

The Proportions of Low- and Intermediate-Molecular-Weight von Willebrand Factor Multimers Are Different in Neonates and Infants Compared to Adults

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von Willebrand factor (vWF) exists as a multimeric glycoprotein, consisting of a series of multimers with varying number of subunits that play a major role in hemostasis, specifically in platelet adhesion and aggregation.¹ The activity of vWF is proportional to the size of the multimers, with high-molecular-weight multimer (HMWM) having increased platelet binding and platelet aggregation capacity compared with the low- and intermediate-molecular-weight multimers (LMWM and IMWM).²

Previous studies have shown that the overall vWF concentration increases with age in healthy children and adults, as well as in individuals with von Willebrand disease. However, there is little known about the changes in the vWF multimer composition across the age spectrum from neonates to adults.³

An understanding of the effect of age on vWF and its multimers is important for accurate diagnosis and management of neonates and children with hematological complications. This study aimed to investigate age-specific changes in vWF concentration and function, as well as the composition of the vWF multimers in a healthy population spanning the age spectrum from neonates to adults.

Plasma samples were obtained from healthy neonates, children, and adults, without previous thromboembolic events (e.g., thrombosis and hemorrhage) and not subjected to any form of anticoagulant therapy, according to the previously established protocol.⁴ This study was approved by the Royal Children's Hospital, Human Research Ethics

Committee (HREC #34183) and the Royal Women's Hospital Human Research Ethics Committee (#2/08). Written informed consent was obtained from parents/guardians of children and from adult participants themselves.

Blood samples were collected in S-Monovette tubes (Sarstedt, Australia), containing 1 volume of citrate per 9 volumes of blood, and were centrifuged at 3,000 rpm for 10 minutes at 10°C (Megafuge 1.0R, Heraeus) to obtain plasma.

vWF concentration and activity were measured using the STA R Max analyzer and Stago reagents, STA vWF antigen (vWF:Ag) and STA ristocetin cofactor activity (vWF:RCO) (Diagnostics Stago, France). vWF collagen binding (vWF:CB) was measured using a commercially available enzyme-linked immunosorbent assay (Diagnostics Stago, France). vWF multimers were analyzed using the Hydrigel 11 vWF Multimer assay (Sebia, France), as previously described.⁵

Data analysis was performed using GraphPad Prism (Version 9.0). Results from the neonatal and pediatric age groups were compared with the results for adults using a one-way ANOVA (analysis of variance), followed by Dunnett's test to correct for multiple comparisons. $p < 0.05$ was considered to be statistically significant.

A representative image of the analyzed Hydrigel vWF multimer gels is shown in ►Fig. 1. vWF activity and its associated multimers were assessed in 80 children and 20 adults, with participant characteristics described in ►Table 1. While vWF concentration and activity, as

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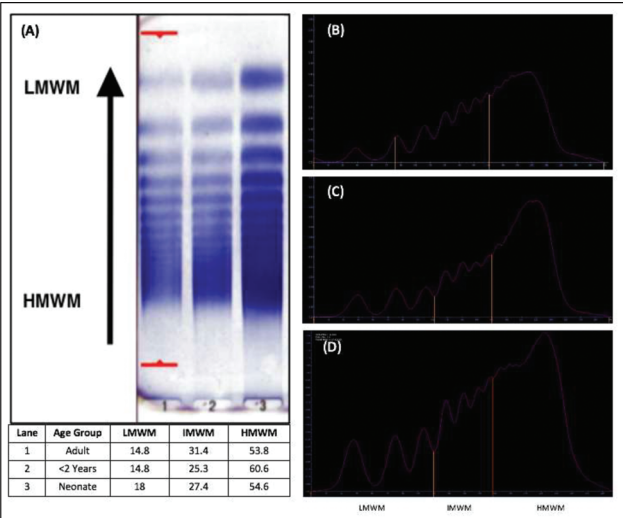


Fig. 1 Representative electrophoresis gel demonstrating vWF multimer patterns in a healthy adult (lane 1), <2 year old (lane 2), and a neonate (lane 3). Representative densitograms for lane 1 (B), lane 2 (C), and lane 3 (D) are also shown. vWF, von Willebrand factor.

represented by vWF:RCo, vWF:Ag, and vWF:CB, in neonates and children were comparable to adults, the proportions of LMWM were significantly higher in neonates compared with adults, before reaching and maintaining adult levels at approximately 6 to 10 years of age (–Table 2). In addition, IMWMs were significantly lower in neonates, and in children up to 5 years of age compared with adults.

The results of our study demonstrate significant age-specific differences in proportions of some of the vWF multimers, with no age-specific differences observed when considering the overall vWF activity and concentration.

Our vWF concentration and activity findings are comparable with previously published work,^{6–10} with no change across the age spectrum. vWF multimers have been previously investigated in critically ill children with varying comorbidities such as febrile seizures and in the setting of extracorporeal membrane oxygenation.^{11,12}

Previous studies investigating vWF multimers have shown that neonates have increased amounts of HMWM.^{13,14} Conversely, we demonstrated that in healthy neonates and children, proportions of LMWM are increased

Table 1 Participant demographic data

	Neonates	≤2 y	>2–5 y	6–10 y	11–17 y	Adults
Participants (n =)	20	22	11	8	19	20
Median age	48.0 h	0.9	3.5	9.5	13.9	32.7
Age range	24.0–96.0	0.3–1.4	2.3–4	8.8–10.3	11.7–17.6	20.5–53.9
Sex	10 M/10 F	18 M/4 F	5 M/6 F	5 M/3 F	10 M/9 F	7 M/13 F
Blood group						
O (n =)	5	6	2	2	4	Not available
A/B/AB (n =)	7	8	5	4	8	

Table 2 Age-specific results for vWF:RCo, vWF:Ag, vWF:CB, and vWF:CB/vWF:Ag ratio, vWF low-molecular-weight multimer (LMWM), vWF intermediate-molecular-weight multimer (IMWM), and vWF high-molecular-weight multimer (HMWM)

	Neonates (h)	≤2 y	>2–5 y	6–10 y	11–17 y	Adults
vWF:RCo						
Mean	102.1	74.0	88.9	86.1	97.2	92.7
95% CI	83.1–121.0	60.7–87.2	71.5–106.3	68.3–103.9	81.2–113.2	78.1–107.3
vWF:Ag						
Mean	112.6	83.8	98.8	91.3	89.8	101.9
95% CI	96.3–128.9	71.0–96.5	82.2–115.4	70.2–112.3	68.9–110.5	88.6–115.2
vWF:CB						
Mean	113.6	89.1	90.5	112.9	78.8	72.6

Table 2 (Continued)

	Neonates (h)	≤2 y	>2–5 y	6–10 y	11–17 y	Adults
95% CI	81.9–145.6	64.9–113.3	35.9–145.2	43.5–182.4	63.1–94.6	51.7–93.5
vWF:CB/vWF:Ag						
Mean	1.0	1.3	0.9	1.2	1.3	0.8
95% CI	0.7–1.3	0.7–1.9	0.4–1.4	0.5–1.8	0.3–2.3	0.5–1.0
vWF LMWM						
Mean	19.9 ^a	17.7	18.3	15.5	15.4	15.5
95% CI	17.1–22.7	15.7–19.7	14.5–22.1	13.9–17.1	12.7–18.1	14.0–17.0
vWF IMWM						
Mean	27.5 ^a	27.7 ^a	26.9 ^a	30.5	28.4	30.5
95% CI	25.5–29.6	26.5–28.8	24.7–29.1	28.3–32.7	26.9–29.8	29.1–31.8
vWF HMWM						
Mean	52.6	54.6	54.8	54.0	56.2	54.0
95% CI	48.3–56.8	52.6–56.7	49.7–59.8	51.4–56.6	53.1–59.3	51.8–56.3

Abbreviations: CB, collagen binding; CI, confidence interval; vWF, von Willebrand factor.

Note: Test results are expressed as percentage (%).

^ap-Value significantly different from adults ($p < 0.05$).

and proportions of IMWM are decreased in neonates and young children compared with adults. However, proportions of HMWM were relatively the same across the age spectrum. Differences between our findings and previous studies could be explained by: (1) differences in the age (e.g., neonates born <36 weeks' gestation) and health status (e.g., thrombotic thrombocytopenic purpura, necrotizing enterocolitis) of the participants used across studies, and (2) different methodological techniques used to investigate vWF multimers. Specifically, the vWF multimer analysis techniques utilized in the studies conducted by Katz et al¹³ and Weinstein et al¹⁴ are in-house, labor-intensive, manual laboratory tests that are operator-dependent. On the other hand, the semi-automated method produced by Sebia reduces operator dependence and has been shown to be reliable and successful in correctly visualizing and interpreting vWF multimers.¹⁵

Differences were seen in this study, across the pediatric age groups when vWF was characterized. Further research is required to gain a better understanding of the effect and interaction of the specific vWF multimers and current therapeutics.

In conclusion, while there are no changes in the overall activity and function of vWF, the sizes specific vWF multimers vary significantly with age in healthy neonates and children up to 5 years of age. Our findings highlight that physiological age-specific differences in hemostatic proteins such as vWF can only be detected using sensitive assays that account for the specific forms of proteins, such as the vWF multimers.

Author Contributions

N.L., S.V.D.H., R.B., and V.K. planned and performed the experiments and analyzed the data. N.L., S.V.D.H., and A.W. wrote the manuscript. V.I. and P.M. oversaw the project

and reviewed the manuscript. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest

None declared.

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