To the editor:

Discrepant activity levels of von Willebrand factor–cleaving protease (ADAMTS-13) in congenital thrombotic thrombocytopenic purpura

We have read with great interest the article of Savasan et al[1] in which the authors describe the confirmation of constitutional thrombotic thrombocytopenic purpura (TTP) due to severe congenital deficiency of the von Willebrand factor (VWF)–cleaving protease, ADAMTS-13, in a pediatric patient. This child had presented with a thrombotic microangiopathy suggestive for constitutional TTP (Upshaw-Schulman syndrome). However, ADAMTS-13 activity was found to be normal (~50%) when first determined in her plasma in 1997 by our laboratory,[2] using the immunoblotting assay of Furlan et al.[3] In contrast, the analysis by Savasan et al,[1] carried out some 5 years later in a different set of plasma samples using the assay of Tsai and Lian,[4] revealed a severely deficient ADAMTS-13 activity (< 10% of that in normal human plasma). A causal homozygous ADAMTS13 gene mutation was identified, confirming a constitutional deficiency. The authors suggest that the different assay methods might be responsible for this discrepancy and attribute the differing result of the immunoblotting assay to either a nonspecific adsorption of the large VWF multimers, to degradation of VWF by plasmin or other proteases generated during the incubation, or to an effect of the barium chloride used for ADAMTS-13 activation.

In our laboratory, the immunoblotting assay[3,5] has so far been applied to the investigation of several hundred patients with thrombotic microangiopathies and consistently identified severe ADAMTS-13 deficiency in a large number of patients with hereditary and acquired TTP. The unequivocal detection of severe ADAMTS-13 deficiency recently has been confirmed by a multicenter assay comparison including several laboratories using different methods for the measurement of ADAMTS-13 activity in plasma.[6]

Dr Savasan kindly agreed to send us a new set of plasma samples of the proposita and her parents. Our analysis of these samples confirmed severe ADAMTS-13 deficiency in the proposita (< 3% of the normal, with an increase to ~8% 2 hours after infusion of fresh frozen plasma) and ~50% activity in both parents, thus confirming constitutional ADAMTS-13 deficiency in the proposita. Results are shown in Figure 1; each sample was analyzed 4 times with identical results.

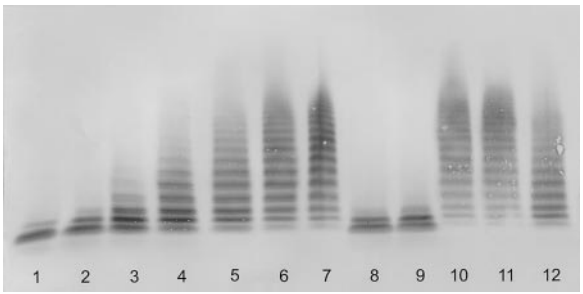

Figure 1. Quantitative immunoblotting of purified VWF substrate degraded by ADAMTS-13 in plasma dilutions. Lanes 1-7 = assay calibration by normal plasma dilutions of 1:20 (100% activity), 1:40 (50%), 1:80 (25%), 1:160 (12.5%), 1:320 (6.25%), 1:640 (3%), and buffer (0%). Lanes 8-12 = plasma dilutions (1:20) of the proposita and her parents. Lane 8 = father (~50% activity), lane 9 = mother (~50%), lanes 10-12 = proposita (lane 10 = March 13, 2003, before plasma infusion, < 3%; lane 11 = March 27, 2003, before plasma infusion, < 3%; lane 12 = March 27, 2003, 2 hours after plasma infusion, ~8%).

We therefore offer an alternative explanation of the discrepant ADAMTS-13 activity values. According to the available information, the sample investigated in 1997 might have been collected after infusion of fresh frozen plasma, which the proposita was receiving at regular intervals. At that time the long half-life of the protease (2-3 days)[7] was still unknown. Thus, the normal ADAMTS-13 activity measured in this sample was most likely originating from the infused plasma. We therefore do not see an indication for a causal relationship between the discrepant ADAMTS-13 activity values and the different assays used for evaluation. Furthermore, we regret that Dr Tsai did not participate in the above-mentioned multicenter assay comparison[6] that allowed for comparison of ADAMTS-13 activity results obtained by different methods on identical plasma aliquots.

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