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Teaching an old dog new tricks?

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Back in the 1950s, researchers at Chapel Hill namely Langdell, Brinkhaus and Wagner were working on a rapid test in order to quantify the effect of various factor VIII (FVIII) concentrates for the management of Haemophilia A. As already observed in 1935 by Armand Quick, the eponymously named Quick time or Prothrombin Time, was not sufficiently sensitive to differentiate normal from haemophilic plasma albeit being able to profile the coagulopathy of liver disease (Quick, 1935). The University of North Carolina group had the idea of using a 'partial' rather than a complete thromboplastin for this purpose (Langdell *et al*, 1953). The partial thromboplastin referred to the fact that the brain extract that was being used at the time had to undergo ultracentrifugation or other processes (e.g. chloroform) in order to remove much of the tissue factor present in the thromboplastin, the former being a substance which was not really well recognised at the time. Eight years later, Proctor & Rapaport (1961) introduced Kaolin in order to activate the assay and hence the Activated Partial Thromboplastin Time (APTT), as we know it today, was born.

In this day and age, such is the importance of this test that it is found in all coagulation laboratories around the world! Its automation made it even more convenient and faster to use, and its popularity has also contributed to it becoming cheaper and more accessible. To a degree, it has become a victim of its own success because its accessibility makes it one of the most commonly requested assays, even when the indication is tenuous. Nowadays, most APTTs are requested for unfractionated heparin monitoring, a drug which is also on its way out, and lupus anticoagulant detection, and it is still the backbone of FVIII, FIX, FXI and FXII monitoring as a one-stage assay.

The endpoint of the APTT is the time to clot formation. However, there is hidden data during the running of this test that had been 'neglected' (Braun *et al*, 1997). We refer to the first and second derivative curves, or the clot waveform, that is generated during every single APTT reaction. This was initially recognised on specific photo-optical analysers such as the MDA® (Trinity Biotech, Bray, Republic of Ireland), but, more recently, this data is also retrievable through dedicated software from the newer optical analysers from other companies. Ten parameters can be derived from the combined graph. This innovation has made these parameters much more easily accessible although still not readily available because they have not been fully standardised and validated. (Toh & Giles, 2002). The waveform is divided into three phases, i.e., pre-coagulation, coagulation and post-coagulation, and it relies on light transmittance.

Initial reports showed the potential utility of this waveform in patients with disseminated intravascular coagulation (DIC). In similar patients, biphasic transmittance waveforms are noticeable even in the early stages of the disease (Downey *et al*, 1997). Others looked at the effect of anticoagulants and other clinical scenarios such as factor deficiencies on this graph (Shima *et al*, 2002).

In this edition of the journal, Nogami *et al* (2019) went a step further in the development of this test by incorporating recombinant human tissue plasminogen activator (rt-PA) with the APTT reagent in a standard automated analyser. The aim was to incorporate data from the often-forgotten fibrinolytic pathway in pathological conditions and not just concentrate on the initial few seconds of coagulation. An ellagic acid and synthetic phospholipid-containing APTT reagent was added to the test plasma and then, together with the standard calcium chloride, they also added 0·63μg/ml of rt-PA to trigger the onset of the reaction. The standard volumes were added to plasma and hence no further dilutional effects were observed. Not surprisingly, the intra- and inter-assay coefficients of variation were somewhat higher for the fibrinolytic parameters but they were all <10%. The maximum reaction time allowed was slightly more than 8 min. The fibrinolytic phase is essentially a much more informative 'post-coagulation' phase with another 3 new parameters, i.e., the fibrinolysis lag-time, maximal fibrinolysis velocity and the endogenous fibrinolysis potential.

Using this modification, one can observe the expected effect of tranexamic acid on coagulation, with no effect being exercised on the initial coagulation part but with a significant block

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to the fibrinolytic pathway, whereas soluble thrombomodulin inhibits coagulation at higher doses and also tends to inhibit fibrinolysis. FVIII- and FIX-deficient plasma, mimicking Haemophilia A and B, showed a prolongation of the initial coagulation times but also enhanced and biphasic fibrinolysis. These parameters improved after the addition of FVIII and FIX, respectively. A positive effect on fibrinolysis in the factor-deficient plasma was also observed after the addition of tranexamic acid. FXIII-deficient plasma revealed the increased susceptibility towards fibrinolysis but no effect on the coagulation part of the curve.

This modification seems to be able to provide useful information on the coagulation part, as well as the fibrinolytic potential, of the plasma sample being tested and is certainly going to be useful for research purposes. But what is the added practical utility of a similar adaptation of the APTT? The authors argue that the incorporation of rt-PA to the standard APTT and using the clot waveform software could be useful in the monitoring of tranexamic acid therapy, the diagnosis of such disorders like plasminogen activator inhibitor-1 (PAI-1) deficiency and the management of bleeding disorders. One could speculate that looking at the fibrinolytic part of coagulation during the treatment of haemophilia patients could lead to further improvements in the personalisation of treatment. This would require further studies to see whether there are significant interindividual differences in the fibrinolytic waveform during treatment. However, we struggle to see its utility in tranexamic acid usage because this drug has a suppressive effect on fibrinolysis even at low doses and probably would not be particularly useful to use in order to modify dosage. In addition, the standardisation of assays of fibrinolysis in general is notoriously difficult (Longstaff, 2018). One would have to look at inter-batch differences and also stability over time. rt-PA tends to lose activity over time at room temperature. This would mean that it would not be possible to keep this modified APTT reagent continuously or on standby in the analyser for routine use and a new mixture of calcium chloride and rt-PA would have to be freshly prepared for each test, resulting in more variability.

Further data on these technical aspects as well as *ex vivo* analyses would be crucial in order to determine whether this modification of a similar assay, which has withstood the test of time, can be incorporated as a clinically useful routine test or whether it will just take its place in coagulation research. We also envisage that it will only be through the introduction of artificial intelligence, with the development of artificial neural networks (Givens *et al*, 1996; Yoon *et al*, 2018), that all this data that a similar test is able to generate can be assimilated in a clinically relevant format. Otherwise, one will always expect this old dog to perform its old trick!

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