Reliability of the Thrombin-Generation Assay in Frozen-Thawed Platelet-Rich Plasma

To the Editor:

The recent introduction of a general coagulation function test, namely the thrombin-generation assay (TGA), has enabled efficient assessment of the global functioning of the hematologic system. By using a fluorogenic substrate, the TGA produces thrombin-generation curves in a fully automated manner that may be useful and sensitive enough to screen for either hypercoagulable states or hemorrhagic diatheses. In the recent report, Hézard et al. (1), concluded that the TGA can be reliably used to screen patients needing further specific thrombophilia testing. Specifically, a thrombin generation lag time ≤1.5 min indicates the need for factor V Leiden genotyping, whereas a peak thrombin concentration >433 nmol/L indicates the need for factor II G20210A genotyping. As reported in that study, the experiments were performed on thawed, previously frozen, platelet-rich plasma (PRP), and little indication is provided on either the collection procedure or the storage conditions of these samples, both of which are essential requisites to enable reliable TGA results (2, 3).

Previous exhaustive evaluations of TGA demonstrated that although the integral amount of thrombin generated in time, expressed by the endogenous thrombin potential (ETP), appears substantially unmodified in frozen-thawed PRP, thrombin generation is accelerated and the maximum amount of generated thrombin is increased, apparently as a result of cold-induced platelet activation, membrane damage, and procoagulant phospholipid exposure (2, 3). Accordingly, in frozen-thawed PRP, the lag time decreases substantially, up to one third, compared with non-frozen specimens (2). The freezing also affects the maximum concentration of thrombin (c_{max}), which is substantially higher in frozen-thawed than in fresh PRP. Thus, it seems likely that assessing thrombin generation in frozen-thawed PRP would introduce a substantial bias in several measurements, especially lag time and peak concentration. Consequently, the ETP would appear to be the single variable that can be assessed in PRP, regardless of the storage conditions (2). However, this is further disputed by Chantarangkul et al. (4), who demonstrated that when the phospholipids are omitted, such as in the experimental conditions of Hézard et al. (1), there is a linear relationship between the ETP value and the number of residual platelets in thawed specimens. Platelets are not an ideal surrogate for exogenous phospholipids, as the fatty acid composition of membrane phospholipids in platelets might be heterogeneous, depending basically on dietary lipid modifications (5). Additionally, the interindividual variability of several TGA indicators measured in PRP is considerably higher, especially in the presence of very low concentrations (≤3 pmol/L) of tissue factor (2). Potential artifacts in thawed specimens, such as platelet debris or the presence of procoagulant material, are detrimental to assay reliability (2). Although ideally TGA should be evaluated on whole blood or PRP, we recommended that frozen plasma is suitable, provided that it is filtered before testing to eliminate the unwanted effect of residual platelets (6). Earlier data showed an increased sensitivity to activated protein C (APC) in frozen-thawed PRP compared with fresh PRP (3). To minimize the influence of using frozen-thawed PRP preparations, Regnault et al. (3) suggested that 6.7 nmol/L exogenous APC be added, instead of 25 nmol/L, the latter being the experimental conditions of Hézard et al. (1).

Because the freezing-thawing effects on ETP, lag time, and c_{max} cannot be anticipated and depend on heterogeneous interindividual functional characteristics of platelets (7), the use of thawed PRP is likely to introduce an unpredictable bias, influencing result comparability and transferability within the same study protocol, especially during assessment of the APC-induced thrombin potential inhibition (2). Additionally, although the use of frozen PRP may be justified to screen for the presence of lupus anticoagulants (3), there are no clear reasons to use PRP to screen for inherited coagulation disorders that do not directly involve platelet pathophysiology, such as factor V Leiden and the factor II G20210A sequence variant (8).

In conclusion, we acknowledge that, on the whole, the TGA might be potentially useful for the laboratory assessment of a large spectrum of clotting abnormalities. Nevertheless, as with other areas of coagulation testing, we suggest that rigorous pre-analytic and experimental conditions for the TGA ought to be fulfilled and standardized (2–4, 6) to provide reliable information on clinically meaningful hypercoagulable states.

References

To the Editor:

Results of serum assays for free triiodothyronine (FT₃) can be falsely increased by cross-reacting drugs and by autoantibodies (e.g., antithyroid hormone autoantibodies, heterophilic antibodies). The FT₃ antibody included in the Vitros FT3 assay (Ortho-Clinical Diagnostics, Inc.) cross-reacts with diclofenac, resulting in falsely high measured FT₃ concentrations. FT₃ and free thyroxine (FT₄) assays that use diiodothyronine (T₂)-gelatin show falsely high FT₃ and FT₄ concentrations attributable to the presence of antibodies to T₂, T₃, or their conjugates.

Concentrations of drugs in blood are in equilibrium between free and protein-bound forms. Therefore, according to the law of mass action, serum concentrations of free hormones (FT₃ and FT₄) do not change upon dilution of the serum. Thus, a concentration change in response to dilution may indicate the presence of an interfering substance, as reported by Westerhuis and Venekamp, who found that FT₄ concentrations decreased when serum containing anti-T₂-gelatin and anti-T₃-gelatin autoantibodies was diluted 5-fold, whereas in other sera, FT₄ concentrations showed no change even at 40-fold dilutions.

We found that measurement of FT₃ concentrations in diluted sera is useful for differentiating between the effects of diclofenac and autoantibodies for the Vitros FT3 assay and the Vitros FT3II assay (Ortho-Clinical Diagnostics, Inc.), which does not show cross-reactivity with diclofenac.

We obtained sera from 2 patients taking diclofenac and 2 patients with antibody against T₂, T₃, or their conjugates. The sera were used for dilution tests in Vitros FT3 and Vitros FT3II assays, along with control sera from 2 healthy individuals and 2 patients with hyperthyroidism. The reference interval for the FT₃ value was 4.6–7.5 pmol/L. We made serial dilutions (1:1, 1:2, 1:4, and 1:8) with 0.01 mol/L phosphate-buffered saline (pH 7.4). The procedures in this study were in accordance with the Helsinki Declaration of 1975 and the amendments of 1996.

Assayed FT₃ concentrations were 5.8 and 5.1 pmol/L in the Vitros FT3 assay and 6.5 and 5.9 pmol/L in the Vitros FT3II assay for the healthy individuals, 9.6 and 14.4 pmol/L in the Vitros FT3 assay and 9.5 and 14.3 pmol/L in the Vitros FT3II assay for patients with hyperthyroidism.

In the 2 patients taking diclofenac, FT₃ concentrations were 14.3 and 12.7 pmol/L for the Vitros FT3 and 6.6 and 4.6 pmol/L for the Vitros FT3II assay, indicating that the presence of diclofenac influenced the Vitros FT3 assay.