To the Editor:

We read with interest the comments of Lippi et al (1) who questioned the reproducibility and difficulty of standardization when we (2) used platelet-rich plasma (PRP) in the thrombin generation assay (TGA). We evaluated the use of TGA to identify patients needing further specific thrombophilic risk factor testing (2). For this, we used frozen-thawed (ft-PRP). PRP was obtained by centrifugation (190 g for 12 min), adjusted to 150 × 10^9/L, and immediately frozen at −80 °C. Samples were immediately thawed before the assay. TGA was triggered by the addition of 0.5 pmol/L of recombinant human tissue factor. To test for thrombophilia, we evaluated the TGA in the presence of activated protein C (APC) (25 nmol/L). This methodology had been described and evaluated earlier by Regnault et al. (3–5).

We agree that the nature of the biological sample is a key point. We also agree that when TGA is performed with platelet poor-plasma (PPP), it is mandatory to avoid residual platelets or platelet debris. This was stressed recently by 2 other groups (6,7). Chantarangkul et al. (6) proposed 2 alternatives to overcome the role of residual platelets when PPP was used: a 0.22-μm filtration before freezing plasma or the use of phospholipids in a concentration >1.5 μmol/L. Gerotziafas et al. (7) also reported the importance of the concentration of phospholipids when PPP was used, which in their experimental condition had to be >4 μmol/L to overcome the bias induced by nonfiltered PPP.

To test for thrombophilia, we chose to use a frozen-thawed (ft-)PRP (2). The 2 theoretical reasons for this choice were to perform TGA in a physiological environment, i.e., in the presence of autologous platelet phospholipids, and to use a biological material compatible with multicenter studies, i.e., a frozen sample. In preliminary experiments, we compared ft-PPP with ft-PRP. When TGA was performed in ft-PPP, synthetic phospholipids were added at a concentration of 4 μmol/L. When APC was used at a concentration of 25 nmol/L, thrombin generation triggered by tissue factor was fully inhibited in ft-PPP, in both normal and factor V Leiden plasma. On the contrary, when the assay was performed in ft-PRP, the test was highly sensitive to APC resistance (Fig. 1). Furthermore, we previously reported on the low imprecision of the assay in ft-PRP (2).

We agree that standardization of the procedure is a crucial point and needs to be evaluated and validated in a large, multicenter scale.

References


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