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White Paper

Development of Coagulation Parameters during Childhood and Puberty

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Disclaimer

This white paper summarizes a research study performed under the responsibility of Dr. Inge M. Appel at the Sophia Children's Hospital in Rotterdam, the Netherlands. Study was sponsored by Siemens by providing reagents, analyzers, performing BCS measurements and paying an honorary. Siemens is not liable for the clinical utility of the results. Sample storage was done according to CLSI Guideline H21-A5. The storage recommendations of this guideline has not been validated by Siemens. Data from this research study must not be misinterpreted as universal reference data. Reference intervals vary from laboratory to laboratory, depending on the population served and the techniques, method, equipment, and reagent lot used. Therefore, each laboratory must establish its own reference intervals or verify them whenever one or more of the aforementioned variables are changed.

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Introduction

The concept of development of the coagulation system during childhood was first introduced by Andrew et al.[1-2] These observations were confirmed in further studies, underlining the need for age-related reference ranges to be determined for each reagent-analyzer combination.[3] Klarmann et al.[4] published pediatric reference data for prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fbg), protein C (PC), antithrombin (AT), coagulation factor VIII (FVIII), and Von Willebrand factor (VWF) established with reagents from Siemens (Marburg, Germany) on the BCS® System from the same company. In the study described here (Appel et al.[5]), pediatric reference data for a full panel of 23 coagulation parameters measured with Siemens reagents are presented. These data were derived from more than 200 samples obtained from healthy children in the age range of 1 month to 18 years investigated on two analyzers: the BCS System and the Sysmex® CA-1500 System (Sysmex Corporation, Kobe, Japan).

Methods

Only blood samples obtained from healthy subjects were included in the study. For comparison purposes, age groups were classified as in earlier studies and grouped as follows: 1) 1–6 months; 2) 7–12 months; 3) 1–5 years; 4) 6–10 years; 5) 11–18 years; and 6) >19 years, as well as <50 years (adults). The study was performed in accordance with the Erasmus Medical Centre—Sophia Children's Hospital Ethics Committee. Written informed consent was obtained from the adult controls and from parents and/or guardians of the children and from the children older than 12 years.

The inclusion criteria were:

- Apparently healthy children and adults without previous thromboembolic or hemorrhagic events or any other coagulation disturbances
- No anticoagulant therapy
- No other interfering disease or clinical apparent infections
- Birth weight of >3000 g for the group 0–6 months old
- No prematurely born children

Additional criteria included children undergoing a minor surgical procedure needing intravenous access for anesthesia and an available written informed consent.

Citrated blood samples were obtained from the children with 18–24 G catheters. From adults and older children, citrated blood samples were obtained by peripheral venipuncture from the antecubital vein employing only light tourniquet to avoid stasis. Standard blood collection tubes containing 0.5 mL of 3.2% (0.105 M) trisodium citrate were used. Immediately after sampling, the blood was centrifuged twice (15 min at 2500 g and 5 min at 10,000 g at room temperature) and frozen at <–70°C within 2 hours of blood withdrawal.

The investigated coagulation parameters and methods are listed in Table 1. All reagents were from Siemens and used according to the instructions for use (IFU), except that frozen sample storage was done according to the recommendations given by the CLSI institute,[6] which include storage recommendations at ≤–70°C, whereas storage claims in the IFUs of the employed reagents are limited to storage at ≤–20°C. All blood samples were thawed within 6 months (CA-1500 measurements) or 10 months (BCS measurements) after blood withdrawal and measured within 4 hours after thawing (except for protein S, which was measured within 2 hours after thawing). Measurements on the Sysmex CA-1500 analyzer were performed at Sophia Children's Hospital in Rotterdam, the Netherlands, and measurements on the BCS instrument were performed in a laboratory at Siemens Healthcare Diagnostics Products GmbH in Marburg, Germany.

Table 1: Reagents employed on BCS and Sysmex CA-1500 systems.

Parameter	BCS Reagents	CA-1500 Reagents
Prothrombin time (PT)	Thromborel® S	Dade® Innovin®
Activated partial thromboplastin time (APTT)	Pathromtin® SL	Dade Actin® FS
Fibrinogen	Multifibren® U	Dade Thrombin
Thrombin time	n.d.	Thromboclotin®
Batroxobin time	n.d.	Batroxobin Reagent
Coagulation factors VIII, IX, XI, and XII	Coagulation factor-deficient plasmas and Pathromtin SL	Coagulation factor-deficient plasmas and Dade Actin FS Activated PTT
Coagulation factors II, V, VII, and X	Coagulation factor-deficient plasmas and Thromborel S	Coagulation factor-deficient plasmas and Dade Innovin
Coagulation factor XIII	Berichrom® Factor XIII	n.d.
Antithrombin (AT)	INNOVANCE® Antithrombin (FXa-based method)	Berichrom AT III (A) (FIIa-based method)
Protein S (PS)	Protein S Ac	Protein S Ac
Protein (PC)	Protein C (clotting method)	Berichrom Protein C (chromogenic method)
Von Willebrand factor antigen (VWF:Ag)	VWF Ag®	VWF Ag
Von Willebrand factor ristocetin cofactor activity (VWF:RCo)	BC von Willebrand Reagent	n.d.
Plasminogen	n.d.	Berichrom Plasminogen
α2-antiplasmin	n.d.	Berichrom α2-Antiplasmin

Statistical analysis and the determination of percentiles were performed using SAS® 9.1 software from SAS Institute Inc., Cary, NC, USA.
The results are presented as median, mean, and the central 90% interval (5th–95th percentile) of the different age groups.
Note VMF: RCo = BC von Willebrand

Table 2: Median, mean, and central 90% interval for **global assays**. The first line shows the median/mean with indication of statistical differences between methods and age groups. The second line shows the limits of the central 90% range.

Assay	Method	1–6 months N=29 [1] (14M/15F)	7–12 months N=25 [2] (19M/6F)	1–5 years N=57 (35M/22F)	6–10 years N=56 (29M/27F)	11–18 years N=50 [3] (24M/26F)	>19 years N=52 (27F/25M)
PT (sec)	Thromborel S	12.5/12.8 [4 5]	12.2/12.4 [4 5]	12.1/12.2 [4 5]	12.6/12.6 [4 5]	12.8/12.6 [4 5]	11.7/11.8 [5]
	BCS	11.2–15.5	11.4–13.5	11.2–13.4	11.5–14.0	11.4–13.8	10.7–12.9
	Innovin	10.7/10.7 [5]	10.6/10.6 [5]	10.6/10.6 [5]	10.9/10.9 [4 5]	10.8/10.9 [4 5]	10.5/10.6 [5]
	CA-1500	10.0–12.7	9.5–12.8	10.0–11.4	10.2–11.6	10.1–11.9	9.7–11.4
PT (%)	Thromborel S	92/89 [4 5]	95/93 [4 5]	97/96 [4 5]	91/91 [4 5]	89/91 [4 5]	101/101 [5]
	BCS	64–108	81–105	81–108	76–104	78–105	88–116
	Innovin	103/104 [5]	106/106 [5]	106/106 [5]	100/100 [4 5]	101/100 [5]	108/108 [5]
	CA-1500	72–122	71–128	89–121	86–116	81–118	89–129
APTT (sec)	Pathromtin SL	41/42 [4 5]	39/39 [4 5]	36/37 [4 5]	37/37 [4 5]	35/36 [4 5]	34/34 [5]
	BCS	33–56	32–49	31–44	31–44	30–43	27–40
	Actin FS	29/29 [4 5]	28/28 [4 5]	27/27 [4 5]	28/28 [4 5]	27/27 [4 5]	25/25 [5]
	CA-1500	21–33	24–33	24–30	25–32	25–30	22–28
TT (sec)	Thromboclotin	19.2/20.0 [4]	18.0/18.0	17.0/17.2	17.5/17.4	17.4/17.8	17.4/17.5
	CA-1500	16.2–24.9	15.4–21.1	15.3–19.7	14.5–19.9	15.2–24.0	15.5–20.5
BT (sec)	Batroxobin	21.0/21.4 [4]	20.2/20.5	20.2/20.3	20.2/20.2	19.8/19.9	20.1/20.1
	CA-1500	19.7–25.0	19.1–24.0	18.8–22.7	19.1–21.5	18.8–21.5	18.7–22.4

[1] N=28 for APTT on BCS (one sample was excluded because of extremely outlying result of 154 seconds obtained with Pathromtin SL)
[2] N=24 for PT, TT, and BT on CA-1500
[3] N=49 for batroxobin time (one sample was excluded because of extremely outlying result of 13.3 sec)
[4] Indicates statistically significant difference between children subgroups and adults in t-test ($p < 0.05$)
[5] Indicates statistically significant difference between devices in t-test ($p < 0.05$)

N=number; M=male; F=female; PT=prothrombin time; sec=seconds; BCS=Siemens BCS System; CA-1500=Sysmex CA-1500 System; APTT=activated partial thromboplastin time; TT=thrombin time; BT=batroxobin time

Results and Discussion

Samples were obtained from 218 healthy children and 52 adults. Results are reported for six different age groups: 1) 1–6 months (n=29); 2) 7–12 months (n=25); 3) 1–5 years (n=57); 4) 6–10 years (n=57); 5) 11–18 years (n=50); and 6) >19 years (n=52). One sample of the 6–10 years group with extremely low results for all single coagulation factors and unmeasurable clotting times for PT and APTT on the BCS was excluded because pre-analytical clotting was suspected.

Children and adults involved had different ethnic backgrounds reflecting the Dutch population. Tables 2–6 summarize results for different age groups by showing the median, mean, and 90% central interval for each parameter and method. For some samples, the sample volume was insufficient to perform all methods; affected age groups and methods are indicated in table footnotes. Figures 1–3 illustrate the development over age for some exemplary parameters.

The immaturity of the coagulation system shortly after birth is reflected by prolonged clotting times of the global screening assays PT and APTT. The initial significantly prolonged APTT's decreased with aging during childhood. The differences observed for PT, thrombin time, and batroxobin time are minimal in the age groups 1–5 years, 6–10 years, and 11–18 years and are likely not clinically relevant. For all global assays, results in the first group (1–6 months) showed a higher inter-individual variability leading to wide ranges for the central 90% interval (Figure 1).

Results for PT and APTT are significantly different between reagent/analyzers used for all age groups. The prolonged APTT values in the youngest age group are explained by the markedly decreased concentrations of the vitamin K-dependent factors IX, VII, X, and prothrombin. For the Pathromtin SL assay, the age dependency was more pronounced compared to Actin FS assay, which may partially be explained by the higher detection rate of transient lupus anticoagulants frequently seen in pediatric patients;[7–9] this may affect Pathromtin SL values more than Actin FS values. The results of the present study are in good agreement with previously published results[4] for the APTT measured by Pathromtin SL assay on the BCS system except for children <1 year, who presented with longer clotting times in our study. This difference may be due to differences in the study population. While Klarmann et al. excluded all individuals with C-reactive protein values beyond the age-specific reference ranges, this study only excluded children with clinically apparent infections.

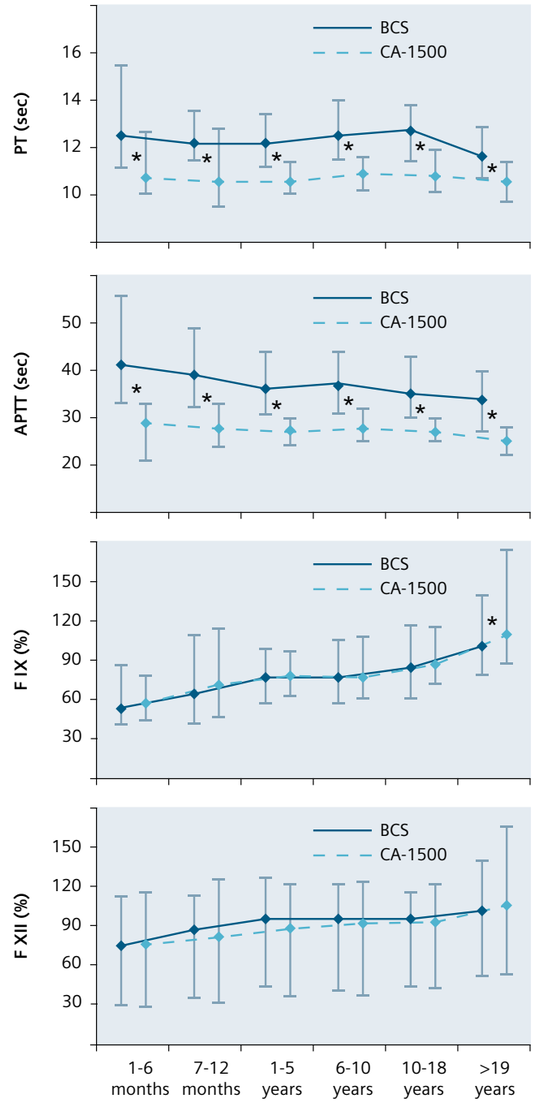

Figure 1: Comparison of PT, APTT, F IX, and F XII data with the BCS and CA-1500 systems.

* Significant differences in t-test between methods for marked age groups ($p < 0.05$).

Table 3: Median, mean, and central 90% interval for **single coagulation factors**. The first line shows the median/mean with indication of statistical differences between methods and age groups. The second line shows the limits of the central 90% range.

Assay	Method	1–6 months N=29 [1] (14M/15F)	7–12 months N=25 [2] (19M/6F)	1–5 years N=57 [3] (35M/22F)	6–10 years N=56 [4] (29M/27F)	11–18 years N=50 [5] (24M/26F)	>19 years N=52 [6] (27F/25M)
Fbg (g/L)	Multifibren U	2.2/2.3 [7]	2.3/2.6 [7]	2.5/2.7 [8]	2.3/2.6 [7]	2.3/2.5 [7]	2.9/3.0
	BCS	1.5–3.8	1.8–4.8	1.9–3.9	2.0–3.9	1.9–3.7	2.1–4.2
	Dade Thrombin	1.9/2.0 [7]	2.2/2.3 [7]	2.4/2.5 [7 8]	2.3/2.4 [7]	2.3/2.4 [7]	2.7/2.8
	CA-1500	1.3–3.3	1.6–4.0	1.7–3.5	1.8–3.6	1.8–3.3	2.0–3.9
F II (%)	Thromborel S	93/91 [7]	98/99 [7]	104/105 [7]	99/99 [7]	96/99 [7]	117/119
	BCS	66–112	83–132	85–126	78–121	78–132	96–147
	Innovin	86/86 [7]	95/97 [7]	102/103 [7]	97/98 [7]	92/94 [7]	114/116
	CA-1500	60–109	77–134	81–126	77–116	70–120	93–151
F V (%)	Thromborel S	114/114	114/117 [8]	108/111	97/99 [7 8]	95/99 [7 8]	112/113 [8]
	BCS	82–145	97–148	85–153	80–123	76–132	84–149
	Innovin	118/110 [7]	102/102 [7 8]	102/104 [7]	93/92 [8]	87/87 [8]	89/91 [8]
	CA-1500	56–148	66–141	68–143	62–127	55–119	57–128
F VII (%)	Thromborel S	98/97 [7]	96/98 [7]	99/99 [7 8]	96/98 [7 8]	100/101 [7 8]	105/108
	BCS	54–126	74–131	81–117	79–119	75–130	86–142
	Innovin	93/91 [7]	89/88	84/85 [7 8]	86/87 [7 8]	86/86 [7 8]	101/101
	CA-1500	38–129	41–148	61–111	61–127	55–115	67–146
F VIII (%)	Pathromtin SL	90/96 [7]	95/100 [7]	109/109 [7 8]	100/101 [7 8]	109/108 [7 8]	123/123 [8]
	BCS	58–144	59–>152	76–143	68–137	70–148	87–>152
	Actin FS	108 /107 [7]	116/119 [7]	124/125 [7 8]	118/119 [7 8]	118/122 [7 8]	133/140 [8]
	CA-1500	67–141	70–213	83–170	75–163	80–166	96–216
F IX (%)	Pathromtin SL	53/57 [7]	64/68 [7]	77/78 [7]	78/80 [7]	84/85 [7]	102/104 [8]
	BCS	41–87	42–109	58–99	57–106	60–117	78–139
	Actin FS	57/57 [7]	71/72 [7]	78/78 [7]	77/80 [7]	87/89 [7]	110/116 [8]
	CA-1500	44–78	46–114	63–97	60–108	72–116	87–174
F X (%)	Thromborel S	90/90 [7]	100/99 [7]	104/104 [7]	95/95 [7]	88/94 [7 8]	112/115
	BCS	66–132	74–124	84–129	74–120	73–128	90–149
	Innovin	88/87 [7]	97/99 [7]	101/100 [7]	92/92 [7]	84/86 [7 8]	110/114
	CA-1500	55–120	67–146	75–124	69–118	66–117	78–159
F XI (%)	Pathromtin SL	83/80 [7]	86/88 [7]	100/100	95/96 [7]	88/91 [7]	104/104 [8]
	BCS	54–101	65–125	72–134	75–127	72–122	77–130
	Actin FS	85/82 [7]	88/91 [7]	104/104 [7]	99/100 [7]	93/95 [7]	115/113 [8]
	CA-1500	57–105	64–129	74–134	78–131	78–122	83–158
F XII (%)	Pathromtin SL	75/72 [7]	88/81 [7]	95/92 [7]	96/90 [7]	96/89 [7]	102/101
	BCS	29–112	35–113	44–127	41–122	44–116	52–140
	Actin FS	76/74 [7]	82/82 [7]	88/87 [7]	92/88 [7]	92/88 [7]	106/108
	CA-1500	28–116	31–126	36–122	37–123	43–122	53–165
F XIII (%)	Berichrom FXIII	96/99 [7]	97/97 [7]	99/100 [7]	104/103 [7]	99/97 [7]	116/115
	BCS	63–152	42–128	71–139	76–133	64–133	68–>156

[1] N=28 for FVII, FVIII, FIX, and FX on BCS; N=27 for FXIII
[2] N=24 for Fbg, FII, FV, FVII, FX, and FIX for CA-1500, N=23 for FXI on BCS; N=18 for FXIII
[3] N= 53 for FXI on BCS; N=50 for FXIII
[4] N=55 for FII on CA-1500; N= 53 for FXI on BCS; N=51 for FXIII
[5] N=48 for FXI on BCS and FXIII,
[6] N=51 for FXI on BCS; N=49 for FXIII
[7] Indicates statistically significant difference between children subgroups and adults in t-test (p<0.0.5)
[8] Indicates statistically significant difference between devices in t-test (p<0.05)

N=number; M=male; F=female; PT=prothrombin time; sec=seconds; BCS=Siemens BCS System; CA-1500=Sysmex CA-1500 System; Fbg=fibrinogen; F=coagulation factor

Age dependency was most distinct in factor IX. Coagulation factors VIII, X, XI, and XII demonstrated considerably lower 5% percentiles in young children (<1 year) as compared to older children and adolescents. Fibrinogen and coagulation factors II, IX, X, XI, and XII demonstrate good comparability between methods performed on BCS and CA-1500 systems (exemplarily shown for factors IX and XII in Figure 1).

Table 4: Median, mean and central 90% interval for **coagulation inhibitors**. The first line shows the median/mean with indication of statistical differences between methods and age groups. The second line shows the limits of the central 90% range.

Assay	Method	1–6 months N=29 [1] (14M/15F)	7–12 months N=25 [2] (19M/6F)	1–5 years N=57 (35M/22F)	6–10 years N=56 (29M/27F)	11–18 years N=50 (24M/26F)	>19 years N=52 (27F/25M)
AT (%)	INNOVANCE AT	105/104 [3]	110/109 [3]	110/109 [3 4]	108/107 [3]	104/104 [3]	116/115
	BCS	81–126	90–132	93–128	92–122	90–119	97–133
	Berichrom AT	106/103 [3]	110/108 [3]	113/113 [4]	110/109 [3]	105/106 [3]	113/114
	CA-1500	78–129	88–132	97–129	97–122	93–122	98–131
PS (%)	Protein S Ac	78/79 [3]	81/80 [3]	85/83 [3]	84/84 [3 4]	82/86 [3]	101/105 [4]
	BCS	60–103	61–95	65–99	63–97	69–119	83–>130
	Protein S Ac	84/83 [3]	85/82 [3]	85/87 [3]	87/89 [3 4]	90/90 [3]	116/114 [4]
	CA-1500	59–99	59–110	60–115	63–116	62–126	86–>130
PC (%)	Protein C	70/71 [3]	83/85 [3]	97/97 [3]	98/97 [3]	100/103 [3]	120/118
	BCS	41–115	60–117	63–133	62–134	71–144	78–148
	Berichrom Protein C	66/67 [3]	76/78 [3]	88/92 [3]	90/92 [3]	93/96 [3]	114/115
	CA-1500	43–102	59–103	71–125	75–120	70–131	83–153

[1] N=28 for PS on CA-1500; N=27 for PC on BCS
[2] N=24 for AT on CA-1500
[3] Indicates statistical significant difference between children subgroups and adults in t-test (p<0.05)
[4] Indicates statistical significant difference between devices in t-test (p<0.05)

N=number; M=male; F=female; PT=prothrombin time; sec=seconds; BCS=Siemens BCS System; CA-1500=Sysmex CA-1500 System; AT=antithrombin

All investigated natural coagulation inhibitors are significantly higher in adults compared to children with lowest levels in the youngest age groups. Age-dependency is most prominent in vitamin K-dependent inhibitors (protein C and protein S). Measurements demonstrate equivalent trends between the two methods investigated (Figure 2).

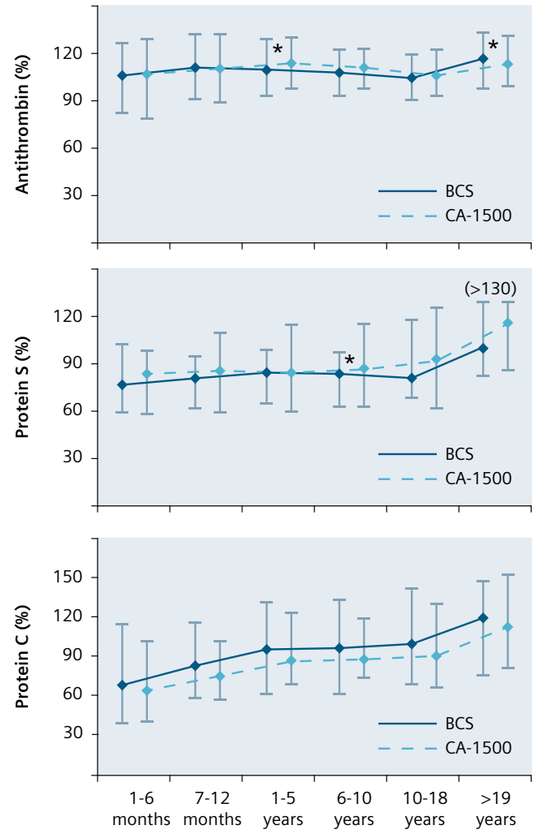

Figure 2: Comparison of antithrombin, protein C, and protein S data with the BCS and CA-1500 systems.

* Significant differences in t-test between methods for marked age groups (p<0.05).

Table 5: Median, mean, and central 90% interval for **α2-antiplasmin and plasminogen**. The first line shows the median/mean with indication of statistical differences between age groups. The second line shows the limits of the 90% range.

Assay	Method	1–6 months N=29 (14M/15F)	7–12 months N=25 [1] (19M/6F)	1–5 years N=57 (35M/22F)	6–10 years N=56 (29M/27F)	11–18 years N=50 (24M/26F)	>19 years N=52 (27F/25M)
α2-antiplasmin (%)	Berichrom α2-Antiplasmin CA-1500	122/121 103–139	123/125 [2] 100–151	128/128 [2] 107–145	119/121 103–140	114/113 [2] 97–126	118/119 103–133
Plasminogen (%)	Berichrom Plasminogen CA-1500	81/79 [2] 56–102	93/94 [2] 66–115	104/106 [2] 84–130	99/99 [2] 75–126	95/99 [2] 83–128	112/117 92–150

[1] N=24 for α2-antiplasmin and plasminogen
[2] Indicates statistical significant difference between children subgroups and adults in t-test (p<0.0.5)

N=number; M=male; F=female; PT=prothrombin time; sec=seconds; BCS=Siemens BCS System; CA-1500=Sysmex CA-1500 System; AT=antithrombin

Plasminogen levels are slightly lower in the first year of life, which agrees with previous findings that plasminogen levels increase to adult levels by approximately 6 months of age.[2] No differences were found for alpha2-antiplasmin between infant and adult levels. It seems that alpha2-antiplasmin levels reach adult levels within the first week of life.[1]

Table 6: Median and central 90% interval for **von Willebrand factor and coagulation factor VIII**.

Assay	Blood Group	1–6 months	7–12 months [2]	1–5 years [2]	6–10 years [2]	11–18 years [1]	>19 years
VWF Ag on BCS (%)	All	106 (N=27) 58–206	82 (N=25) 53–153	86 (N=57) 52–140	91 (N=56) 58–145	93 (N=50) 57–147	111(N=50) 65–182
	AB/A/B	106 (N=13) 77–215	80 (N=12) 64–155	97 [1] (N=27) 66–141	100 (N=27) 59–150	98 (N=30) 52–142	118 [1] (N=30) 65–196
	O	102 (N=14) 56–192	86 (N=10) 50–122	71 [1] (N=29) 45–152	80 (N=27) 45–144	90 (N=17) 61–152	99 [1] (N=20) 59–161
VWF Ag on CA-1500 (%)	All	109 (N=28) 63–223	96 (N=25) 60–158	90 (N=57) 60–140	94 (N=56) 60–142	99 (N=50) 60–159	112 (N=52) 72–188
	AB/A/B	110 (=14) 76–243	87 (N=12) 67–163	101 [1] (N=27) 71- 140	98 (N=27) 63–153	102 (N=30) 56–160	119 [1] (N=30) 72–199
	O	104 (=14) 61–192	100 (N=10) 59–141	77 [1] (N=29) 50–158	86 (N=27) 46–141	98 (N=17) 63–165	103 [1] (N=22) 62–162
VWF:RCO on BCS (%)	All	98 (N=27) 56–>150	73 (N=25) 51–>150	74 (N=57) 51–128	77 (N=56) 46–138	85 (N=50) 51–147	93 (N=50) 56–>150
	AB/A/B	94 (N=13) 62–>150	68 (N=12) 56–>150	82 [1] (N=27) 57–138	83 (N=27) 47–>150	90 (N=30) 43–147	106 [1] (N=30) 61–>150
	O	103 (N=14) 55–>150	88 (N=10) 52–114	66 [1] (N=29) 41–122	71 (N=27) 38–127	84 (N=17) 51–>150	82 [1] (N=20) 50–116
FVIII on BCS (%)	AB/A/B	98 (N=13) 72–>152	114 [1] (N=12) 77–>152	121 [1] (N=27) 89–148	105 [1] (N=27) 71–138	110 (N=30) 69–>152	131 [1] (N=30) 83–>152
	O	86 (N=15) 50–130	87 [1] (N=10) 59–115	100 [1] (N=29) 65–132	90 [1] (N=27) 52–143	103 (N=17) 70–134	116 [1] (N=22) 86–145
FVIII on CA-1500 (%)	AB/A/B	113 (N=14) 82–142	129 [1] (N=12) 93–232	128 [1] (N=27) 102–171	122 (N=27) 84–172	124 (N=30) 79–190	142 [1] (N=30) 92–221
	O	104 (N=15) 67–134	100 [1] (N=10) 69–129	115 [1] (N=29) 76–158	107 (N=27) 59–172	116 (N=17) 93–147	127 [1] (N=22) 96–160

[1] Indicates statistically significant difference between non-O and O blood groups in Student's t-test
[2] Blood group is not known for three individuals of the 7–12 months group, one individual of the 1–5 yrs group; two individuals of the 6–10 yrs group; and three individuals of the 11–18 yrs group.

Values for coagulation FVIII of all individuals are given in Table 4.

N=number; BCS=Siemens BCS System; CA-1500=Sysmex CA-1500 System

All VWF assays showed for children > 1years and adults the well-known blood-group dependency with 10–20% lower VWF levels in blood group 0 individuals compared to blood group non-0. VWF antigen and activity levels were higher in the youngest age group compared to older children independent of blood group. Antigen and activity levels in the non-O blood groups reached a nadir at about 12 months and then gradually increased towards adulthood. For blood group 0, this nadir was reached later in the age group of 1–5 years (Figure 3). FVIII demonstrated the same trend regarding the differences between blood group non-O versus O. It is remarkable that the high VWF levels in the youngest children are not accompanied by a parallel increase in FVIII; however, similar observations have been published previously.[2,4]

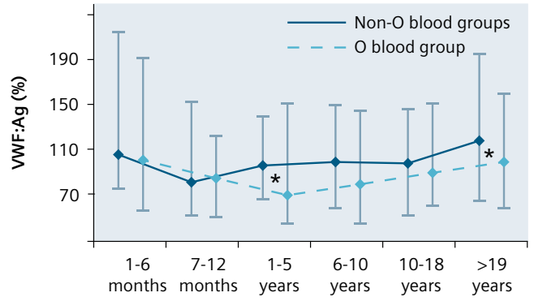

Figure 3: Comparison of von Willebrand factor in blood group non-O and O with BCS system.

* Significant differences between age groups (p<0.05).

Summary

The understanding of physiological age-dependent changes in the coagulation system is crucial to an accurate diagnosis in case of coagulation disorders, especially in the very young infant. Recent studies have provided reference ranges that delineate age-dependent characteristics of global coagulation assays and single coagulation factors and inhibitors. Reference ranges vary considerably with the reagents and analyzers used.

The comparison of the results obtained by Appel et al.[5] and Klarmann et al.[4] demonstrate that reference intervals depend not only on the method used but also on the reference population included and the laboratory environment. The study by Appel et al.[5] is the first to compare two different methods (analyzers and reagents) in the same study population. The correlation between these methods is remarkably high for single procoagulant factors (except for factor V), coagulation inhibitors, and von Willebrand factor. Most age groups do not demonstrate significant differences between methods, even though methods do differ with regard to the analyzer reagent used (e.g., the method for AT is FXa-based on the BCS system and FIIa-based on the CA-1500 system).

The largest differences between methods were found for PT and APTT; however, most of these differences were not more pronounced in children than in adults for PT. APTT is the only parameter for which age dependency seemed to be different between methods. The youngest age group demonstrated a considerably higher 95% percentile (140% of adult value) with Pathromtin SL reagent on the BCS system compared to the 95% percentile with Actin FS (120% of adult value). All other age-dependent parameters (e.g., protein C, FIX, FXII) demonstrated equivalent trends for the different methods; the percentage differences between children groups and adults for the biological decision points/medians were nearly identical between methods.

The data presented here have certain limitations; especially that the younger age groups were too small to allow a statistically valid reference range calculation. However, the findings of this study are in good agreement with previous, similar studies and may provide guidance for the age dependent trends to be expected for coagulation parameters.

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