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LETTER TO THE EDITOR

Reply to "Comment on: A comparative study in type 2 von Willebrand disease patients using four different platelet-dependent von Willebrand factor assays"

Dear Editor,

We would like to thank Favaloro [1] for his interest in our manuscript and for sharing the data of the Royal College of Pathologists of Australasia Quality Assurance Program. We agree that this quality control survey can be very useful in establishing the sensitivity and specificity of diverse assays evaluating samples from patients with different types of von Willebrand disease (VWD) [1]. In our manuscript [2], we focused only on the assays that measure the platelet-dependent von Willebrand factor (VWF) activity using either platelets and ristocetin (VWF:RCo) or gain-of-function recombinant glycoprotein (GP) Ib molecules (VWF:GPIbM), with the goal to investigate the possible advantages or disadvantages of these 2 methods among a heterogeneous group of patients with type 2 VWD. All investigated cases have been characterized at both biochemical and molecular levels and classified following the International Society on Thrombosis and Haemostasis guidelines. In particular, we focused on patients with VWD-carrying variants in the A1 domain (type 2B with/without high molecular weight multimers (HMWM), 2M, and 2M/2A), which plays a key role in VWF binding to the GPIb platelet receptor as well as to ristocetin. Our work pointed out that the VWF:RCo assays overestimate type 2B patients (with/without HMWM) vs the VWF:GPIbM commercial assay, whereas the VWF:GPIbM assays appear to be less effective in detecting the type 2M and 2M/2A variants vs the VWF:RCo assays.

Moreover, we take the opportunity to evaluate our results by applying the cutoff of 0.7 for the platelet-dependent VWF activity/VWF:Ag ratio as suggested to discriminate type 1 from type 2 patients with VWD in the new guidelines [3]. The graphical abstract [2] showed the percentage of the correct diagnosis and misdiagnosis obtained in each VWD type group with the 4 different assays using either 0.6 or 0.7 as cutoff. Contrary to what was reported by Favaloro [1], it is clear that the misidentification does not concern only the type 2B without HMWM but also the type 2A, 2M, and 2M/2A patients. Hence, the choice of 0.6 or 0.7 as a cutoff affects all groups of type 2 evaluated in our study, with the obvious exception of the 2B with HMWM, behaving as type 1 VWD [2]. This finding emphasized this critical

TABLE Platelet-dependent von Willebrand factor activity/VWF:Ag ratios in patients with type 2M von Willebrand disease.

ID patient	VWF:Ag (IU dL^{-1})	VWF:RCo automated/ VWF:Ag ratio	VWF:GPIbM automated/ VWF:Ag ratio	Amino acid change
P1	26	0.23	0.38	p.Ala1377Val-Arg1379Cys/WT
P2	19	0.32	1.05	p.Ala1377Val-Arg1379Cys/WT[a]
P3	44	0.14	0.23	p.Asp1277_Leu1278delinsGlu/WT
P4	31	0.35	0.42	p.Ile1343Val-Val1360Ala-Phe1369Ile-Ser1378Phe-Arg1379Cys/WT
P5	62	0.10	0.42	p.Phe1369Ile-Ser1378Phe-Arg1379Cys/WT[a]
P6	22	0.27	0.59	p.Phe1369Ile-Ser1378Phe-Arg1379Cys/WT[a]
P7	26	0.23	0.35	p.Leu1278Pro/WT
P8	19	0.32	0.53	p.Phe1369Ile-Ser1378Phe-Arg1379Cys/WT[a]
P9	32	0.19	0.38	p.Ala1377Val-Arg1379Cys/WT[a]

GPIb, platelet receptor glycoprotein Ib; VWD, von Willebrand disease; VWF, von Willebrand factor; VWF:Ag, IU, International Units; VWF antigen; VWF:GPIbM, VWF gain-of-function mutant GPIb binding; VWF:RCo, VWF ristocetin cofactor activity; WT, wild-type.

[a]Patients carrying the same variant are related. The assays used to evaluate type 2M patients were performed as reported previously [2].

aspect and how the diagnosis of type 1 or type 2 VWD in patients with "borderline" platelet-dependent VWF activity/VWF:Ag ratios should not rely only upon this feature. Indeed, the authors of the new guidelines [3] recommend a cutoff of 0.7, aware that type 1 patients, who could be falsely included among the type 2, will be correctly classified following additional investigations routinely performed on type 2 patients.

Since only 9 type 2M patients were evaluated in our study [2], we herein investigated 9 additional patients (Table) to further examine the limitations of the commercial VWF:GPIbM assay with this VWF variant often being misdiagnosed as type 1. At variance with our previous findings [2], all investigated cases, with a single exception, were correctly diagnosed by both assays. Nevertheless, the VWF:GPIbM assay appears to marginally overestimate these patients compared with the VWF:RCo assay.

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AUTHOR CONTRIBUTIONS

P.C. and L.B. wrote the manuscript. P.C., G.C., and M.T.P. performed the assays. E.B. enrolled the patients. F.P. critically reviewed the manuscript. All authors approved the final version of the manuscript.

RELATIONSHIP DISCLOSURE

F.P. reports participation at educational meetings and is on the advisory board of CSL Behring, Biomarin, Roche, Sanofi, Sobi, and Takeda/Spark. The other authors report no conflicts of interest.

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