Medical University) or by specialized laboratory personnel from the Central Laboratory (Zurich University Children's Hospital), all using original reagents purchased from Pentapharm, Munich, Germany. Defined standard operating procedures included warming sampling tubes to 37°C and gently inverting these three times to re-suspend any sediment before starting analysis. According to the manufacturer's instructions, extrinsically activated (ExTEM), intrinsically activated (InTEM), and fibrinogen polymerization (FibTEM) tests were performed. Data were collected for 60 min, and the ROTEM[®] parameters coagulation time (CT, s), clot formation time (CFT, s), α angle (ALP, °), amplitude recorded at 10 min (A10), and maximum clot firmness (MCF, mm) were registered (Fig. 1). The clot lysis index after 60 min (CLI60, %) describes the ratio between the MCF and amplitude at 60 min. To assess fibrin polymerization, the relevant parameters A10 and MCF were analysed.

In addition, an ApTEM test (aprotinin-containing extrinsically activated test) was performed in the last 196 children if signs of fibrinolysis were detected.

Data analysis

The primary study objective was to estimate reference ranges for standard ROTEM[®] assays in the six age groups. Secondary study endpoints were to (i) search for possible differences between age groups, (ii) analyse gender-related differences, (iii) compare ROTEM[®] parameters with standard coagulation tests and concentrations of FXIII, and (iv) compare clot strength at 10 min (A10) with MCF.

The SPSS software package (Version 18.0; SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Descriptive methods were used to calculate the 2.5% and 97.5% percentiles according to the NCCLS guidelines for establishing reference ranges.¹³ Data are presented as median

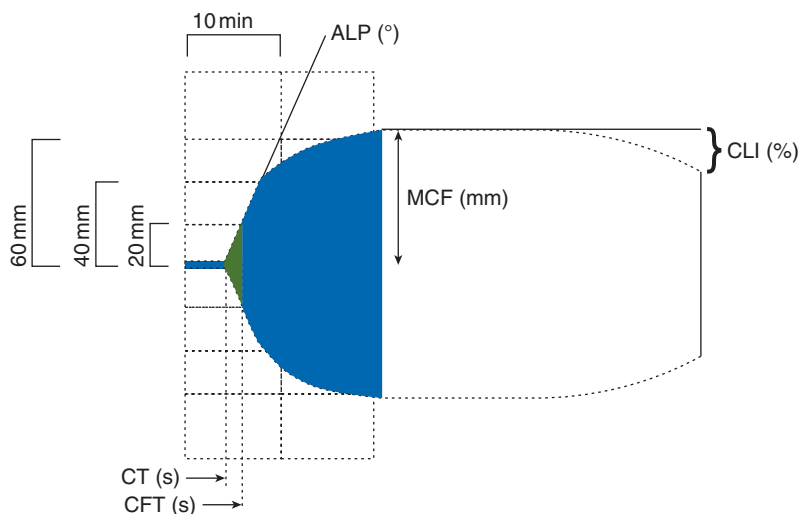


Fig 1 Representative thromboelastometric tracing (ROTEM®) showing the dynamics of initiation of coagulation (CT), propagation of coagulation (CFT and ALP), maximum clot firmness (MCF), and clot lysis index (CLI).

values with Q1 and Q3 quartiles, if not otherwise indicated. The Kolmogorov–Smirnov test was applied to test for Gaussian distribution of study variables. Since several parameters investigated showed statistically significant departure from normality (at an α level of 0.05), the non-parametric Kruskal–Wallis test, the Mann–Whitney *U*-test (to investigate age- and gender-related differences), and the Spearman correlation were used for analysis. Owing to multiple testing, Bonferroni’s adjustment was applied with a more conservative level of significance set at <0.005 .

Results

Study population

A total of 407 subjects were recruited; 48 were excluded *post hoc* because of difficulties in blood sampling or because standard laboratory test results were outside the reference ranges, leaving 359 subjects (325 Innsbruck and 34 Zurich) eligible for analysis. Each age group encompassed at least 50 children.

Demographic data are listed in Table 1. Included subjects underwent the following types of surgery: general ($n=214$), orthopaedic ($n=43$), plastic ($n=53$), removal of osteosynthesis material ($n=11$), ear, nose, and throat ($n=3$),

ophthalmological ($n=5$), urological ($n=21$), and dental ($n=9$). Data on standard laboratory parameters and concentrations of FXIII are given in Table 2.

Intrinsically activated ROTEM® assay

All parameters of the intrinsically activated assay differed significantly among all age groups (Table 3 and Fig. 2). CT range was wider than reported for adults, and highest values were observed in children aged 0–3 months ($P=0.001$). CFT was shortest ($P<0.001$) and α angle ($P<0.001$) and MCF ($P<0.001$) strongest in children aged 0–3 months when compared with children >3 months. A10 strongly correlated (correlation coefficient 0.95; $P<0.001$) with MCF, with the median difference being 1.5 mm (inter-quartile range 3–5 mm).

Extrinsically activated ROTEM® assay

CT, CFT, and α angle differed significantly, whereas MCF and CLI60 were comparable among all age groups (Table 3 and Fig. 3). CT was shortest in children 0–3 months ($P<0.001$), CFT was shortest ($P<0.001$), and α angle ($P<0.001$) and MCF showed highest degrees in children 0–3 months. A10

Table 1 Demographics of paediatric age groups (median and inter-quartile range)

Age group	n	Age	Sex (n) (male, female)	Height (cm)	Body weight (kg)
0–3 months	51	1.0 (1.0–2.0) months	39, 12	56.0 (53.0–59.0)	5.0 (4.2–5.5)
4–12 months	55	7.0 (6.0–10.0) months	38, 17	70.0 (66.0–75.5)	8.3 (7.2–9.4)
13–24 months	54	18.0 (15.8–20.0) months	42, 12	81.0 (75.8–85.0)	11.0 (9.9–12.0)
2–5 yr	70	3.3 (2.6–4.3) yr	52, 18	103.5 (92.8–108.5)	15.0 (13.4–17.0)
6–10 yr	79	7.3 (6.0–9.3) yr	59, 20	128.0 (116.8–136.5)	24.3 (20.0–30.0)
11–16 yr	50	13.7 (11.8–14.9) yr	30, 20	158.0 (147.5–167.8)	52.0 (41.5–69.5)

Table 2 Median and first and third quartiles of cell count and coagulation parameters. aPTT, activated partial thromboplastin time; PT, prothrombin time; AT, antithrombin; FXIII, coagulation factor XIII; Hb, haemoglobin (n=359)

	n	aPTT (s)	PT (s)	AT (%)	Fibrinogen (mg dl ⁻¹)	FXIII (%)	Hb (g dl ⁻¹)	Platelets (× 10 ⁶ litre ⁻¹)
Reference ranges in adults (Central Lab)		26–37	11.4–14.0	75–125	210–400	70–140	13.0–17.7	150–380
0–3 months [median (Q1–Q3)]	51	42 (40–46)	13.2 (12.6–13.6)	70 (63–78)	228 (206–251)	92 (81–101)	10.2 (9.4–10.8)	402 (312–445)
4–12 months [median (Q1–Q3)]	55	37 (33–40)	12.8 (12.1–13.6)	94 (87–100)	235 (196–264)	88 (76–99)	11.1 (10.4–11.6)	339 (288–425)
13–24 months [median (Q1–Q3)]	54	34 (32–37)	12.4 (12.0–13.1)	100 (95–107)	253 (224–307)	90 (80–104)	11.6 (11.2–12.4)	312 (244–357)
2–5 yr [median (Q1–Q3)]	70	34 (32–36)	12.7 (12.2–13.4)	98 (92–103)	273 (249–325)	93 (81–105)	11.9 (11.4–12.3)	294 (274–363)
6–10 yr [median (Q1–Q3)]	79	34 (31–35)	12.7 (12.2–13.1)	95 (90–100)	262 (240–310)	88 (80–99)	12.8 (12.2–13.4)	270 (239–311)
11–16 yr [median (Q1–Q3)]	50	32 (31–35)	12.6 (12.0–13.2)	97 (88–103)	274 (239–359)	89 (82–106)	13.9 (13.2–14.9)	262 (239–283)

Table 3 Median and reference ranges (2.5% and 97.5% percentiles) for ROTEM[®] InTEM and ExTEM assay. CT, coagulation time; CFT, clot formation time; ALP, α angle; A10, clot strength at 10 min; MCF, maximum clot firmness; CLI60, clot lysis index at 60 min. The Kruskal–Wallis test showed overall age-related differences for InTEM CT (P=0.002), CFT, ALP, A10, and MCF (all P<0.001), but no differences for ExTEM MCF or ExTEM CLI60 (n=359)

ROTEM [®] parameter	InTEM CT (s)	InTEM CFT (s)	InTEM ALP (°)	InTEM A10 (mm)	InTEM MCF (mm)	ExTEM CT (s)	ExTEM CFT (s)	ExTEM ALP (°)	ExTEM A10 (mm)	ExTEM MCF (mm)	ExTEM CLI60 (%)
Median	184	63	77	55	61	55	95	72	53	60	No data
Reference ranges in adults ⁵	137–246	40–100	71–82	44–68	52–72	42–74	46–148	63–81	43–65	49–71	
0–3 months (n=51)											
Median	184	44	81	62	66	48	57	78	60	62	87
Reference range	105–285	27–88	74–85	50–72	54–73	38–65	30–105	69–84	51–72	54–74	71–94
4–12 months (n=55)											
Median	172	60	78	59	63	53	72	76	57	60	86
Reference range	76–239	37–100	73–83	47–70	52–73	37–77	44–146	68–82	46–68	46–71	71–95
13–24 months (n=54)											
Median	161	61	78	59	64	55	75	75	56	60	88
Reference range	99–207	42–112	70–82	45–67	50–72	37–73	46–139	64–81	41–68	46–72	77–94
2–5 yr (n=70)											
Median	170	60	78	59	63	56	72	75	58	61	86
Reference range	99–239	40–94	72–82	49–68	53–73	46–97	41–109	69–82	49–68	52–70	74–93
6–10 yr (n=79)											
Median	168	64	77	57	62	57	77	74	56	60	87
Reference range	97–212	48–93	72–80	49–66	53–69	43–74	49–114	67–80	49–65	53–68	70–97
11–16 yr (n=50)											
Median	171	68	77	56	62	59	81	74	57	62	88
Reference range	128–206	45–106	70–81	48–67	54–71	44–91	53–115	67–80	49–67	53–72	76–94

strongly correlated with MCF (correlation coefficient 0.91; P<0.001) and the median difference was 2 mm (inter-quartile range 2–5 mm).

Eighty-six subjects showed CLI60<85%, and 27 subjects even showed values <80%. In 27 subjects with enhanced lysis, an ApTEM test was available showing improved lysis

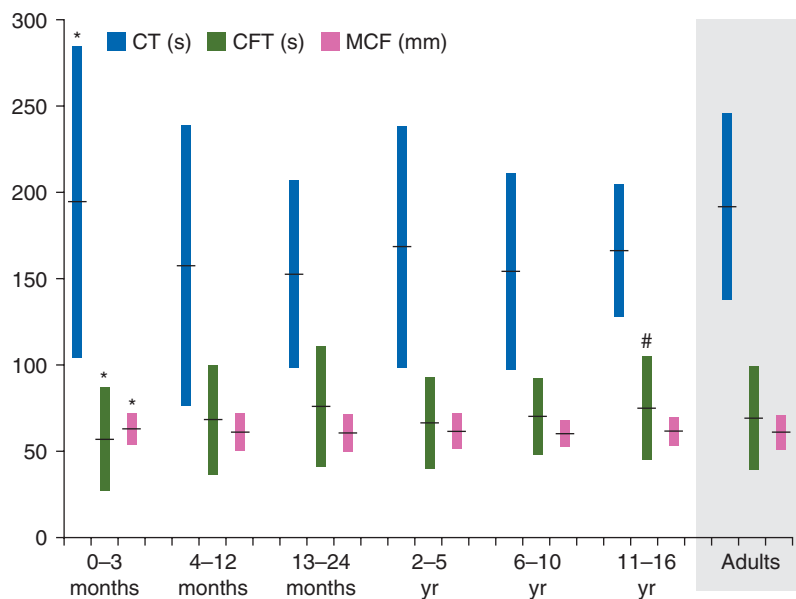


Fig 2 ROTEM® InTEM reference ranges and median of all age groups and adults showing maturation of CT, CFT, and MCF. * $P < 0.005$ vs children > 3 months; # $P < 0.005$ vs children < 11 yr.

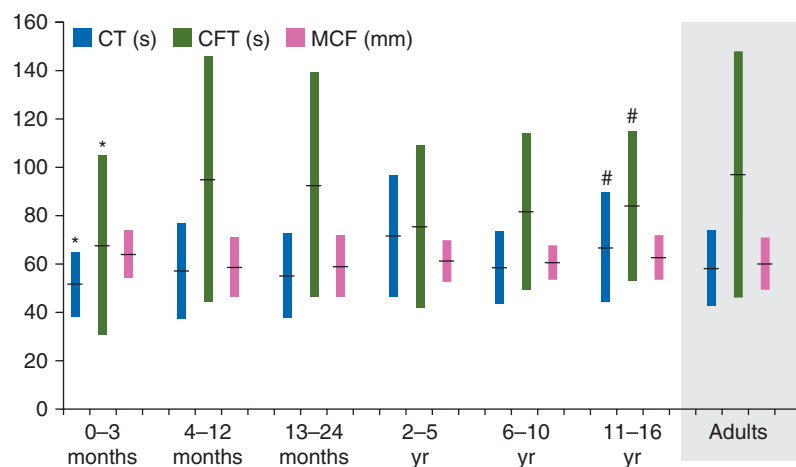


Fig 3 ROTEM® ExTEM reference ranges and median of all age groups and adults showing maturation of CT, CFT, and MCF. * $P < 0.005$ vs children > 3 months; # $P < 0.005$ vs children < 11 yr.

index in 12 (data not shown). In subjects exhibiting enhanced fibrinolysis, FXIII levels were within reference ranges [88% (inter-quartile range 80–97%)].

Fibrinogen/fibrin polymerization

Results for fibrinogen/fibrin polymerization differed significantly among all age groups (Table 4), with lowest 2.5% percentiles observed in children aged 13–24 months. A10 strongly correlated with MCF (correlation coefficient 0.98;

$P < 0.001$), the difference being 0.6 mm (inter-quartile range 0–1 mm).

Correlation between ROTEM® parameters and standard laboratory tests and FXIII levels

For the entire study population, aPTT values correlated weakly (correlation coefficient 0.34; $P < 0.001$) with intrinsically activated CT, whereas no correlation was found between PT and extrinsically activated CT. FXIII, fibrinogen concentration,

Table 4 Median and reference ranges (2.5% and 97.5% percentiles) for ROTEM[®] FibTEM assay. A10, clot strength at 10 min; MCF, maximum clot firmness. The Kruskal–Wallis test showed overall age-related differences for A10 ($P=0.03$) and MCF ($P=0.042$) ($n=355$)

ROTEM [®] parameter	A10 (mm)	MCF (mm)
Median	14	16
Reference ranges in adults ⁵	9–24	9–25
0–3 months ($n=51$)		
Median	13	14
Reference range	8–22	8–23
4–12 months ($n=53$)		
Median	12	12
Reference range	6–24	7–25
13–24 months ($n=52$)		
Median	11	12
Reference range	5–24	6–24
2–5 yr ($n=70$)		
Median	12	13
Reference range	7–22	7–23
6–10 yr ($n=79$)		
Median	11	12
Reference range	7–21	7–22
11–16 yr ($n=50$)		
Median	12	13
Reference range	7–22	8–24

and platelet count showed stronger correlations with MCF (correlation coefficient FXIII <0.4 ; fibrinogen and ExTEM MCF 0.52, and platelets and InTEM MCF 0.592; $P<0.001$) and fibrinogen concentrations significantly correlated with MCF (correlation coefficient FibTEM MCF 0.52; all $P<0.001$), which was also observed in children aged under 12 months (correlation coefficient FibTEM MCF 0.50; $P=0.001$). Children aged 0–3 months showed the lowest median levels of AT and highest platelet count. Both AT and platelet count showed no correlation with CTs, but platelet count showed significant correlation with accelerated clot formation (CFT: correlation coefficient -0.507 , $P<0.001$; α angle: correlation coefficient 0.477, $P<0.001$) and MCF (correlation coefficient 0.535; $P<0.001$). We found no correlation between haemoglobin values and any ROTEM[®] parameter.

Gender dependency of ROTEM[®] parameters in children

Although no exact balance between male and female children was established, no sex-related differences in ROTEM[®] parameters were observed (data not shown).

Discussion

Investigating a large cohort of otherwise healthy children, we found that the ROTEM[®] parameters measuring initiation and propagation of coagulation and fibrinogen/fibrin polymerization showed significant age dependency, whereas extrinsically

activated clot firmness and fibrinolysis were similar among all age groups.

The most striking finding was that children aged 0–3 months, despite showing prolonged standard plasma coagulation test results, exhibited accelerated coagulation and strong clot firmness. Coagulation factors are independently synthesized by the fetus, and newborns show concentrations of factors II, VII, IX, X, XI, and XII below adult values, whereas concentrations of fibrinogen, factors V, VIII, XIII, and the von Willebrand factor are within the range of or above adult values.^{14–15} The fetal fibrinogen molecule differs from that of the adult by exhibiting fewer fractions of high molecular fibrinogen, and increased content of sialic acid and phosphorus.^{16–18} In addition, concentrations of antithrombin and protein C are lower than in adults, whereas levels of $\alpha 1$ antiplasmin, C1 esterase inhibitor, and $\alpha 2$ macroglobulin are above those of adults.^{14–15} Consequently, standard coagulation tests, which strongly depend on concentrations of procoagulant proteins, are prolonged in early childhood, although healthy newborns show no increase in bleeding tendency. In contrast, and as also observed by others,¹⁹ viscoelastic whole-blood analyses showed accelerated initiation of coagulation after extrinsic activation and enhanced propagation of coagulation, whereas clot strength was within the range of adults. In the youngest age group, intrinsically activated parameters also showed a higher upper limit of the reference range for initiation of coagulation, clot formation was accelerated, as in extrinsically activated assays, and median clot firmness was even stronger than that reported for adults. To ensure that findings were not influenced by the physiologically low haemoglobin level in the youngest age group, we looked for relationships between haemoglobin and ROTEM parameters, but found no significant correlation.

It was not clear why in the youngest age group activation by the intrinsic pathway (InTEM) showed prolonged CTs, but the shortest CTs were observed in the ExTEM assay. As no correlation was found between CT and platelet count or between CT and antithrombin, other factors, such as a slower onset of maturation of contact activators (FXI and FXII) when compared with a faster maturation of FVII, might be responsible for this observation.

Differences in study design and reagents used make a direct comparison of our results with those of other studies somewhat difficult. Using either modified ROTEM[®] or TEG[®] analysis in term and preterm infants, three other studies reported data similar to ours.^{6–9–10} Haizinger and colleagues⁷ and Osthaus and colleagues⁸ used standard ROTEM[®] assays showing ROTEM[®] parameters in the range observed in the present study, although their age groups differed. Edwards and colleagues⁹ performed classical TEG[®] in neonates and reported shorter r times (corresponding to ROTEM[®] CT), but also reduced clot firmness in neonates.²⁰ In contrast, another study using kaolin-activated TEG[®] to investigate children between 1 month and 16 yr found no relation with age,¹¹ whereas Miller and colleagues²¹ using native TEG[®] also found shorter r times and k values (corresponding to ROTEM[®] CFT) below 12 months when compared with those

of older children and adults. Cvirn and colleagues¹⁰ also measured the endogenous thrombin potential in cord blood and showed shorter lag times for thrombin generation, thereby confirming the ROTEM[®]/TEG[®] results with regard to faster initiation of coagulation.

PT and aPTT values showed only weak or no correlation with ROTEM[®] CTs. A similar finding was also reported in orthopaedic patients showing decreased levels of coagulation factors during haemodilution⁴ and during liver transplantation.²² Sufficient clot firmness is a prerequisite for cessation of bleeding from wound sites and microvessels. For example, clot firmness is massively reduced in Glanzmann's thrombasthenia²³ and in congenital fibrinogen deficiency,²⁴ and these patients show increased bleeding tendency even without exposure to surgical stress.

Strauss and colleagues⁶ demonstrated that clot firmness correlates with gestational age, reaching median levels of 60 mm in full-term neonates, which is in perfect concordance with our findings. In addition, we demonstrate that children aged 4–24 months showed the lowest 2.5% percentiles for clot strength, indicating low reserve when exposed to haemodilution and blood loss. We also show that the amplitude measured at 10 min highly significantly correlated with final clot firmness. Thus, data obtained at 10 min might be suitable as a means of guiding therapy. In our study, amplitude of fibrin/fibrinogen polymerization as measured with a cytochalasin D-containing assay (FibTEM) differed among age groups, and some children aged <24 months showed low 2.5% percentiles, thereby explaining the low total clot firmness. In addition, fibrinogen concentrations correlated significantly with fibrin polymerization and total clot strength, also in the group of children younger than 12 months, whereas Miller and colleagues²⁵ using TEG[®] and abciximab found no such correlations. The reason for these contrasting results is not entirely clear, but might be related to the different types of assays.

Two other studies in 59 healthy newborns and 100 healthy children showed no enhanced fibrinolytic activity in children.^{9 11} As in the present study and without relation to bleeding tendency, Miller and colleagues²¹ observed that low clot lysis indices (<85%), usually interpreted as hyperfibrinolysis, frequently occur in children.^{21 26} Fibrinolysis as measured with ROTEM[®] has been shown to correlate with levels of tissue plasminogen activator and also factor XIII concentrations.^{27 28} Although we did not analyse molecular markers of fibrinolysis in the present study, factor XIII levels were comparable in children with and without enhanced fibrinolysis. We speculate that the observed reduction in clot firmness may result from enhanced contraction of formed clots, namely in those cases showing identical indices after addition of aprotinin. This hypothesis, however, needs to be confirmed by further investigations.

In adults, ROTEM[®] assays showed a slight trend towards faster coagulation activation and clot strength in females.⁵ When compared with male newborns, significantly higher levels of coagulation factor VII were reported in female newborns, who also showed a trend to increased levels of

coagulation factor VIII.²⁹ However, confirming other data, we found no gender-related changes in ROTEM[®] parameters during childhood.⁹

Excluding all children <4 months, overall Kruskal–Wallis's test revealed no significant changes for all ROTEM[®] parameters.

Limitations of our study need to be considered. Adequate blood sampling can be cumbersome in small children. Great care was taken to avoid the known influences of pre-analytic factors. Nevertheless, the i.v. catheters used needed to be small in size. Thus, a degree of artificial coagulation activation cannot be excluded. However, our findings on accelerated coagulation in the youngest age groups conform to the described physiology of haemostasis in childhood and agree with results obtained in other studies.^{6 9 10 15} The finding that fibrinogen levels correlate with fibrin polymerization and clot firmness is of clinical importance, because reduced clot firmness in the presence of microvascular bleeding usually triggers substitution therapy. However, these correlations can be altered in disease and during haemodilution. It is well known that measurements of fibrinogen concentration are overestimated in the presence of colloids³⁰ and that fibrinogen/fibrin polymerization depends on various factors that are not reflected by measuring fibrinogen concentration.^{31–34} Critics may argue that our results might be influenced by the fact that blood samples were drawn after induction of anaesthesia, and preoperative fluid deficit might be a potential confounding factor. However, the majority of blood collections were performed immediately after inhalation induction of anaesthesia and children were also allowed to receive clear fluids up to 2 h before surgery. Available data show that propofol, fentanyl, and sevoflurane exhibit no effect on platelets.³⁵ Thus, we believe that the remaining influences on test results should be rather small.

In conclusion, we show that reference ranges for ROTEM[®] parameters in children are age-dependent. We also show that, similar to adults, in children, fibrinogen concentrations, platelet count, and FXIII contribute to clot firmness as measured with ROTEM[®] assays. The strong association between the 10 min value for clot firmness and final results at 60 min allows rapid diagnosis and treatment. Because age dependency of clinically relevant ROTEM[®] parameters was most obvious in children 0–3 and 4–24 months, we suggest that institutional age-related reference ranges be defined, at least for these groups of children. Although initiation of coagulation in children <3 months analysed with standard plasma coagulation test was prolonged, ROTEM[®] showed accelerated clot formation and strong clot firmness, which might be partly attributed to increased platelet count. The results of this study should be helpful in understanding developmental haemostasis and facilitating haemostasis management in children.

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Conflict of interest

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