The clinical usefulness of measuring thrombin generation

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In medicine there are hardly any universally valid rules, but the first law of hemostasis and thrombosis might be among the exceptions: *The more thrombin the less bleeding but the more thrombosis*; *the less thrombin the more bleeding, but the less thrombosis.*

What is loosely called *thrombin* here is the amount of enzymatic work that thrombin potentially can do in the time between its generation and its disappearance in a clot or thrombus. We called it the Endogenous Thrombin Potential (ETP) and it is represented by the surface under the thrombin generation curve.[1]

Thrombosis and bleeding bring an inestimable burden of death and disease. The exact numbers are impossible to assess because thrombosis, as *Nobody's disease but everybody's complication* [Lord Ajay Kakkar dixit] does not, as such, appear in the mortality statistics. A rough estimate attributes around half of all causes of death in Europe to thrombosis (Table 1).[2]

The mortality due to bleeding-by-lack-of-thrombin is even more difficult to estimate; the severity of a bleeding being determined by the extent of the wound and the adequacy of the hemostatic reaction. The impressive figures on bleeding due to over-anticoagulation that we will encounter below set a lower limit.

Nobody will deny that being able to gauge the mechanism behind so much pathology is a major asset. For centuries we had to deal with clotting times. They are good to explore severe hypocoagulability but fail in hypercoagulability and in modest hypocoagulability. The reason is that a clotting time is inversely related to the amount of thrombin formed so that, *grosso modo*, the whole spectrum of ETP values >50% of normal is represented by clotting times that are less than twice prolonged.

The superiority of the ETP as a function test is well illustrated in the specialised field of hemophilia. It is well known that severe and moderate bleeding is only loosely related to the native factor VIII level, let alone to a clotting time in any of its variations. There is, however, a perfect correlation of the native ETP with clinical severity.[2] The adequacy of substitution therapy as well as of alternatives, such as bypass with other (activated) clotting factors, downregulation of antithrombin, factor VIII replacing antibodies etc. can also be judged by the ETP and not by clotting times or factor VIII tests. It therefore is a useful guide during surgical interventions in hemophiliacs with inhibitors.[3]

Another, and much more common use of the ETP is in parenchymatous liver disease, where the synthesis of plasma proteins is impaired. The prothrombin time then is usually prolonged, but the ETP will be hardly diminished – if at all. This is because the anticoagulant proteins – antithrombin *e.g.* – are decreased to the same extent as the clotting factors are, but have no influence on the prothrombin time. According to some protocols procoagulant

treatment is given to cure the prothrombin time, which increased the risk of thrombosis as an immediate result.[4]

By far the most common disturbance of the clotting system is iatrogenic, *viz.* prophylaxis of thrombosis. All anticoagulant treatment amounts to decreasing thrombin generation capacity, either by decreasing procoagulant factors - vitamin K antagonists (AVK) or direct oral anticoagulants (DOACs) - increasing antithrombin activity (heparins) or direct inhibition (DOACs).

In the normal population the between-person variation is high.[5] In the highest quartile the relative risk of venous thrombosis (RRVT) is seven times as high as in the lowest.[6] In non-O blood groups the ETP is 108% and the RRVT 2.2, in the blood group O it is 92% and 0.7.[7] In another study the risk is 0.57 in the group with a below average and 2.2 in the group with an above average ETP.[8] So in the broad range of normal ETPs there is a strong variation of thrombotic risk between the higher and the lower ones.

In studies on bleeding in orally anticoagulated populations and in patients with congenital deficiencies of clotting factors it has been found that the risk of bleeding hardly increases unless the ETP falls below of 30% of normal.[9,10] In patients on an INR of 2-3, corresponding to an ETP of ~ 40±6%, the RRVT is ~0.2.[10]

It follows that stable anticoagulation to an ETP of about 35-65% of normal (Figure 1) will keep a population on a minimal risk of thrombosis with a hardly increased risk of bleeding.

The modern DOACs open promising horizons to achieve this. Clinical trials have shown that, given in standard doses they protect slightly better than vitamin K antagonists do. Recent studies have shown that there are obviously good and bad responders to the drug: a fixed concentration of 300 nM rivaroxaban inhibits the ETP in normal persons by 45±10.6%.[11] Such variations superimpose upon the considerable interindividual variation to result in a very broad distribution of ETP after inhibition and that only partly lies in the ideal zone (Figure 1).

In real life the distribution curve of the treated population will be still broader because this *in vitro* experiment only reflects the pharmacodynamic response, the direct influence of the drug on its target, and variation due to variability in uptake and clearance are not considered. Also, the distribution curve will be shifted to the right because patients under anticoagulant treatment will not be a random representation of the normal population, like in Figure 1, because the higher quartiles of the normal population, with their larger risk of thrombosis, will be overrepresented.

From these experiments one may surmise that administration of a standard dose of DOAC causes a highly variable response of the ETP in a population of patients, so that only a fraction of it will be optimally anticoagulated. This, by the way, has also been found for two other DOACs, for heparin and for heparin likes.[12]

Now any decrease of the ETP, optimal or not, will bring about an important attenuation of the risk of thrombosis. A person that normally has an ETP of 120% of normal may, even if he/she is a bad responder to a DOAC, attain an ETP of 75% and thus reduce his/her relative risk from 2 to 0.6, thus contributing significantly to the favorable statistics in the treated group. Nevertheless, much is to be gained if the dosage would be individualised so as to be maintained in the 35-65% zone.

Why is this not envisaged for the moment?

The first and foremost reason is that before the advent of thrombin generation measurement, it was not possible

Table 1. Death in Europe per 100,000 inhabitants (2017).

	Total	Thrombosis	%
Coronary infarction	277	277	100
Stroke	189	151	80
Cancer	304	101	33
Miscellaneous	283	>35	>12
Total	1053	>564	>53

Source: Eurostat (ec.europe.eu).

Miscellaneous: e.g. pulmonary and other embolisms, diffuse intravascular micro-embolization. If thrombosis due to cancer is not counted the total % drops to >44.
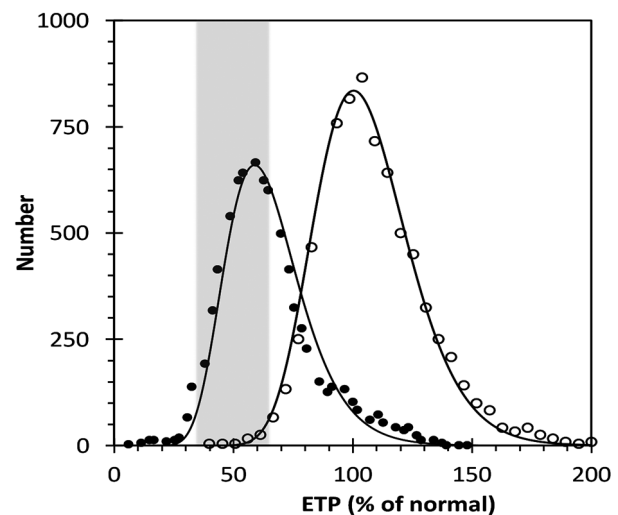

Figure 1. Distribution of the endogenous thrombin potential before and after inhibition with a direct oral anticoagulant. White dots: Normal volunteers (N>7000). Black dots: Same plasmas in the presence of IC50 of a DOAC. Gray zone: Zone of optimal anticoagulation.

to adequately measure the effect of DOACs on the clotting system. When the trials on DOACs were planned, thrombin generation measurement was only known as a research tool and no reliable laboratory automaton was as yet available for its large-scale application.

A second reason is that discarding laboratory control is a tremendous advantage over anti-vitamin K treatment sparing both money and hassle. It thus compensates in part for the much higher costs of DOACs over AVK and is an important selling argument, not ready to be dropped by pharmaceutical firms.

Mind however that continuous monitoring, as in AVK treatment is not what would be needed, rather dose adjustment, is used, like blood-pressure to tailor antihypertensive drugs and blood sugar that of antidiabetics.

There is another reason to consider dose adjustment. Bleeding due to overdosage of DOAC is not a rare occurrence. The incidence is ~3 per 100 patient-years.[13] That makes 100.000 bleeds per year on the 3.5 million patients on DOACs in the United States.[14] One might consider spotting over-responders before they start bleeding, be it for the profit of pharmaceutical firms. As an example, the German drug maker Bayer and the Johnson & Johnson subsidiary Janssen Pharmaceuticals will jointly pay $775m (£587m; €687m) to settle more than 25 000 lawsuits in US federal and state courts, representing almost all of the outstanding claims against the bestselling anticoagulant rivaroxaban (Xarelto).[15]

If one wants patient-based evidence to see whether dose adjustment would pay of, one does not need to start large, costly, and cumbersome trials. It would suffice to collect samples from a sufficiently large number of bleeding and thrombotic events in patients on DOAC prophylaxis and see whether their basic ETP will indeed be in the low range for those who bleed and high in case of thrombosis.

Finally: Above we have preferred to give three examples of the clinical use of thrombin generation. One where the use of ETP is recognised and used in actual practice, *i.e.* hemophilia; another where it is in the process of being accepted in the clinic, *i.e.* in liver disease and one where the future use of thrombin generation would cause a tremendous health benefit once it were adopted: DOAC medication.

It is likely that in the future, Thrombin Generation will ameliorate diagnosis and treatment everywhere where now clotting times are used.

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