Clinical Biochemistry 75 (2020) 78–82

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Establishing reference intervals of coagulation indices based on the ACL Top 700 system for children in Southwestern Fujian, China

Huifen Zhang[a], Jiming Li[b,*], Haichen Chen[c], Xingdong Wu[a]

[a] Department of Pediatric Internal Medicine, Women and Children's Hospital, School of Medicine, Xiamen University, Xiamen 361003, PR China
[b] Department of Medical Laboratory, Women and Children's Hospital, School of Medicine, Xiamen University, Xiamen 361003, PR China
[c] Department of Pediatric Surgery, Women and Children's Hospital, School of Medicine, Xiamen University, Xiamen 361003, PR China

ARTICLE INFO

Keywords:
Coagulation
Reference interval
Children

ABSTRACT

Background: Till date, China has not issued industry standards for reference intervals (RIs) of pediatric blood coagulation indices. Here, we evaluated changes in the coagulation indices in the venous blood of healthy children aged 29 days to 12 years derived using the ACL Top 700 system and established appropriate RIs.
Methods: We analyzed venous blood from 1770 healthy children for five coagulation indices. RIs were established according to the Clinical and Laboratory Standards Institute C28-A3c guideline.
Results: The coagulation indices were grouped by age. For prothrombin time (PT) and international normalization ratio (INR), the RIs of infants and toddlers were identical; preschool children had the same RI as school-age children. Pediatric RIs for PT and INR were slightly lower than those for adults. The RIs of activated partial thromboplastin time (APTT), thrombin time (TT), and fibrinogen (FIB) in childhood were divided into two groups by age (1 month to 1 year and 1–12 years). The RI of APTT in infants was the widest; the overall level of FIB in infants was the lowest; children's APTT and FIB RIs were lower than those of adults. The pattern of TT values and RI trends in childhood were similar to those of APTT.
Conclusions: There were minor changes in the RIs of coagulation indices for children. The RIs of PT, INR, APTT, TT, and FIB must be grouped by age. The RIs of coagulation indices for children were different from those for adults; therefore, establishing separate RIs for children is necessary.

1. Introduction

Coagulation function screening is often required in clinical practice, such as during preoperative preparation and for treating infectious and hemorrhagic diseases [1–3]. The accuracy of coagulation tests has greatly improved with the development of testing technology and widespread use of the coagulation analyzer. The clinical interpretation of coagulation test results largely depends on established reference intervals (RIs) derived from clinical studies in healthy populations. Most countries or regions do not have RI initiatives and sufficient resources to establish RIs for their local population. This remains a critical gap in the field. Coagulation test results are known to depend on the analyzers / reagents used. Therefore, laboratories should technically establish their own RIs according to the guideline. Children cannot be merely considered miniature adults, at least for the purpose of hemostasis [4]. Researchers in some countries have established RIs for coagulation parameters in local children. For instance in France, ACL analyzers and supporting reagents were used to define the age-specific RIs for coagulation parameters in pediatric populations [5]; Austrian researchers used Siemens and Stago analyzers and supporting reagents to set up dynamic RIs for coagulation parameters from infancy to adolescence [6]. It appears that laboratories using the same analyzers and reagents may share RIs; however, the applicability to different ethnic groups needs to be verified. At present, there is no uniform industry standard in China for coagulation screening indicators for children; the RIs of coagulation indices used in different children's hospitals are either those recommended by the "National Guide to Clinical Laboratory Procedures in China" [7], those provided by manufacturers of clinical laboratory analysis systems, or those that are self-established and validated by each hospital laboratory. However, differences caused by race, age, and sex and differences in methodology, detection systems, and reagents may affect the applicability of selected RIs [5,6].

In this study, we screened venous blood samples from apparently healthy children (aged > 28 days to 12 years old) in southwest Fujian,

* Corresponding author at: Department of Medical Laboratory, Women and Children's Hospital, School of Medicine, Xiamen University, No. 10, Zhenhai Road, Siming District, Xiamen 361003, PR China.
E-mail address: lijm2030@sina.com (J. Li).

https://doi.org/10.1016/j.clinbiochem.2019.11.005
Received 10 August 2019; Received in revised form 6 November 2019; Accepted 7 November 2019
Available online 23 November 2019
0009-9120/

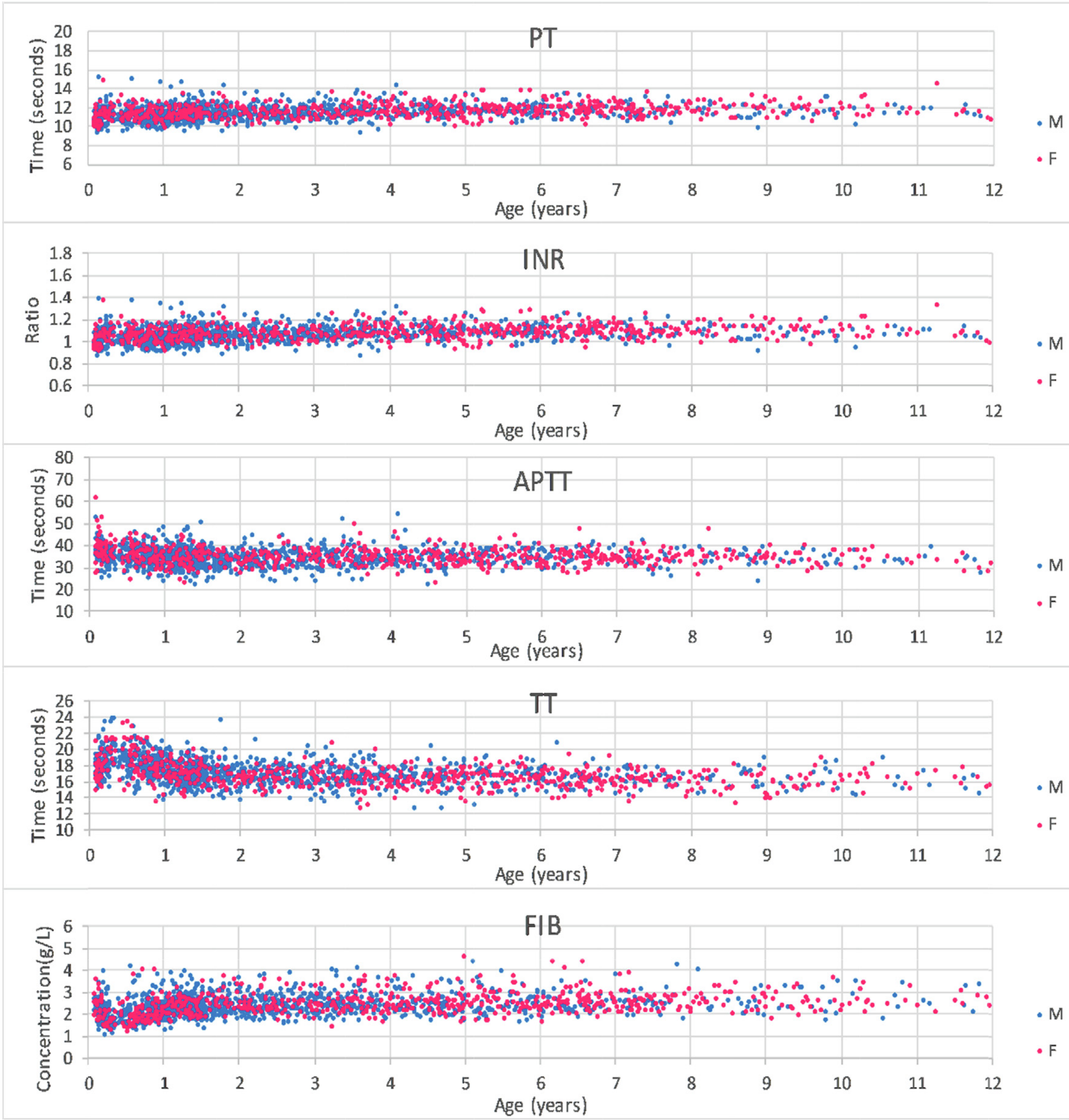

Fig. 1. Scatterplots of the five coagulation indices. Note: M, male; F, female.

China, for coagulation indices according to the C28-A3c guidelines [8] and established RIs of blood coagulation indices suitable for local children.

2. Materials and Methods

2.1. Study population

In this present study, the coagulation tests of the study subjects were obtained retrospectively, and laboratory information system results were analyzed. Apparently healthy children were selected as study subjects. These children were either diagnosed with a finger or toe deformity or underwent preparations for elective hernia surgery at the Department of Pediatric Surgery of the Women and Children's Hospital, School of Medicine, Xiamen University. All study subjects were from the Xiamen, Quanzhou, Zhangzhou, or Longyan areas. All study subjects underwent detailed physical examinations and disease history consultation during hospitalization. The exclusion criteria were as follows: positive history of heart, liver, kidney, hematological diseases and allergic or autoimmune diseases, acute and/or chronic infections within 1 month prior to enrolment, intake of any drugs and/or supplements within the 2 weeks prior to enrolment, height and/or weight out of corresponding ranges (mean value plus or minus two standard deviations for reference population of same age and sex), and the presence of any secondary sexual characteristics [9]. A total of 1770 children aged > 28 days to 12 years [as the numbers of selected neonates (≤28 days) and children aged ≥ 12 years were too small, these age groups were excluded] tested between January 1, 2017 and May 31, 2019 were selected; this included 416 infants (≥29 days– < 1 year of age, Ag1), 626 toddlers (≥1– < 3 years of age, Ag2), 440 preschool-age children (≥3– < 6 years of age, Ag3), and 288 school-age children (≥6– < 12 years of age, Ag4). The study design was approved by the hospital Institutional Review Board Committee; the Declaration of Helsinki was adhered to throughout the study.

2.2. Sample collection and processing

Venous blood was collected using a 21–23 gauge needle into evacuated glass tubes (Fuzhou Chang Geng Medical Devices Co. Ltd, China) containing 0.109 M sodium citrate (1 vol/9 vol) according to international recommendations [10]. Each evacuated tube was filled with exactly 2 ml of anticoagulated blood, which was gently inverted back

Table 1
The partitions of five coagulation indices by sex groups.

Indices	Group	Sex	Number	Mean	Standard deviation	Z	Z*
PT, sec	Ag1	Male	247	11.17	0.81	1.52	3.95
		Female	169	11.29	0.78		
	Ag2	Male	391	11.45	0.73	1.07	4.85
		Female	235	11.51	0.65		
	Ag3	Male	238	11.71	0.70	1.03	4.06
		Female	202	11.78	0.72		
	Ag4	Male	139	11.77	0.70	1.96	3.29
		Female	149	11.93	0.69		
INR	Ag1	Male	247	1.03	0.074	1.39	3.95
		Female	169	1.04	0.071		
	Ag2	Male	391	1.06	0.066	1.96	4.85
		Female	235	1.07	0.059		
	Ag3	Male	238	1.08	0.064	1.61	4.06
		Female	202	1.09	0.065		
	Ag4	Male	139	1.09	0.063	1.35	3.29
		Female	149	1.10	0.062		
APTT, sec	Ag1	Male	247	35.62	4.26	2.14	3.95
		Female	169	36.68	5.38		
	Ag2	Male	391	33.95	4.02	0.96	4.85
		Female	235	34.25	3.63		
	Ag3	Male	238	34.43	4.04	0.44	4.06
		Female	202	34.59	3.66		
	Ag4	Male	139	34.39	3.38	0.03	3.29
		Female	149	34.38	3.24		
TT, sec	Ag1	Male	247	18.28	1.62	0.50	3.95
		Female	169	18.37	1.91		
	Ag2	Male	391	16.92	1.31	1.52	4.85
		Female	235	16.77	1.13		
	Ag3	Male	238	16.63	1.28	1.80	4.06
		Female	202	16.42	1.16		
	Ag4	Male	139	16.43	1.15	1.98	3.29
		Female	149	16.17	1.07		
FIB, g/L	Ag1	Male	247	2.12	0.51	0.94	3.95
		Female	169	2.07	0.54		
	Ag2	Male	391	2.40	0.46	1.39	4.85
		Female	235	2.45	0.42		
	Ag3	Male	238	2.50	0.50	1.50	4.06
		Female	202	2.57	0.49		
	Ag4	Male	139	2.55	0.50	1.08	3.29
		Female	149	2.61	0.44		

Note: Ag1, infant (≥29 days– < 1 year); Ag2, toddler (≥1 year– < 3 years); Ag3, preschool-age children (≥3 years– < 6 years); Ag4, school-age children (≥6 year– < 12 years).

Table 2
The partitions of five coagulation indices by age groups.

Indices	Program	Group	Number	Mean	Standard deviation	Z	Z*
PT, sec	Ag1 VS Ag2	Ag1	416	11.20	0.80	5.42	6.25
		Ag2	626	11.46	0.71		
	Ag2 VS Ag3	Ag2	626	11.46	0.71	6.36	6.32
		Ag3	440	11.74	0.71		
	Ag3 VS Ag4	Ag3	440	11.74	0.71	2.45	5.22
		Ag4	288	11.87	0.70		
INR	Ag1 VS Ag2	Ag1	416	1.04	0.07	4.76	6.25
		Ag2	626	1.06	0.06		
	Ag2 VS Ag3	Ag2	626	1.06	0.06	8.11	6.32
		Ag3	440	1.09	0.06		
	Ag3 VS Ag4	Ag3	440	1.09	0.06	2.22	5.22
		Ag4	288	1.10	0.06		
APTT, sec	Ag1 VS Ag2	Ag1	416	35.92	4.63	6.92	6.25
		Ag2	626	34.03	3.93		
	Ag2 VS Ag3	Ag2	626	34.03	3.93	1.96	6.32
		Ag3	440	34.50	3.87		
	Ag3 VS Ag4	Ag3	440	34.50	3.87	0.41	5.22
		Ag4	288	34.39	3.28		
TT, sec	Ag1 VS Ag2	Ag2	416	18.30	1.71	14.49	6.25
		Ag3	626	16.88	1.27		
	Ag2 VS Ag3	Ag3	626	16.88	1.27	4.36	6.32
		Ag4	440	16.54	1.23		
	Ag3 VS Ag4	Ag4	440	16.54	1.23	3.08	5.22
		Ag5	288	16.27	1.11		
FIB, g/L	Ag1 VS Ag2	Ag2	416	2.11	0.52	7.95	6.25
		Ag3	626	2.42	0.45		
	Ag2 VS Ag3	Ag3	626	2.42	0.45	3.69	6.32
		Ag4	440	2.53	0.50		
	Ag3 VS Ag4	Ag4	440	2.53	0.50	1.64	5.22
		Ag5	288	2.59	0.47		

Note: Ag1, infant (≥29 days– < 1 year); Ag2, toddler (≥1 year– < 3 years); Ag3, preschool-age children (≥3 years– < 6 years); Ag4, school-age children (≥6 years– < 12 years).

and forth several times after blood collection. Plasma was obtained by centrifugation at 3000*g* and +20 °C for 10 min, and the coagulation test was completed within 1 h after blood collection.

2.3. Laboratory assays

An automatic coagulation analyzer (ACL TOP 700, Instrumentation Laboratory Company, USA) and auxiliary reagents, calibration plasma, and quality control materials were used. Sample testing was performed by an ISO 15189 accredited laboratory in strict accordance with operating instructions. The following coagulation indices were assessed: prothrombin time (PT), international normalization ratio (INR), activated partial thromboplastin time (APTT), thrombin time (TT), and fibrinogen (FIB). The following reagents were used: PT Reagent, APPT Reagent, TT Reagent, Fibrinogen Reagent, Calibration plasma, normal control, low abnormal control, and high abnormal control; all were used within their respective expiration dates.

2.4. Statistical analysis and determination of RIs

All data were analyzed according to the CLSI C28-A3c guidelines [8]. Statistical analysis was performed using SPSS 22.0 software (IBM, Armonk, New York, United States). First, we inspected data using boxplots and removed outliers. Second, we statistically confirmed the age and sex grouping using the Harris and Boyd method [11]; when $Z > Z^*$, partitioning was considered necessary (Note: s_1 is the standard deviation of the first group; s_2 is the standard deviation of the second group; $\bar{x}_1$ is the mean of the first group; $\bar{x}_2$ is the mean of the second group; n_1 is the number of subjects in the first group; and n_2 is the number of subjects in the second group, $Z=\frac{\bar{x}_1-\bar{x}_2}{\sqrt{\frac{s_1^2}{n_1}+\frac{s_2^2}{n_2}}}$; $Z^*=3\sqrt{\frac{n_1+n_2}{240}}$). Third, the data in each partition were transformed by the Box–Cox transformation method, and Q-Q plots were used to assess the normality of each partition. We used the Tukey and adjusted Tukey tests twice to remove outliers in normally distributed and skewed partitions, respectively. Finally, we used the non-parametric rank method to calculate the RI for all partitions as all groups included > 120 participants. We also calculated 90% confidence intervals (CIs) for the upper and lower limits of each RI with the weighted data.

3. Results

A total of 1770 subjects were included in the present study. Among the five indices, PT, APTT, TT, and FIB were directly measured by the instrument; INR was obtained from instrument calculation. Scatterplots of the five coagulation indices are shown in Fig. 1.

According to scatterplots of the parameters, we divided the children into the following groups: infants (≥29 days– < 1 year of age, Ag1); toddlers (≥1– < 3 years of age, Ag2), preschool-age children (≥3– < 6 years of age, Ag3), and school-age children (≥6– < 12 years of age, Ag4). Age and sex partitions of each coagulation parameter were determined using the Harris and Boyd method. See Tables 1 and 2 for details.

As depicted in Table 1, none of the five coagulation indices needed sex-specific partitioning. For male–female comparisons in each age

Table 3
Age-specific reference intervals for five coagulation indices.

Indices	Reference interval						
	Age range (y,m,d)	Samples, n	Median	Lower limit	Upper limit	Lower 90% CI	Upper 90% CI
PT, sec	≥29 d– < 3 y	1022	11.29	10.00	12.67	9.93–10.07	12.60–12.74
	≥3 y– < 12 y	710	11.75	10.60	13.10	10.40–10.70	13.02–13.12
INR	≥29 d– < 3 y	1021	1.05	0.93	1.17	0.92–0.93	1.16–1.18
	≥3 y– < 12 y	709	1.09	0.98	1.21	0.97–0.99	1.20–1.22
APTT, sec	≥29 d– < 1 y	406	35.69	27.43	43.68	26.84–28.21	42.87–44.67
	≥1 y– < 12 y	1320	34.15	27.87	40.80	27.44–28.07	40.34–41.18
TT, sec	≥29 d– < 1 y	404	18.12	15.40	21.27	15.16–15.61	21.11–21.41
	≥1 y– < 12 y	1326	16.60	14.42	18.90	14.30–14.50	18.80–19.00
FIB, g/L	≥29 d– < 1 y	399	1.99	1.34	2.94	1.29–1.40	2.86–3.06
	≥1 y– < 12 y	1312	2.42	1.73	3.36	1.70–1.75	3.33–3.39

Note: y, year; m, month; d, day; n, the net sample number; ≥29 d– < 1 y, infants; ≥1 y– 3 y, toddlers; ≥3 y– < 6 y, preschool-age children; ≥6 y– < 12 y, school-age children.

group, the Z value was less than the Z* value. As presented in Table 2, all five of the coagulation indices required partitioning by age, as the indices indicated that the Z value was greater than the Z* value among different age groups.

We used the non-parametric rank method to calculate the RIs for all partitions and then calculated 90% CIs for the upper and lower limits of each RI with the weighted data. The RIs of the five coagulation indices are presented in Table 3.

4. Discussion

Coagulation screening (PT, INR, APTT, TT, and FIB) is necessary for preoperative preparation, anticoagulation therapy, thrombolytic therapy, infectious disease evaluation, etc. The coagulation system in childhood is immature and developing [12]. At the same time, the plasma concentrations of most pro- and anticoagulant proteins in childhood are lower than those in adults [13]. As a result, the RIs for coagulation indices in children are different from those in adults. On the basis of these factors, this study retrospectively analyzed the results of venous blood coagulation screening in apparently healthy children in southwest Fujian province, China, and established RIs for coagulation indices suitable for local pediatric populations.

In this study, we observed that each coagulation index measured at the same age range (> 28 days to 12 years) did not need sex-specific RIs; however, all indices measured required grouping by age, as reported by Toulon et al. [5]. We found that the test results of PT were consistent throughout childhood; however, the Harris and Boyd method [11] suggested establishing RIs in two age groups, namely, 1 month to 3 years and 3–12 years. According to Weidhofer's et al., there is little difference in the RIs of PT between children and adults [6]; our study showed similar results. In 1985, the International Council for Standardization in Hematology published guidelines regarding the monitoring of oral anticoagulants; it recommended the use of INR to report PT results [14]; the indices of INR and PT were correlated. INR is calculated from PT and the international sensitivity index (ISI) of measuring reagent. INR = (patient PT/normal control PT) ISI. Our study showed that the RIs established for INR and PT were consistent; this verified the correlation between PT and INR. The age partitions determined for APTT, TT, and FIB were different from those determined for PT. The RI of APTT in the 1 month to 1 year age group was wider than that of the 1–12-year age group, with more extreme upper and lower limits; overall, the RI for the 1–12-year age group shifted downward compared to that of adults [7]. In this study, the trend of the APTT level was consistent with that of the study by Toulon in France [5], which also used the ACL Top 700 system. However, the overall APTT level of the Chinese population was longer than that of the French population, irrespective of whether they were adults or children; this may be due to racial differences. Kanaji et al. and Endler et al. showed that the allele frequency of the frequently occurring C → T polymorphism in the factor XII promoter region at nucleotide 46 was higher in Orientals than in Caucasians and Europeans [15,16]. This led to lower plasma factor XII activity in Orientals; this may explain the prolongation of APTT in Chinese populations [17]. The pattern of TT values and RIs established were similar to those for APTT, and the RI of TT in childhood was wider than that of adults (16–18 s) [7]. TT was considered abnormal if exceeded that of the normal control in a clinical setting by at least 3 s. If no separate RIs of TT are established in childhood, some TT test results may be misjudged, particularly during infancy. Human plasma fibrinogen is synthesized in the liver [18]. The overall level of FIB in the 1 month to 1-year age group was lower than that in the 1–12-year age group, and the RIs of both were lower than those of adults [7]. This may be explained by the fact that FIB synthesis is lower during childhood. Compared with the Austrian cohort studied by Weidhofer et al. [6], the RIs of FIB in this Chinese population were narrower, showing a lower upper limit; this difference may be attributable to different detection instruments and reagents, or ethnic differences.

In summary, our study has established RIs of PT, INR, APTT, TT, and FIB for children in southwest Fujian, China, and is the first of its kind to be performed in this region. The obtained data confirmed that the RIs of PT, INR, APTT, TT, and FIB for children (from 1 month to 12 years) differed from the RIs defined for adults. In this study, the RIs of coagulation indices also differed across various age groups among children. Our study has a limitation; the sample sizes of the neonatal and adolescent age groups were relatively small. Therefore, the corresponding RIs could not be established. Differences caused by race, age, and sex and differences in methodology, detection systems, and reagents may affect the applicability of selected RIs. We recommend that laboratories capable of investigating coagulation function should establish RIs suitable for themselves. Laboratories that cannot establish their own RIs should select those that have been established by other laboratories in their own conditions, and quote this after verification.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to thank pediatric surgeons who offered their help in screening for study subjects, as well as all the participants involved in the study and the staff of the Medical Laboratory, Women and Children's Hospital, School of Medicine, Xiamen University. We would like to thank Editage (www.editage.cn) for English language editing.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] E.A. McGillicuddy, M.W. Maxfield, B. Salameh, L.H. Stein, U. Ahmad, W.E. Longo, Bleeding diatheses and preoperative screening, J. Surg. Educ. 70 (2013) 423–431, https://doi.org/10.1016/j.jsurg.2012.12.006.

[2] T. Van der Poll, H. Herwald, The coagulation system and its function in early immune defense, Thromb. Haemost. 112 (2014) 640–648, https://doi.org/10.1160/th14-01-0053.

[3] J.B. Larsen, A.M. Hvas, Predictive value of whole blood and plasma coagulation tests for intra- and postoperative bleeding risk: a systematic review, Semin. Thromb. Hemost. 43 (2017) 772–805, https://doi.org/10.1055/s-0037-1602665.

[4] G. Lippi, M. Franchini, M. Montagnana, G.C. Guidi, Coagulation testing in pediatric patients: the young are not just miniature adults, Semin. Thromb. Hemost. 33 (2007) 816–820, https://doi.org/10.1055/s-2007-1000373.

[5] P. Toulon, M. Berruyer, M. Brionne-François, F. Grand, D. Lasne, C. Telion, et al., Age dependency for coagulation parameters in paediatric populations. Results of a multicentre study aimed at defining the age-specific reference ranges, Thromb. Haemost. 116 (2016) 9–16, https://doi.org/10.1160/th15-12-0964.

[6] C. Weidhofer, E. Meyer, R. Ristl, H. Wiedemann, J. Cadamuro, U. Kipman, et al., Dynamic reference intervals for coagulation parameters from infancy to adolescence, Clin. Chim. Acta 482 (2018) 124–135, https://doi.org/10.1016/j.cca.2018.04.003.

[7] Ministry of Health of the People's Republic of China. National guide to clinical laboratory procedures, 4th ed. Beijing: People's Health Publishing House, 2015:96–115.

[8] G.L. Horowitz, S. Altaie, J.C. Boyd, Defining, establishing and verifying reference intervals in the clinical laboratory; approved guideline, 3rd ed. C28-A3c. Wayne, PA: Clinical and Laboratory Standards Institute, 2010.

[9] J. Li, H. Zhang, X. Huang, J. Zhang, X. Wu, Establishment of reference intervals for complete blood count parameters in venous blood for children in the Xiamen area, China, Int. J. Lab. Hematol. 41 (2019) 691–696, https://doi.org/10.1111/ijlh.13095.

[10] WHO, Guidelines for thromboplastin and plasma used to control oral anticoagulant therapy. WHO Tech. Rep. Ser., 889 (1999) 64–93.

[11] K. Ichihara, J.C. Boyd, An appraisal of statistical procedures used in derivation of reference intervals, Clin. Chem. Lab. Med. 48 (2010) 1537–1551, https://doi.org/10.1515/cclm.2010.319.

[12] M. Andrew, Developmental hemostasis: relevance to hemostatic problems during childhood, Semin. Thromb. Hemost. 21 (1995) 341–356, https://doi.org/10.1055/s-2007-1000655.

[13] S. Kuhle, C. Male, L. Mitchell, Developmental hemostasis: pro- and anticoagulant systems during childhood, Semin. Thromb. Hemost. 29 (2003) 329–338, https://doi.org/10.1055/s-2003-42584.

[14] ICSH/ICTH, Recommendations for reporting prothrombin time in oral anticoagulant control, J. Clin. Pathol. 38 (1985) 133–134. https://doi.org/10.1136/jcp.38.2.133.

[15] T. Kanaji, T. Okamura, K. Osaki, M. Kuroiwa, K. Shimoda, N. Hamasaki, et al., A common genetic polymorphism (46 C to T substitution) in the 5'-untranslated region of the coagulation factor XII gene is associated with low translation efficiency and decrease in plasma factor XII level, Blood 91 (1998) 2010–2014.

[16] G. Endler, M. Exner, C. Mannhalter, S. Meier, K. Ruzicka, S. Handler, et al., A common C– > T polymorphism at nt 46 in the promoter region of coagulation factor XII is associated with decreased factor XII activity, Thromb. Res. 101 (2001) 255–260, https://doi.org/10.1016/s0049-3848(00)00404-7.

[17] J. Li, X. Lai, C. Yan, A. Xu, L. Nie, Y. Zhou, et al., Age-associated developmental changes in the activated partial thromboplastin time (APTT) and causes of prolonged APTT values in healthy Chinese children, Clin. Chem. Lab. Med. 47 (2009) 1531–1537, https://doi.org/10.1515/cclm.2009.339.

[18] G.A. Tennent, S.O. Brennan, A.J. Stangou, J. O'Grady, P.N. Hawkins, M.B. Pepys, Human plasma fibrinogen is synthesized in the liver, Blood 109 (2007) 1971–1974, https://doi.org/10.1182/blood-2006-08-040956.