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LETTER TO THE EDITOR

Letter in response to Othman & Favaloro "Comparison of two ways of performing ristocetin-induced platelet agglutination (RIPA) mixing study for diagnosis of type 2B VWD"

To the Editor,

Platelet type-von Willebrand disease (PT-VWD) is one of the rarest inherited bleeding disorders and can be misdiagnosed as subtype 2B VWD (2B-VWD) because they both share similar laboratory profile and clinical manifestations [1]. The differential diagnosis between 2B-VWD and PT-VWD is of utmost importance to avoid misdiagnosis and ultimately mistreatment. Typically, when there is excessive agglutination with low-concentration ristocetin (<0.5-0.7 mg/mL), a 2B-VWD or PT-VWD should be suspected. The International Society on Thrombosis and Haemostasis (ISTH) guidelines for platelet function disorders and the multisociety American Society of Hematology/ISTH/National Hemophilia Foundation/World Federation of Hemophilia guidelines for VWD diagnosis suggest additional tests, such as von Willebrand factor multimer analysis, genome sequencing, and ristocetin-induced platelet agglutination (RIPA) mixing studies, for the differential diagnosis [1,2]. However, neither the former nor the latter guidelines offer a RIPA mixing precise methodology. Indeed, the RIPA mixing study enables us to investigate the von Willebrand factor and platelet GpIb receptor interaction, but in the absence of methodological standardization, genome sequencing is considered as the gold standard for the differential diagnosis [1–3]. As genotyping is expensive, time-consuming, laborious to implement and requires some expertise, its use is restricted to proficient molecular genetic laboratories. Recently, Othman and Favaloro [1] challenged the use of RIPA mixing studies, outlining the lack of applicability in laboratories and challenging interpretation for laboratories and physicians [1]. Since 2020, we have performed concurrently the RIPA mixing studies using 2 different methodologies, known as volume–volume (Argentinean method) and phosphate-buffered saline-ethylenediaminetetraacetic acid (PBS-EDTA; Westmead method) dilution methods, which are considered to be reliable based on genotype testing results [3].

Hereby, we present 2 cases of excessive low-concentration ristocetin agglutination and RIPA mixing studies (Figure) suggesting 2B-VWD in patients with no family history of bleeding disorder. The first patient was a 21-year-old woman with excessive mucocutaneous bleeding presenting as easy bruising and menorrhagia, and the second patient was a 32-year-old woman with a prolonged postpartum hemorrhage requiring massive transfusions and hysterectomy. The ISTH Bleeding Assessment score was 4 for both patients. The preanalytical phases, testing procedure, and result interpretation were carried out following manufacturer's instructions and ISTH guidelines [1,2]. Details of methodologies, their advantages and disadvantages, and laboratory test results are summarized in the Supplementary Material. Although sample preparation in the volume–volume method is a 2-step process using 2 citrated blood samples, the PBS-EDTA method requires multiple preanalytical and analytical steps using a large volume of citrated blood samples. In comparison with the molecular genetic results that confirmed 2B-VWD, we have observed a complete agreement between phenotyping up to RIPA mixing studies and genotyping, suggesting the reliability of both ways to perform RIPA mixing studies. The volume–volume method offers the advantage of requiring a small blood volume compared with the PBS-EDTA dilution method, enabling us to investigate VWD in children, particularly in neonates, while being faster, robust, and using fewer reagents. The volume–volume method can also be easily implemented in hemostasis laboratory. We also believe as previously stated by Othman and Favoloro [1] that the issues with RIPA mixing studies are not so much in the validity or poor applicability but rather in the lack of standardization, the limited awareness of its value, and its challenging interpretation among laboratory personnel and/or physicians. These results should however be verified on a larger scale, particularly in thrombocytopenic subjects. In conclusion, we think that RIPA mixing studies have the potential to be a valuable tool for clinical laboratories to differentiate, at the phenotypic level, 2B-VWD and PT-VWD.

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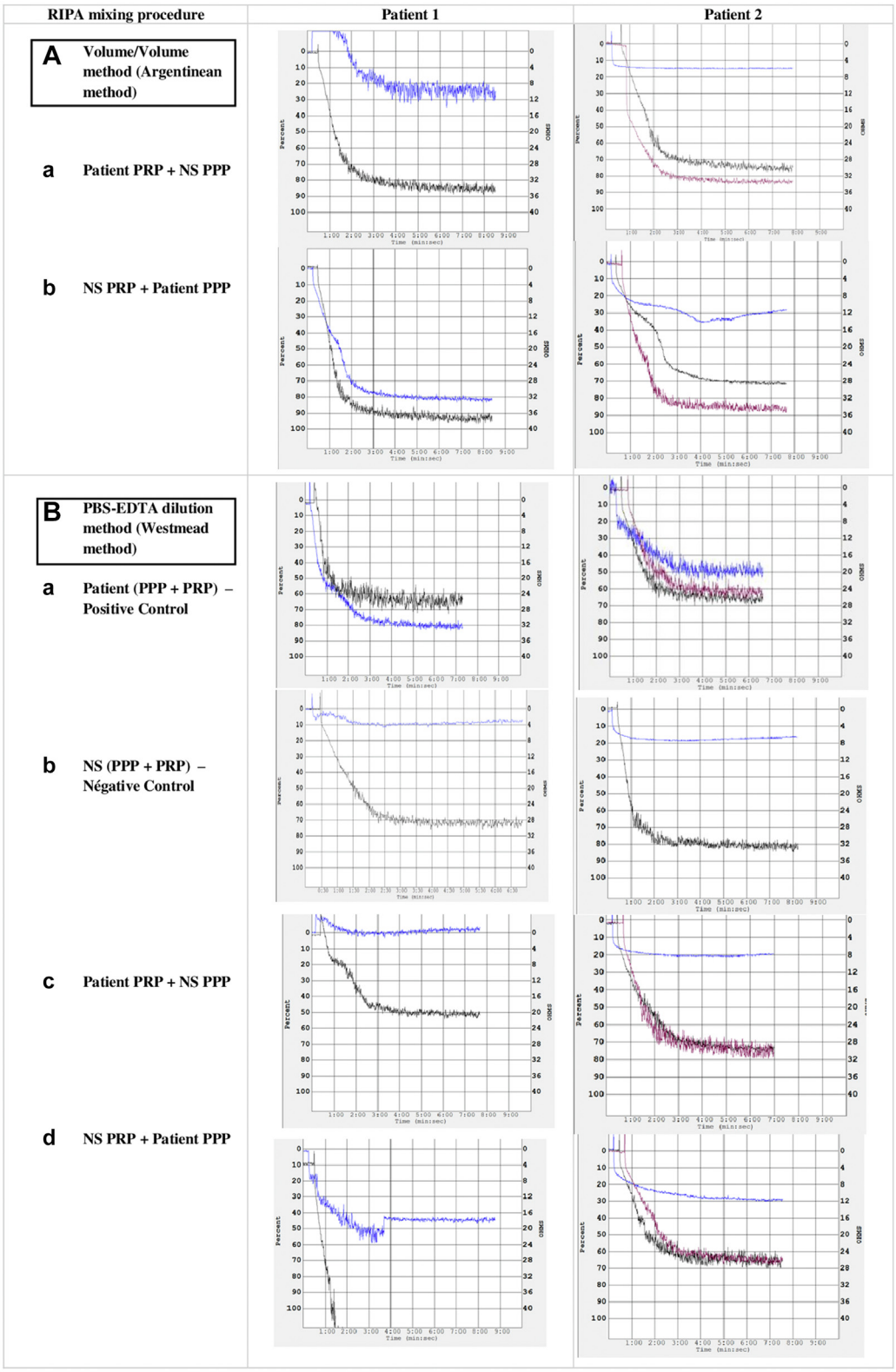

FIGURE RIPA-based mixing study to identify plasma-based defect (ie, 2B-VWD) using (A) volume-volume RIPA mixing procedure (Argentinean method) using (a) patient's PRP and NS PPP, (b) NS PRP and patient's PPP. RIPA mixing study using the following final concentrations of ristocetin: 0.7 mg/mL (blue curve), 1.25 mg/mL (black curve) and 2.0 mg/mL (red curve). (B) PBS-EDTA RIPA mixing procedure (Westmead method) using (a) P1: patient platelets/patient plasma, (b) NS1: control platelets/control plasma, (c) P2: patient platelets/control plasma, and (d) NS2: control platelets/patient plasma. RIPA mixing study using the following final concentrations of ristocetin: 0.7 mg/mL (blue curve), 1.25 mg/mL (black curve) and 2.0 mg/mL (red curve). Platelet counts of patient 1: 232 × 109 /L, patient 2: 130 × 109 /L and NS: 350 × 109 /L. 2B-VWD, type 2 von Willebrand disease; EDTA, ethylenediaminetetraacetic acid; NS, normal subject; PBS, phosphate-buffered saline; PPP, platelet-poor plasma; PRP, platelet-rich plasma; RIPA, ristocetin-induced platelet agglutination.

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AUTHOR CONTRIBUTIONS

All the authors have approved the entire content of the submitted manuscript and any subsequent revised version and have accepted responsibility for the entire work.

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RELATIONSHIP DISCLOSURE

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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REFERENCES

[1] Othman M, Favaloro EJ. 2B von Willebrand disease diagnosis: considerations reflecting on 2021 multisociety guidelines. *Res Pract Thromb Haemost*. 2021;5:e12635. https://doi.org/10.1002/rth2.12635
[2] James PD, Connell NT, Ameer B, Di Paola J, Eikenboom J, Giraud N, et al. ASH ISTH NHF WFH 2021 guidelines on the diagnosis of von Willebrand disease. *Blood Adv*. 2021;5:280–300.
[3] Frontroth JP, Favaloro EJ. Ristocetin-induced platelet aggregation (RIPA) and RIPA mixing studies. *Methods Mol Biol*. 2017;1646:473–94.

SUPPLEMENTARY MATERIAL

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