Age dependency for coagulation parameters in paediatric populations

Results of a multicentre study aimed at defining the age-specific reference ranges

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Summary

Understanding of developmental haemostasis is critical to ensure optimal prevention, diagnosis, and treatment of haemorrhagic and thrombotic diseases in children. As coagulation test results are known to be dependent on the reagents/analysers used, it is recommended for each laboratory to define the age-dependent reference ranges by using its own technical condition. That study was carried out in seven centers to establish age-specific reference ranges using the same reagents and analyser. Plasma samples were obtained from 1437 paediatric patients from the following age groups: 15 days-4 weeks (n=36), 1–5 months (n=320), 6–12 months (n=176), 1–5 years (n=507), 6–10 years (n=132) and 11–17 years (n=262). Indication of coagulation testing was pre-operative screening for non-acute diseases in most cases. PT values were similar in the different age groups to those in adults, whereas longer aPTTs were demonstrated in the younger children. Plasma levels of all clotting factors, except for FV, were significantly decreased ($p<0.0001$) in the youngest children, adult values being usually reached before the end of the first year. The same applied to antithrombin, protein C/S, and plasminogen. In contrast, FVIII and VWF levels were elevated in the youngest children and returned to adult values within six months. The same applied to D-dimer levels, which were found elevated, particularly until six months of life, until puberty. These data suggest that most coagulation test results are highly dependent on age, mainly during the first year of life, and that age-specific reference ranges must be used to ensure proper evaluation of coagulation in children.

Keywords

Coagulation, childhood, paediatrics, reference ranges, developmental haemostasis

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Introduction

Children are not just miniature adults (1), at least for haemostasis. Actually, the paediatric haemostatic balance is different from that in adults, and is an evolving process as shown by Andrew et al. in the late 1980s and early 1990s (2–4). The concept of developmental haemostasis (5), suggesting that the haemostatic system changes and matures throughout the time from fetal to adult life, particularly in the early months of life, was confirmed by several studies in various technical conditions (6–11). The understanding of that concept, which is now universally accepted, is critical to ensure optimal prevention, diagnosis, and treatment of haemorrhagic and thrombotic diseases in children (5, 12). As a consequence, it becomes mandatory to use age-specific reference ranges for coagulation parameters. However, if the global trend is consistent across the studies (2–4, 6–11), differences in absolute values are reported that are likely due to differences in the reagents/instruments used to measure these parameters (10). This could be particularly obvious for global coagulation tests such as the prothrombin time (PT) or the activated partial thromboplastin time (aPTT) (10). Indeed, with the development of novel reagents and analysers, it becomes crucial to have specific data for its own technical environment (13). So, it is recommended by the Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (ISTH) for each laboratory to define the age-dependent reference ranges by using its own technical condition (14).

To comply with the Clinical and Laboratory Standards Institute (CLSI) C28A3 guideline (15), reference ranges have to be established by testing at least 30 different individuals, in each age-group. However, collection of samples obtained from such patients that were withdrawn into the containers used in a specific institution raises a logistical problem that would be far beyond the capabilities of many laboratories. In order to circumvent that difficulty, the

present multicentre study was carried out in seven centres using the same technical conditions, i. e. reagents and instruments.

Subjects, materials and methods

Studied population

Blood samples were collected prior to the surgery from 1437 presumably healthy children during the preoperative workup for minor day surgery in most cases. Actually, despite numerous international and national guidelines aimed at limiting preoperative testing (16, 17), such investigations, particularly laboratory tests, are still widely performed (18, 19). None had acute infection, particularly affecting the respiratory track, had any personal or family history of thromboembolism or bleeding, or was on anticoagulant treatment. Residual plasma was used and no additional blood samples were drawn specifically for the present study. The study was carried out according to the Principles of the Declaration of Helsinki, after being approved by the local Ethics Committees. There were 1437 paediatric patients: 959 males (M) and 478 females (F), aged between 15 days and 17 years. They were divided into six age groups: from 15 days to 4 weeks (median=3 weeks, n=36, 24 M and 12 F, all but three were full-term newborns), from 1-5 months (median=3 months, n=320, 244 M and 76 F), from 6-12 months (median=8.5 months, n=176, 118 M and 58 F), from 1-5 years (median=2 years, n=511, 373 M and 138 F), from 6-10 years (median=8 years, n=132, 85 M and 47 F) and from 11-17 years (median=13 years, n=262, 115 M and 147 F). Vitamin K was routinely administered, usually at a dose of 2 mg orally, or 1 mg intravenously or intramuscularly, at birth that was repeated at day 3 or day 4. An adult population (n=64), sampled in the same conditions, was also studied for comparison purpose. There were 30 M and 34 F, with a mean age of 37 years (range: 19–58). They were otherwise healthy and not taking any medication.

Evaluated samples

Venous blood was collected, through a 21–23 G-needle, into polymer evacuated tubes containing 0.109 M sodium citrate (1 vol/9 vol) according to international recommendations (20). Depending of the age of the children, sampling was performed using different collection tubes i. e. Monovette-S (Sarstedt, Nümbrecht, Germany) in neonates and youngest children, the so-called paediatric tubes, which had the same external dimensions as the adult ones, allowing the withdrawal of limited blood volume, or adult tubes from different manufacturers (Becton-Dickinson, Le-Pont-de-Claix, France, Greiner Bio-0ne, Kremsmünster, Austria, and Terumo Europe, Leuven, Belgium). The routine coagulation test results were previously found to be comparable when patients who not on unfractionated heparin were drawn in these tubes (21–23). Blood samples were routinely handled according to the current recommendations for preanalytical phase (24, 25). Accordingly, plasma was obtained by centrifugation at 2,000–2,500 × g and +18 °C for 15 minutes, within 4 hours after sampling. The remaining plasma, if any, was stored frozen in aliquots at –80 °C until evaluated after having undergone a second cycle of centrifugation.

Table 1: Employed reagents on the ACL TOP 500/700 analysers (all were from Instrumentation Laboratory), i. e. reagent brand names, assays principles, and units (for details, see Subjects, materials and methods).

Parameters	Reagents	Principles	Units
Prothrombin Time	HemosIL RecombiPlasTin 2G	Clotting	sec, ratio, %
Activated Partial Thromboplastin Time	HemosIL SynthASil HemosIL APTT SP	Clotting Clotting	sec, ratio sec, ratio
Fibrinogen	HemosIL Fibrinogen C HemosIL QFA	Clotting Clotting	g/l g/l
Coagulation factors II, V, VII, X	HemosIL Deficient, and RecombiPlasTin 2G	One-stage (PT-based) clotting assay	IU/ml
Coagulation factors VIII, IX, XI, XII*	HemosIL deficient, and HemosIL SynthASil	One stage (aPTT-based) clotting assay	IU/ml *In U/ml
Coagulation factor XIII	HemosIL Factor XIII Antigen	Latex agglutination	IU/ml
Antithrombin	HemosIL Liquid Antithrombin	Chromogenic	IU/ml
Protein C	HemosIL Protein C HemosIL ProClot	Chromogenic (PT-based) Clotting	IU/ml IU/ml
Protein S	HemosIL Free Protein S HemosIL Protein S Activity	Latex agglutination (PT-based) Clotting	IU/ml IU/ml
VWF	HemosIL von Willebrand Factor Ristocetin Cofactor Activity HemosIL von Willebrand Factor Activity HemosIL von Willebrand Factor Antigen	Latex agglutination Latex agglutination Latex agglutination	IU/ml IU/ml IU/ml
Plasminogen	HemosIL Plasminogen	Colourimetric	U/ml
D-dimer	HemosIL D-dimer HS 500	Latex agglutination	ng/ml (FEU)

Laboratory assays

The technical condition was identical at each participating center, particularly regarding reagents (►Table 1) and assay procedures on the ACL TOP 500/700 analysers. All were from Instrumentation Laboratory (IL, Bedford, MA, USA).

All routine coagulation tests i.e. PT, aPTT, fibrinogen, coagulation factor (F) II, FV, FVII, FVIII, FIX, FX, FXI, and FXII were locally performed at each participating center. Esoteric assays were analysed in a single center on frozen plasma samples i.e. FXIII, antithrombin, protein C (PC), protein S (PS), von Willebrand factor (VWF), plasminogen, and D-dimer.

PT and aPTT were routinely evaluated using the HemosIL RecombiPlasTin 2G and the HemosIL SynthASil reagents respectively. PT test results were expressed as the clotting time (in second), as the patient-to-control ratio, and as the percentage activity (%), whereas aPTT test results were expressed as the clotting time (in second) and as the patient-to-control ratio. In a subset of patients, aPTT was also evaluated using another reagent, HemosIL APTT SP. Fibrinogen (in g/l) was evaluated according to Clauss (26) using two reagents, HemosIL Fibrinogen C and HemosIL QFA.

Plasma levels of coagulation FII, FV, FVII, and FX were evaluated by one-stage PT-based clotting assays using the RecombiPlasTin 2G reagent and the corresponding specific factor-deficient plasmas. Plasma levels of coagulation FVIII, FIX, FXI, and FXII were evaluated by one-stage aPTT-based clotting assays using the SynthASil reagent and the corresponding specific factor-deficient plasmas. All coagulation factor levels were expressed in International unit per ml (IU/ml), except FXII in U/ml. FXIII (in IU/ml) was evaluated using an automated latex microparticle-based immunoassay.

VWF ristocetin cofactor activity (in IU/ml) was measured using a ristocetin-induced binding of VWF to a recombinant wild-type GP1b fragment (glycocalicin) by a latex-based immunoassay, abbreviated as VWF:GP1bR according to the last nomenclature of the SSC of the ISTH (27). Evaluation of VWF "activity" (VWF:Ab, in IU/ml) was based on the spontaneous binding of a monoclonal antibody directed against the VWF A1 domain (glycoprotein Ib binding site) using an automated latex particle-based immunoassay. VWF antigen concentration (VWF:Ag, in IU/ml) was used using an automated latex microparticle-based immunoassay.

Antithrombin (in IU/ml) was evaluated using an anti-Xa chromogenic substrate based assay. PC activity (in IU/ml) was evaluated using both a chromogenic substrate-based assay and a PT-based clotting assay. PS anticoagulant activity was evaluated using a PT-based clotting assay, whereas the antigen concentration of the free form of PS was evaluated using an automated latex-based immunoassay (both expressed in IU/ml). Plasminogen activity (in IU/ml) was evaluated using a chromogenic-substrate based assay. D-dimer levels (expressed in ng/ml fibrinogen equivalent unit: FEU) were measured using an automated latex-based immunoassay. Individuals methods were detailed subsequently (►Table 1).

When needed, assays were calibrated using the HemosIL Calibration Plasma that was calibrated against a secondary standard traceable to current World Health Organisation (WHO) Inter-

Table 2: Median values and age-specific reference ranges defined, according to the CLSI-C28-A3 guideline, as the 95 % CI in the different age-groups, for routine coagulation tests in the different age-groups. Prothrombin time (PT) was expressed as the clotting time, the patient-to-control ratio, and the percentage activity. Activated partial thromboplastin time (aPTT), evaluated using two reagents, was expressed as the clotting time and the patient-o-control ratio. Fibrinogen, evaluated according to Clauss using two reagents, is expressed in g/l. The number of evaluated samples (n) in each age groups is between brackets.

Parameter	15 days – 4 weeks	1–5 months	6–11 months	1–5 years	6–10 years	11–17 years	Adults
PT (sec)	11.2 [9.5–12.6]	11.0 [9.7–12.8]	11.0 [9.8–13.0]	11.3 [9.9–13.4]	11.7 [10.0–14.6]	11.8 [10.0–14.1]	10.9 [9.2–12.2]
(ratio)	0.98 [0.83–1.13]	0.97 [0.84–1.17]	0.98 [0.85–1.13]	0.99 [0.86–1.20]	1.04 [0.90–1.30]	1.04 [0.90–1.24]	0.95 [0.81–1.07]
(%)	100.0 [83–109] (n=36)	100.0 [78–108] (n=320)	100.0 [81.9–108.3] (n=176)	100.0 [76.0–103.8] (n=507)	94.5 [66.6–111.2] (n=132)	94.0 [72.0–109.0] (n=262)	109.5 [90.0–148.8] (n=64)
aPTT SynthASil (sec)	35.4 [27.6–45.6]	33.5 [24.8–40.7]	32.4 [25.1–40.7]	31.6 [24.0–39.2]	31.6 [26.9–38.7]	31.0 [24.6–38.4]	31.7 [27.8–36.3]
(ratio)	1.16 [0.91–1.43] (n=36)	1.10 [0.82–1.30] (n=311)	1.07 [0.82–1.32] (n=174)	1.06 [0.80–1.31] (n=490)	1.04 [0.85–1.28] (n=124)	1.02 [0.81–1.28] (n=249)	1.04 [0.86–1.20] (n=64)
aPTT APTT SP (sec)	39.0 [33.2–45.6]	33.3 [25.0–43.3]	34.3 [31.7–45.3]	32.4 [25.7–38.4]	32.8 [25.5–42.4]	32.6 [26.1–47.4]	32.8 [26.9–39.5]
(ratio)	1.28 [1.12–1.43] (n=36)	1.10 [0.86–1.38] (n=32)	1.12 [1.04–1.49] (n=31)	1.07 [0.84–1.26] (n=34)	1.08 [0.87–1.39] (n=34)	1.06 [0.86–1.55] (n=34)	1.08 [0.88–1.30] (n=44)
Fibrinogen Fib C (g/l)	2.54 [1.43–4.02] (n=34)	2.26 [1.50–3.76] (n=289)	2.33 [1.57–3.60] (n=163)	2.73 [1.88–4.13] (n=453)	2.78 [1.89–4.75] (n=113)	2.66 [1.77–4.20] (n=209)	2.75 [2.17–3.42] (n=44)
Fibrinogen QFA (g/l)	2.40 [1.36–3.00] (n=30)	2.10 [1.41–4.37] (n=96)	2.30 [1.48–3.67] (n=53)	2.60 [1.64–4.97] (n=105)	2.76 [1.71–5.37] (n=36)	2.48 [1.68–5.29] (n=63)	2.86 [2.13–4.22] (n=44)

Parameter	15 days – 4 weeks	1–5 months	6–11 months	1–5 years	6–10 years	11–17 years	Adults
FII (IU/ml)	56.3 [44.8–74.3] (n=21)	75.0 [46.7–110.6] (n=81)	91.5 [73.9–117.2] (n=37	99.0 [49.4–130.0] (n=64)	90.0 [68.4–132.0] (n=29)	93.5 [47.6–119.2] (n=44)	101.0 [75.2–132.0] (n=46)
FV (IU/ml)	100.0 [69.0–123.7] (n=21)	99.5 [59.5–147.0] (n=79)	102.0 [59.0–159.8] (n=37)	110.5 [73.2–188.1] (n=64)	101.0 [82.0–140.6] (n=27)	97.0 [61.7–124.8] (n=41)	99.0 [60.7–141.6] (n=45)
FVII (IU/mL)	75.6 [55.0–108.0] (n=21)	88.0 [43.0–141.1] (n=71)	88.0 [55.2–128.0] (n=33)	82.0 [47.8–124.2] (n=44)	77.0 [55.0–135.4] (n=21)	81.5 [55.4–133.1] (n=38)	95.0 [59.0–151.0] (n=43)
FVIII (IU/ml)	95.5 [65.2–153.4] (n=21)	84.5 [50.3–187.3] (n=134)	75.0 [53.4–132.2] (n=51)	95.0 [59.0–167.0] (n=98)	86.5 [60.6–154.4] (n=31)	93.0 [42.8–154.6] (n=45)	97.0 [56.0–145.9] (n=44)
FIX (IU/ml)	43.5 [30.0–77.0] (n=21)	53.0 [29.0–105.1] (n=134)	76.5 [50.5–106.8] (n=50)	84.0 [52.6–128.9] (n=97)	80.0 [55.3–156.0] (n=31)	96.5 [60.2–138.4] (n=52)	112.0 [69.5–131.0] (n=40)
FX (IU/ml)	85.0 [66.0–92.0] (n=21)	89.0 [67.5–122.2] (n=78)	100.0 [75.8–134.4] (n=36)	99.0 [59.7–152.8] (n=58)	99.0 [71.3–161.5] (n=23)	93.0 [64.0–130.5] (n=40)	106.0 [72.8–150.4] (n=46)
FXI (IU/ml)	56.0 [32.9–75.0] (n=21)	64.0 [27.6–126.4] (n=124)	86.0 [60.9–125.6] (n=51)	92.0 [58.0–154.0] (n=92)	83.0 [31.8–154.0] (n=30)	84.0 [55.4–139.4] (n=54)	105.0 [49.1–139.2] (n=43)
FXII (U/ml)	69.2 [25.0–81.0] (n=21)	76.0 [38.0–136.6] (n=73)	109.2 [48.0–156.1] (n=32)	107.0 [50.0–174.7] (n=40)	83.7 [49.4–153.5] (n=21)	91.7 [49.4–153.5] (n=37)	108.0 [46.5–157.3] (n=36)
FXIII (IU/ml)	86.0 [78.4–100.0] (n=21)	82.9 [55.3–133.2] (n=77)	92.0 [51.1–136.8] (n=35)	97.4 [49.4–137.2] (n=97)	96.5 [53.5–142.4] (n=36)	106.0 [64.4–133.1] (n=60)	100.4 [68.1–138.2] (n=46)

Table 3: Median values and age-specific reference ranges defined, according to the CLSI-C28-A3 guideline, as the 95 % CI in the different age-groups, for coagulation factors (F), expressed in International Unit per ml (IU/ml) or in U/ml when the calibration plasma was not calibrated against any international standard. Coagulation FV, FVII, FX and FII were evaluated using one stage PT-based clotting assays. FVIII, FIX, FXI, and FXII were evaluated using one stage aPTT-based clotting assays. FXIII was evaluated using a Latex microparticle-based agglutination assay. The number of evaluated samples (n) in each age groups is between brackets.

national Standards when available. Quality controls were performed using both normal and abnormal control plasmas (Normal Control, Low Abnormal Control, and Special Test Control Level 2). These lyophilized calibration and control plasmas were obtained from IL.

Statistical analysis

The reference ranges were defined, according to the CLSI C28-A3 guideline (15), as the boundaries encompassing 95 % of the population in each age-group (95 % confidence interval: CI). Test results in specific age groups were compared to those in adults using either the t-test or the Mann-Whitney U test, depending of the population sizes. A p value smaller than 0.05 was considered to be significant. Statistical analysis was performed using the MedCalc software version 15.2.2 (MedCalc Software bvba, Ostend, Belgium).

Results

The data obtained in the different centers were not significantly different, and the same applied when data were analysed according to the collection tubes used (data not shown). Accordingly, test results were pooled before being further analysed.

PT was found unchanged throughout childhood and similar to that in adults. In contrast, aPTT was significantly longer in the youngest children, with a correlatively higher patient-to-control ratio. It then shortened reaching adult values at the end of the first year of life. The plasma levels of all clotting factors, except FV, were significantly decreased in the youngest children ($p<0.0001$), when compared to adults, and then reaching adult values usually at the end of the first year. The same applied to antithrombin, PC, PS, and plasminogen. Fibrinogen levels, evaluated using two different reagents, demonstrated a U-shaped curve across the age-groups, with relatively higher levels in the youngest children, then decreasing to reach a nadir during the first months of life and then reaching adult values between 1 and 5 years. The same applied to FVIII and VWF levels that were found significantly elevated in the youngest children. Their plasma levels then decreased to reach a nadir value between the 6th and 12th month of life and then increasing to reach adult values. Antithrombin, PC, PS activity and

Table 4: Median values and age-specific reference ranges defined, according to the CLSI-C28-A3 guideline, as the 95 % CI in the different age-groups, for von Willebrand (VWF) ristocetin cofactor activity (VWF:GP1bR), activity (VWF:Ab), and antigen concentration (VWF:Ag). Test results are expressed in International Unit per ml (IU/ml). The number of evaluated samples (n) in each age groups is between brackets (for details see Subjects, materials and methods).

Parameter	15 days – 4 weeks	1–5 months	6–11 months	1–5 years	6–10 years	11–17 years	Adults
VWF:GP1bR (IU/ml)	99.6 [87.8–121.5] (n=21)	89.0 [33.2–154.1] (n=81)	67.1 [37.1–118.6] (n=37)	83.3 [40.8–131.8] (n=99)	89.1 [42.1–162.6] (n=35)	92.8 [45.0–139.1] (n=54)	87.7 [46.8–131.4] (n=48)
VWF:Ab (IU/ml)	121.5 [73.7–188.9] (n=21)	104.0 [40.9–191.0] (n=73)	86.0 [42.7–176.0] (n=36)	82.4 [43.6–155.8] (n=93)	83.0 [41.2–128.9] (n=35)	83.5 [54.0–136.9] (n=58)	93.9 [41.6–152.5] (n=46)
VWF:Ag (IU/ml)	163.3 [46.0–219.5] (n=21)	101.5 [35.5–217.0] (n=74)	78.6 [48.4–199.4] (n=36)	89.1 [41.0–185.7] (n=92)	80.0 [44.8–141.1] (n=35)	92.0 [55.6–123.4] (n=58)	90.8 [42.5–144.4] (n=46)

Table 5: Median values and age-specific reference ranges defined, according to the CLSI-C28-A3 guideline, as the 95 % CI in the different age-groups, for coagulation inhibitors. Antithrombin activity was evaluated using a factor Xa-based chromogenic assay, and protein C (PC) activity was evaluated using a chromogenic assay (chromo) and a PT-based clotting assay (clot). Protein S (PS) was evaluated using a PT-based clotting assay (clot) and antigen concentration of the free form (free Ag). Test results are expressed in International Unit per ml (IU/ml). The number of evaluated samples (n) in each age group is between brackets (for details see Subjects, materials and methods).

Parameter	15 days – 4 weeks	1–5 months	6–11 months	1–5 years	6–10 years	11–17 years	Adults
Antithrombin (IU/ml)	41.0 [32.8–62.8] (n=20)	80.1 [29.0–120.0] (n=60)	96.0 [63.0–121.8] (n=34)	96.5 [60.5–128.3] (n=54)	97.0 [64.2–136.4] (n=29)	97.0 [69.1–135.9] (n=42)	111.5 [83.2–125.7] (n=46)
PC chromo (IU/ml)	39.1 [27.2–48.0] (n=20)	51.2 [22.8–95.0] (n=80)	79.9 [46.6–150.9] (n=36)	92.6 [59.1–147.5] (n=101)	100.5 [45.9–153.5] (n=36)	99.0 [72.3–155.1] (n=56)	108.0 [70.2–144.0] (n=46)
PC clot (IU/ml)	37.5 [29.7–114.6] (n=20)	82.0 [28.1–127.8] (n=80)	85.0 [43.7–151.3] (n=37)	86.3 [61.0–143.5] (n=100)	91.0 [39.3–170.3] (n=39)	95.1 [65.8–126.6] (n=58)	119.0 [68.8–149.0] (n=37)
PS free Ag (IU/ml)	83.8 [61.0–108.0] (n=20)	94.9 [48.0–126.5] (n=80)	86.0 [63.0–138.9] (n=37)	86.4 [53.0–134.9] (n=102)	95.1 [61.5–141.7] (n=40)	93.5 [61.4–130.7] (n=63)	89.9 [63.1–127.2] (n=48)
PS clot (IU/ml)	90.1 [29.0–115.2] (n=20)	81.6 [33.3–153.9] (n=78)	88.3 [51.8–138.4] (n=35)	97.6 [60.2–148.8] (n=94)	104.8 [66.5–161.5] (n=34)	99.3 [52.5–147.1] (n=48)	101.9 [57.9–137.5] (n=47)

Table 6: Median values and age-specific reference ranges defined, according to the CLSI-C28-A3 guideline, as the 95 % CI in the different age-groups, for fibrinolytic parameters plasminogen (in unit per ml, U/ml) evaluated using a chromogenic substrate-based assay and D-dimer (in ng/ml FEU) evaluated using a latex microparticle agglutination-based assay. The number of evaluated samples (n) in each age group is between brackets (for details see Subjects, materials and methods).

Parameter	15 days – 4 weeks	1–5 months	6–11 months	1–5 years	6–10 years	11–16 years	Adults
Plasminogen (U/ml)	52.6 [41.0–82.7] (n=20)	69.2 [37.6–109.6] (n=72)	80.7 [49.3–126.4] (n=37)	91.8 [59.6–178.0] (n=93)	92.0 [52.4–158.1] (n=30)	91.8 [58.1–130.6] (n=43)	94.0 [63.4–134.8] (n=47)
D-dimer (ng/ml)	530 [445–1200] (n=20)	515 [90–878] (n=44)	270 [133–844] (n=37)	280 [88–780] (n=72)	275 [60–567] (n=30)	245 [69–580] (n=53)	277 [109–560] (n=41)

plasminogen were significantly decreased in the youngest children (<1 month) and then increased to reach adult values during the first months or first years of life, whereas free PS antigen concentration was not significantly different from that in adults. Interestingly, D-dimer levels were found elevated during childhood, particularly during the first six months of life and remained slightly elevated before reaching adult values at puberty. The corresponding reference ranges, defined according to the CLSI-C28-A3 guideline, as the 95 % CI in the different age groups, were reported in the ▶ Tables 2–6.

Discussion

The concept of developmental haemostasis, suggesting that the haemostatic system changes and matures throughout the time from the fetal life to adulthood, is critical to ensure optimal prevention, diagnosis, and treatment of haemorrhagic and thrombotic diseases in children (5, 12). The data presented here, confirm that, in the described technical conditions, most coagulation test results are highly dependent on age, mainly during the first year of life, and that age-specific reference ranges must be used to ensure proper evaluation of coagulation in children. Our results were congruent with those of previous studies evaluating mostly activity assays (2–4, 6–10). Nevertheless it could be noticed that if the global age-related trends were also similar to those reported for antigen assays (11), the test results for specific analytes showed marked discrepancies that deserve to be further investigated.

We were unable to demonstrate any significant change in PT values in the different age-groups, despite significantly lower levels of various extrinsic pathway clotting factors FVII and FII in the youngest children, adult values being usually reached before the age of one year. As no samples from neonate before two weeks of age was evaluated in the present study, and as vitamin K was routinely administered just after delivery, we cannot speculate for prolonged PT (with higher PT ratio) in the early days of life as previously reported (2, 3, 8). However, to address that issue, such a study is currently carried out. As the practical consequence, since PT result could be normal even in the case of low factor levels, it should be recommended to evaluate plasma levels of extrinsic pathway factors rather than the PT for evaluating the coagulation system in youngest children, particularly before one month of age.

The prolongation of aPTT in youngest children is likely to be multifactorial (28), but mainly related to decreased levels of various clotting factors either vitamin K–dependent as FIX, and/or involved in the contact phase such as FXI, FXII, or prekallikrein and high-molecular-weight kininogen that were not measured in the present study. If an age-related trend to longer aPTTs in youngest patients was found for the two tested reagents, the prolongation was found to be more pronounced with the HemosIL APTT SP than with the HemosIL SynthASil, which could be related to increased sensitivity to lower factor levels (29). However, the prolongation of aPTT in children could also be due to the presence of transient lupus anticoagulant (LA) (30–34). In our series, the vast majority of sampled children was not suffering from any acute illness or infection, making unlikely that LA could account to a significant point to the reported aPTT prolongation in youngest children. However, we did not screen for LA in our series.

As previously demonstrated, fibrinogen levels, evaluated according to Clauss (26), remained below adult values during the first year of life (2–4, 6–10). The results obtained using two different fibrinogen reagents were congruent, as expected since the assay principle was identical, i. e. activation of fibrinogen into fibrin by thrombin in excess (35). VWF levels were increased in the youngest children before decreasing to a nadir at six months and finally increasing to adult values between one and five years, depending on the assay used. Such an evolution of VWF:RCo and VWF:Ag was previously reported by Klarmann et al., with a significant difference between O and non-O blood groups (36). Natural coagulation inhibitors, i. e. antithrombin, PC and PS (activity) levels, were low in the youngest children and reached adult values at one year of age or latter. D-dimer levels were elevated during whole infancy, particularly during the first months of life, and reached adult values only at puberty. This could be of critical importance, even if it should be noted that the cut-off value used for the exclusion of venous thromboembolism (VTE) has to be defined using clinical studies carried out in patients with suspicion of VTE, and not using the upper limit of the reference range (37).

There could be some potential limitations to our conclusions. Even if there may be some differences in coagulation parameters based on racial origins, we decided to define reference ranges in a population reflecting the heterogeneous mixture of races in the global population in Western Europe, and not to focus on a single ethnical origin. In addition, we did not evaluate samples from neonates aged below two weeks, in whom the variability was found to be more pronounced (2, 3, 9, 38–42). Actually it appeared to be very difficult to establish "true" reference ranges in healthy newborns, as they are not routinely sampled for coagulation testing. Moreover, in a previous report, it appeared that less than 20 % of the parents gave their inform consent to allow blood collection from their newborn children (9). Finally, we achieved the requirement of the CLSI C28A3 guideline (15) of having at least 30 samples evaluated for the different parameters in all the different age-groups for all parameters except in the neonates between day

What is known about this topic?

- Coagulation test results vary throughout childhood, leading to the concept of developmental haemostasis.
- The use of age-adjusted reference values is critical to ensure proper management of children with thrombosis or bleeding disorders.

What does this paper add?

- First large-scale multicenter study aimed at defining the reference ranges for the most commonly used coagulation parameters in paediatric populations.
- Prothrombin time remains within the normal adult range in younger patients despite low levels of clotting factor.

Abbreviations

aPTT: activated partial thromboplastin time; CI: confidence interval; CLSI: Clinical and Laboratory Standards Institute; F: (coagulation) factor; FEU: fibrinogen equivalent unit; ISTH: International Society on Thrombosis and Haemostasis; IU: international unit; LA: lupus anticoagulant; PC: protein C; PS: protein S; PT: prothrombin time; VTE: venous thromboembolism; WHO: World Health Organization; VWF: Von Willebrand Factor.

15 and four weeks of age, and for some parameters in the children between six and 10 years.

In conclusion, these results, obtained using IL reagents and instruments, are globally congruent with previous studies obtained using other technical conditions (2–4, 6–10), despite some discrepancies in the absolute values of reference ranges for some coagulation assays, particularly for global assays. Such differences are likely to be related to differences in reagents/analysers sensitivity (13) and/or in studied populations. These results confirm the need for coagulation laboratories to develop and to implement age-related reference ranges specific to their own testing system.

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Conflicts of interest

None declared.

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