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Paediatric reference intervals for common coagulation assays in Chinese children as performed on the STA-R coagulation analyzer

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Abstract

Introduction: In order to correctly manage the paediatric patients affected with haemostatic disorders, age-appropriate reference intervals should be used. The purpose of this study was to establish age-specific reference intervals for prothrombin time (PT), thrombin time (TT), activated partial thromboplastin time (aPTT) and fibrinogen (Fg).

Methods: In this study, a total of 34 234 apparently healthy children and adolescents aged 0-15 years were chosen as reference individuals. PT, TT, aPTT and Fg were performed on the STA-R coagulation analyzer. Outliers were eliminated using the Dixon *D/R* ratio rule. Partitioning by age was achieved using Harris and Boyd's standard normal deviate test. The lower (2.5th percentiles) and upper (97.5 percentiles) reference intervals were established using the nonparametric method.

Results: Compared with the adult group, the median time of PT was significantly different in the groups consisting of children aged 0-15 days, 15 days-1 month, 1-6 months and 11-15 years. The median time of APTT and TT was significantly prolonged in all paediatric age groups than in the adult group ($P < .05$). Compared with the adult group, the median values of Fg were significantly different in the groups consisting of children aged 0-15 days and 2-15 years. Our results showed that all coagulation assays required partitioning by age.

Conclusion: Our results suggest that results of coagulation assays are highly dependent on age, and that age-specific reference intervals must be used to ensure proper evaluation of paediatric coagulation assays.

KEYWORDS

age groups, blood, haemostasis, paediatrics, reference values

1 | INTRODUCTION

Haemostasis is a complex physiological mechanism involving procoagulant and anticoagulant factors. Ultimately, it can enable the blood to avoid both excessive bleeding by promoting clot formation after endothelial injury and excessive clotting by restricting clot formation at the injury site. Children are not simply miniature adults, at least for homeostasis.[1] Actually, the paediatric haemostatic balance differs from that in adults due to continuous changes of biological development. The concept of developmental homeostasis suggesting that the haemostatic system changes and matures throughout the time from foetal to adult life, particularly in the first few months of

life, has been universally accepted.[2-4] Accurate and reliable paediatric reference intervals play an important role in clinical diagnosis and therapy of haemorrhagic and thrombotic diseases. Especially in children, reference intervals may vary significantly with age. In addition, paediatric reference intervals are influenced by race, ethnicity, gender, analyzer, reagent, methodology, etc[5,6] It is therefore recommended by the Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (ISTH) for clinical laboratories to establish their own reference intervals.[7]

However, establishing reference intervals can be challenging because it requires a large number of plasma samples from healthy individuals. This challenge is further expanded in paediatrics because the dynamic changes caused by child growth and development markedly affect circulatory levels of disease biomarkers. Many clinical laboratories use reference intervals provided by manufacturers or reported in the literature. However, they were mostly from Western populations and might have been obtained from an insufficient number of healthy individuals. This may lead to misinterpretations and inadequate clinical decisions.

In this study, we reported the age-dependent reference intervals for common coagulation assays from a generally healthy population of children aged 0-15 years.

2 | MATERIALS AND METHODS

2.1 | Study population

The study was approved by the institutional ethics committee of the Third Affiliated Hospital of Zhengzhou University. We enroled a total of 35 560 apparently healthy children and adolescents (0-15 years of age) between February 2015 and February 2019 from our hospital. Those subjects were scheduled to undergo minor elective surgery in Outpatient Departments of Paediatric Surgery, Otorhinolaryngology, and Urology. The following inclusion criteria were used for the subjects: (a) subjects who came to the Department of Pediatric Surgery for elective surgical operations such as inguinal/umbilical hernia repair, posterior urethral valve resection, circumcision, congenital imperforate hymen opening and benign rectal polyp excision; (b) subjects who came to the Department of Otolaryngology for elective surgical operations such as tympanoplasty, deviated septum surgery, tonsillectomy, and adenoidectomy; (c) subjects who came to the Urology Department for elective surgical operations such as urodynamic interventions, hydrocele surgery, varicocele surgery and circumcision; (d) a birth weight of >2500 g and delivery at full-term for children aged 0-6 months. Exclusion criteria were the followings: (a) having a history of bleeding or thrombotic disorders; (b) undergoing anticoagulation treatment; (c) taking medications for at least 2 weeks; (d) acute infection; (e) a disease that could affect the coagulation system such as malignancies and liver cirrhosis, (f) blood samples that were icteric, hemolyzed or lipemic; (g) blood samples that were not at the desired level. A total of 1326 individuals were excluded, and finally, 34 234 apparently healthy children and adolescents were included in this study. A total of 6994 healthy adults aged 18-50 years were also recruited for this study. Exclusion criteria are the same as those for children.

2.2 | Samples collection and handing

All blood samples were obtained by aseptic venipuncture into vacutainer tubes and anticoagulated with 1:9 volume of 109 mmol/L trisodium citrate. Vacutainer tubes were purchased from Shandong Aosaite Medical Devices Co., Ltd. All samples were centrifuged immediately at 2000 × g for 15 minutes at room temperature (25°C). The following coagulation assays were performed on the STA-R coagulation analyzer (Diagnostica Stago): prothrombin time (PT), thrombin clotting time (TT), activated partial thromboplastin time (aPTT) and fibrinogen (Fg). The four blood coagulation assays (PT, TT, aPTT and Fg) were performed by using the clotting method. The remaining plasma specimens were stored frozen in aliquots at −70°C. All assays were performed according to the manufacturer's instruction. PT, TT, aPTT and Fg were measured using commercially available kits from Diagnostica Stago. The stability of PT, TT, aPTT and Fg was 48 hours, 8 hours, 24 hours, 120 hours at room temperature, respectively. Samples with preanalytic processing times longer than the stability of analytes in

TABLE 1 The imprecision and accuracy for the method used to calculate the reference intervals

	Level 1		Level 2			
Tests (units)	Mean value	CV%	Mean value	CV%	Mean value	Bias (%)
PT (s)	14.07	1.90	22.87	2.14	34.02	0.43
TT (s)	16.18	2.36	–	–	16.60	−1.23
aPTT (s)	33.22	1.48	53.65	2.32	49.84	−0.92
Fib (g/L)	3.12	3.25	1.16	2.83	1.85	−2.63

Note: Level 1, normal concentration; level 2, pathological concentration. The imprecision for the method used to determine the reference intervals is given for each assay as an average coefficient of variation (CV%) of internal controls through 4 year. Accuracy (expressed as bias%) was calculated from external quality assessment (EQA) programmes organized by the Chinese National Center for Clinical Laboratories.

Abbreviations: aPTT, activated partial thromboplastin time; PT, prothrombin time; TT, thrombin time.

blood at room temperature were excluded from the calculations. Normal and abnormal control plasmas were run daily and used to calculate the precision (expressed as CV %). Accuracy (expressed as bias %) was calculated from external quality assessment (EQA) programs organized by the Chinese National Center for Clinical Laboratories. The imprecision and accuracy of the assays are presented in Table 1.

2.3 | Statistical methods

Statistical analyses were performed using SPSS 17.0 software (SPSS Inc). According to the CLSI EP28-A3 guidelines, outliers were eliminated using the Dixon *D/R* ratio rule.[8] Partitioning by age and/or gender was first examined by visually inspecting the scatter plots and distribution for overall trends. And then, it was justified with the Harris-Boyd method.[8,9] The lower (2.5th percentiles) and upper (97.5 percentiles) reference intervals were established using the nonparametric method. The 90% confidence intervals of the lower and upper limits were also calculated. The assay results in specific age groups were compared with those in adults using the Mann-Whitney *U* test. $P < .05$ was considered to be statistically significant.

3 | RESULTS

The reference population started with 35 560 apparently healthy children and adolescents aged 0-15 years. After applying these exclusion criteria, our study included 34 234 subjects. Subsequently, six outliers were detected in PT and aPTT. Eight outliers were detected in TT and Fg. These outliers were removed from further analysis. Paediatric reference intervals for common coagulation assays are summarized in Table 2, partitioned by age, as per the Harris-Boyd method.

The 90% confidence intervals of the lower and upper limits were also calculated. The profiles and trends for reference intervals are shown in Figure 1.

Compared with the adult group, the median values of PT were significantly different in the groups consisting of children aged 0-15 days, 15 days-month, 1-6 months and 11-15 years. They

TABLE 2 Age- and gender-specific paediatric reference intervals for common coagulation assays

Analye (Unit)	Age	N	Outliers	Medians	Lower limit (CI)	Upper limit (CI)
PT (s)	0-≤15 d	3785	1	16.5*	12.7 (12.5-12.8)	22.2 (21.3-23.6)
	>15 d-≤1 mo	3749	1	15.1*	11.9 (11.8-12.0)	21.0 (20.7-22.0)
	>1-≤6 mo	4354	0	13.6*	11.3 (11.3-11.3)	15.8 (15.7-16.0)
	>6 mo-≤11 y	21 098	4	14.2	11.7 (11.7-11.8)	17.1 (16.3-18.7)
	>11-≤15 y	1242	0	14.9*	12.1 (12.0-12.2)	18.7 (18.0-19.7)
	Adults	6992	2	14.3	11.7 (11.7-11.8)	17.3 (17.3-17.4)
TT (s)	0-≤15 d	3783	3	18.9*	15,0 (14.6-15.2)	24.7 (23.7-24.9)
	>15 d-≤1 mo	3749	1	18.1*	14.8 (14.7-14.9)	22.7 (21.9-23.3)
	>1m-<1y	7558	0	17.6*	14.6 (14.5-14.7)	21.6 (21.3-22.0)
	≥1-≤2 y	8282	2	16.8*	14.5 (14.5-14.6)	19.7 (19.4-20.0)
	>2-<15 y	10 854	2	16.4*	14.2 (14.1-14.3)	18.9 (18.8-19.1)
	Adults	6994	0	15.9	14.2 (14.1-14.2)	17.5 (17.5-17.6)
aPTT (s)	0-≤15 d	3785	1	49.5*	36.4 (35.7-37.5)	66,8 (64.4-68,3)
	>15 d-≤1 mo	3749	1	46.1*	34.4 (33.8-35.1)	63.1 (60.7-65.8)
	>1-≤6 mo	4354	0	42.1*	31,8 (31.1-32.4)	58.6 (57.4-60.9)
	>6 mo-≤2 y	11 484	4	39.4*	30.2 (30.2-30.5)	56.4 (55.3-57.8)
	>2-≤14 y	10 586	0	38.7*	30.1 (30.0-30.4)	52.4 (51.4-53.4)
	15 y	270	0	36.3*	30.0 (29.9-30.3)	45.2 (43.9-45.2)
	Adults	6992	2	35.4	29.9 (29.8-30.1)	42.3 (41.9-42.6)
Fg (g/L)	0-≤15 d	3785	1	1.91*	0.65 (0.53-0.76)	2.93 (2.78-3.37)
	>15 d-≤ 1 mo	3749	1	2.07	0.77 (0.70-0.86)	3.32 (3.00-3.48)
	>1-≤6 mo	4352	2	2.14	0.82 (0.74-091)	3.59 (3.43-3.76)
	>1 mo-<1 y	3204	0	2.16	0.96 (0.82-1.04)	3.78 (3.51-4.03)
	≥1-≤2 y	8282	2	2.30	1.29 (1.22-1.33)	3.98 (3.84-4.17)
	>2-≤15 y	10 854	2	2.60*	1.31 (1.25-1.37)	4.51 (4.39-5.03)
	Adults	6990	4	2.23	1.23 (1.19-1.31)	3.90 (3.72-4.06)

Note: Compared with adults,*$P<.05$.

Abbreviations: aPTT, activated partial thromboplastin time; CI, confidence intervals; Fg, fibrinogen; PT, prothrombin time; TT, thrombin time.

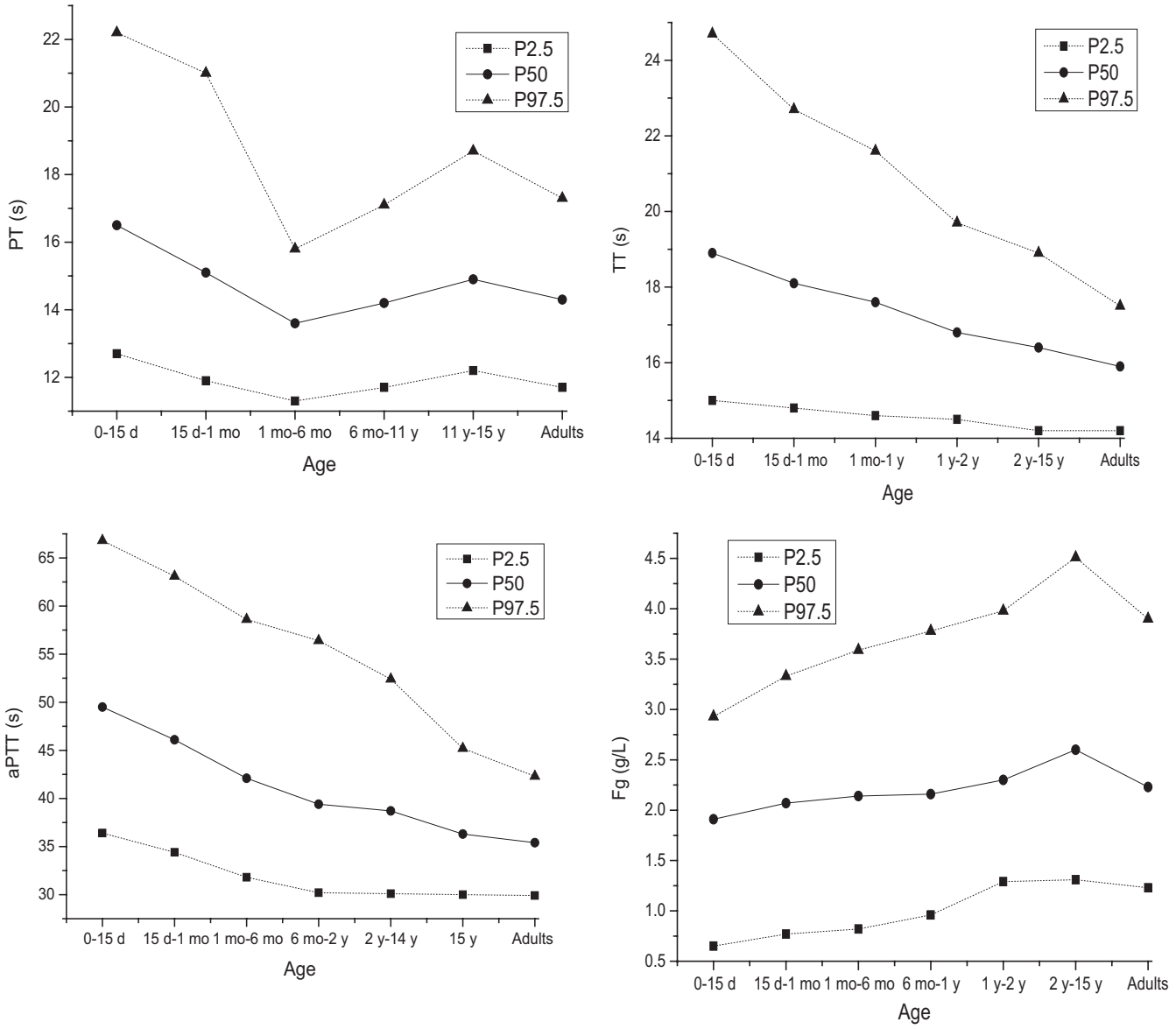

FIGURE 1 The profiles and trends of paediatric reference intervals for PT, aPTT, TT and Fg. For all graphs, the bottom dotted line is the lower limit (the 2.5th percentile). The middle solid line is the median level (the 50th percentile). The top dotted line is the upper limit (the 97.5th percentile). On horizontal axis, d, day; y, year

required five age partitions. The median time of APTT and TT was significantly prolonged in all paediatric age groups than in the adult group ($P < .05$). APTT and TT shortened gradually with increasing age. They required 5-6 age partitions. Compared with the adult group, the median values of Fg were significantly different in the groups consisting of children aged 0-15 days and 2-15 years. Fg values increased progressively with age, and reached their peaks at 2-15 years of age. They required six age partitions.

4 | DISCUSSION

The concept of developmental haemostasis was first introduced in 1987 by Andrew et al[10] and has been universally accepted. The understanding of age-related physiological changes in the coagulation system plays an important role in accurate diagnosis and therapy of haemorrhagic and thrombotic diseases. Recent studies have reported age-specific reference intervals of coagulation assays and coagulation factor activities. However, these may vary considerably with the use of different analyzers and reagents.[11]

In this study, we found age-related differences between children and adults for PT. The major reason we found these differences and the earlier studies[12] did not may be the larger number of subjects and the more restrictive age groups in our study. For aPTT, all paediatric age groups had higher median values than those of adults. The prolonged aPTT values in the paediatric population may be explained by markedly decreased levels of the vitamin K-dependent clotting factors such as Factor IX (FIX).[3] The prolonged

aPTT values may also be related to FXI, FXII, and prekallikrein.[13] In Weidhofer et al's study,[14] almost no fluctuations can be found for TT during life. In this study, TT values were reduced during birth to 5 years, which were consistent with the report by Appel et al[3] In this study, TT values were reduced during 1-15 years, which were consistent with the report by Li et al[15] This discrepancy may be due to differences in the study population, sample size and age groups. The Fg values did not exhibit a statistically significant increase until adulthood in Andrew et al's study.[10] Our study discovered a statistically significant difference in Fg values from birth to 15 days and from 2 years to 15 years when compared with adulthood. Our finding of Fg values increasing progressively with age is in line with other studies.[16]

Our findings have implications for the interpretation of paediatric coagulation tests. For example, a PT value that is prolonged by 4.6 seconds in a newborn, based on paediatric reference intervals, may be physiologic and does not indicate a disease state.

A potential limitation of this study is that it consists of the skewed distribution of individual age.

In conclusion, we found several statistically significant differences between adult and paediatric coagulation reference intervals, supporting the need to determine new paediatric reference intervals. Our study has established reference intervals based on the use of instrumentation, reagents and methodologies currently used in the clinical laboratory. These findings may improve the diagnosis and treatment of haemostatic disorders in paediatric patients.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Study concept and design: JL, YD. Acquistiion of data: YL, EY. Interpretation of data/results: EY, YL, QW. Data analysis: LW, YS. Drafting of the manuscript: JL, YD. Ctitical revision of the manuscript: EY. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

ETHICAL APPROVAL

This study was approved by the Ethics Committee of the Third Affiliated Hospital of Zhengzhou University on 11-12-2014, number 2014/721. All the subjects gave informed written consent.

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