

probably facilitate the specificity of the interactions. It is not unlikely that on the surface of endothelial cells, apoER2 also acts in combination with a coreceptor. Toll-like receptors 2 and 4 are possible candidates. It would be of great interest to see whether the presence of additional receptors influences the signal transduction pathway activated.

Sacharidou et al focus on the down-regulation of NO synthesis. It has been shown in mouse models that activation of endothelial cells by anti- $\beta$ 2 glycoprotein I antibodies results in more changes in cell metabolism, regulated by the translocation of NF- $\kappa$ B.<sup>9</sup> Upregulation of tissue factor expression and downregulation of thrombomodulin could also be involved in the loss of antithrombotic properties of endothelial cells. It is clear that the binding of anti- $\beta$ 2 glycoprotein I antibodies to endothelial cells strongly influences the metabolism of the cells. An important issue is whether this happens continuously when the antibodies circulate or whether it is induced after a trigger as yet unknown.

The experiments in which Sacharidou et al attenuate thrombus formation induced by the antibodies by inhibiting PP2A are interesting. The identification of apoER2 as a receptor for anti- $\beta$ 2 glycoprotein I antibodies did not lead to the identification of possible therapeutic targets, because reelin signaling in the brain is important for brain development, dendritic spine remodeling, and synaptic plasticity.<sup>7</sup> apoER2 is obviously not a primary target for treatment. The signaling pathway underlying apoER2 in the brain is different from the pathway now described for endothelial cells.<sup>7</sup> PP2A has not been identified in the brain as an intermediary in apoER2 signaling. As shown by Sacharidou et al, this opens new avenues through which to specifically inhibit apoER2 signaling in endothelial cells. However, we do not know the physiological function of apoER2 signaling in endothelial cells. Additional studies with long-lasting inhibition of PP2A are needed to identify this pathway as a target for prophylactic treatment of patients with APS.

**Conflict-of-interest disclosure:** The author declares no competing financial interests. ■

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## THROMBOSIS AND HEMOSTASIS

Comment on Kearon et al, page 2151

# “Antiphospholipids”: the more, the worse?

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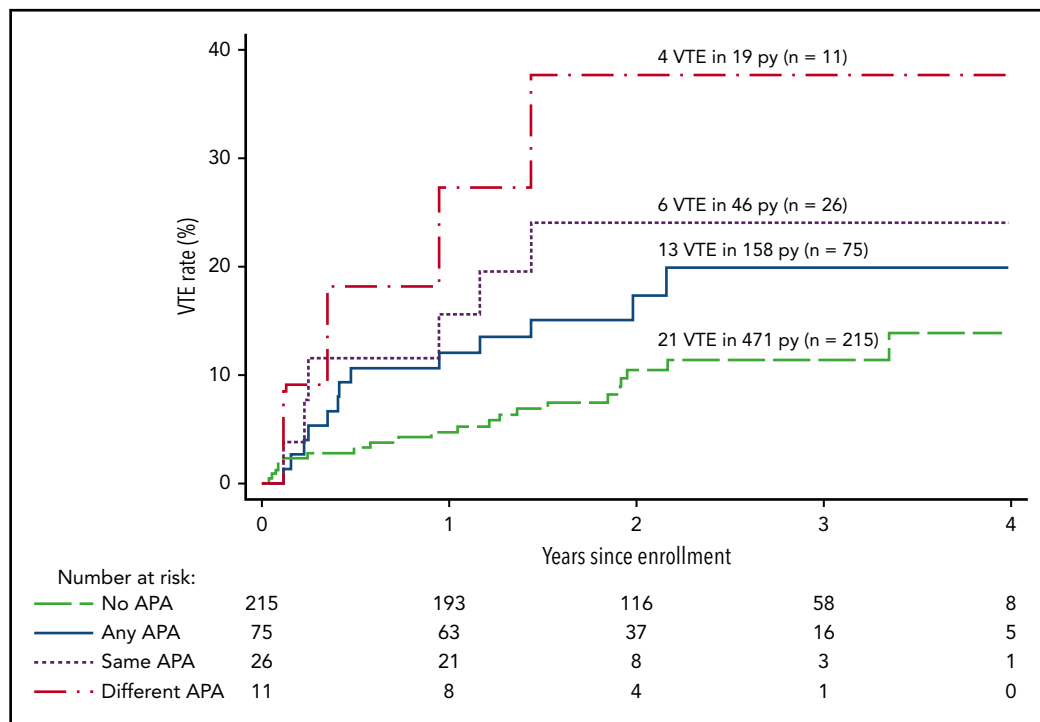
**In this issue of *Blood*, Kearon et al show that the risk of recurrent venous thromboembolism (VTE) is significantly increased when several tests for antiphospholipid antibodies (APAs) of different types are positive.<sup>1</sup>**

So-called APAs are a heterogeneous group of autoantibodies directed at plasma proteins such as prothrombin or  $\beta$ 2 glycoprotein I.<sup>2</sup> Routine tests to detect APAs include the lupus anticoagulant, anticardiolipin antibodies, and anti- $\beta$ 2 glycoprotein I antibodies. Although a significant association between APA and VTE had been previously demonstrated,<sup>3</sup> the risk of recurrent VTE remained uncertain and debated.<sup>4</sup>

According to classification criteria, antiphospholipid syndrome (APS) is present when at least 1 of the defined clinical manifestations is documented (vascular thrombosis, pregnancy morbidity) and 1 routine test for APA is positive.<sup>5</sup> Previous data indicate that all APS patients do not share the same risks; in particular, patients with triple positivity (all routine tests positive) seem to be at high risk for thrombotic events.<sup>6</sup> Further risk stratification is obviously important to identify patients who are candidates for long-term anticoagulants.

Kearon and colleagues now report the characteristics of APA associated with an increased risk of recurrent thrombosis after a first unprovoked episode of VTE (see figure). The authors take advantage of the prospective D-dimer Optimal Duration Study,<sup>7</sup> in which plasma samples were tested for APAs. Decisions about duration of anticoagulation were made without knowledge of the presence of APAs or not.

The results of the current study show the risk of recurrent thrombosis in 3 categories of patients. Although patients with any APA positivity at any time did not have a significant increase in the risk of recurrence, recurrence risk was significantly increased in 2 different laboratory profiles. Patients with the same type of APAs measured on 2 separate time points and thus consistent with Sapporo-Sydney laboratory classification criteria<sup>5</sup> had almost a threefold higher risk for recurrence than patients without persistent APAs. In addition, patients with



Recurrent VTE after stopping anticoagulants in patients with different APA profiles: patients with multiple tests positive and patients with persistent positivity are at increased risk for recurrent VTE. No APAs (all tests negative), any APAs (at least 1 test positive once), same APAs (positive test persistent for the same assay on 2 occasions), different APAs (multiple tests positive). See Figure 3 in the article by Kearon et al that begins on page 2151.

multiple APA positivity (Sapporo-Sydney laboratory category I) either at the same time or at different time points have a threefold higher risk than patients with only 1 test positive and a 4.5 higher risk than patients without APAs. These results may explain the reasons for previous controversies about the risk of recurrence because some earlier studies had performed only a single APA measurement, whereas others took into account persistent antibodies or multiple positivity.<sup>4,6</sup>

One other interesting result of this study is that patients with APAs did not have higher D-dimer levels than patients without APA. Furthermore, D-dimer levels were not significantly higher in patients with APAs or any specific type and recurrent VTE compared with patients with APA and without recurrence. These results confirm earlier results suggesting that D-dimers are not associated with an increased thrombotic risk in patients with APA.<sup>8</sup>

Some important issues are still to be considered. First, as indicated in this report, cutoff levels for APA positivity remain a matter of debate. In this study, cutoffs were indicated by manufacturers of commercially available tests, whereas Sydney-Sapporo suggest to use either the 99th

percentile of a control population or the 40 immunoglobulin G (IgG) or IgM phospholipid units for the anticardiolipin assay to define cutoffs. The risk associated with these different cutoffs must be investigated in further studies, and sensitivity analyses should be performed to determine the risk of recurrence according to varying cutoffs. Second, it was not possible in this study to identify the risks associated with different types of APA: lupus anticoagulant compared with enzyme-linked immunosorbent assay (ELISA) tests or IgG and IgM isotypes of anticardiolipin and anti- $\beta 2$  glycoprotein I antibodies. Finally, new ELISA tests and new techniques (chemiluminescence) are now available and have been evaluated in preliminary, mainly cross-sectional reports. Antiphosphatidylserine/prothrombin ELISA assays have been advocated because prothrombin is 1 of the main targets of APA.<sup>9</sup> Tests for detection of antibodies against domain I of  $\beta 2$  glycoprotein I, in particular those recognizing the G40-R43 epitope of  $\beta 2$  glycoprotein I, are also being developed to identify pathogenic APA.<sup>9,10</sup> These new tests should be included in future prospective studies and compared with classical APA tests.

The better characterization of APA and identification of profiles (persistent APA

and multiple positivity) associated with a risk of recurrence in patients with a first unprovoked episode of VTE will help select candidates for indefinite prolongation of anticoagulant therapy. It is also important to consider these profiles for randomized controlled trials, in particular, when testing direct oral anticoagulants compared with traditional vitamin K antagonists in patients with APS.

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## THROMBOSIS AND HEMOSTASIS

Comment on Ebert et al, page 2161

# Tissue factor and oxidative stress

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**In this issue of *Blood*, Ebert et al conclude that endothelial cell (EC) tissue factor (TF) activity induces a prothrombotic state in mice that lack the antioxidant paraoxonase-2 (PON2).<sup>1</sup>**

Because of its potent procoagulant activity, TF gene expression and activity are tightly regulated to maintain hemostasis

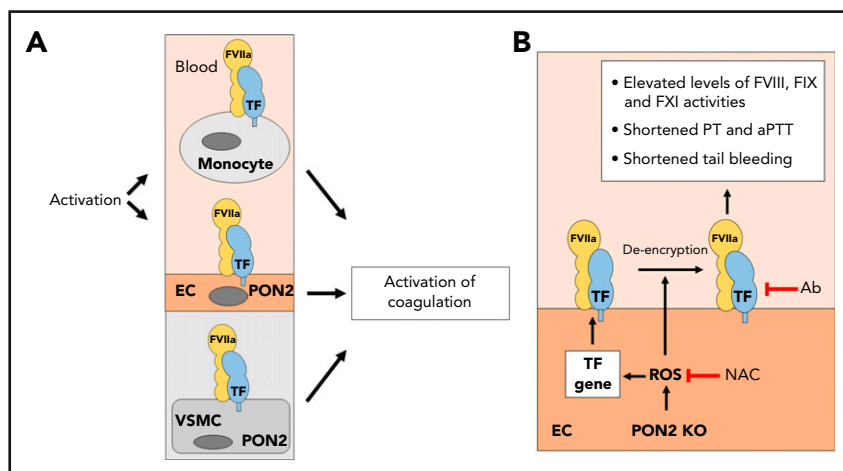
while preventing thrombosis. TF is constitutively expressed by perivascular cells, such as vascular smooth muscle cells (see

figure panel A).<sup>2</sup> In contrast, unstimulated blood monocytes and ECs do not express TF. However, they express TF after activation as part of the host defense system. TF activity is also regulated posttranslationally with several proposed mechanisms of de-encryption, including interaction with phosphatidylserine and oxidation of an allosteric disulfide bond (see figure panel B).<sup>2</sup>

The PON family of antioxidants consists of 3 members: PON1, PON2, and PON3.<sup>3</sup> PON1 and PON3 are expressed by the liver and bind to high density lipoprotein and prevent its oxidation. In contrast, PON2 is expressed by macrophages, vascular smooth muscle cells, and ECs (see figure panel A).<sup>3</sup> Interestingly, PON2-deficient mice developed atherosclerosis when fed a Western diet, and this was attributed to a deficiency of PON2 in macrophages.<sup>4</sup>

Ebert and colleagues observed numerous abnormalities in PON2<sup>-/-</sup> mice, including increased reactive oxygen species in the aorta; increased levels of interleukin-6 (IL-6) in the plasma; elevated plasma levels of factor VIII (FVIII), FIX, and FXI activities; shortened prothrombin time; shortened activated partial thromboplastin time; and a shortened tail bleeding time. Furthermore, they found that platelets expressed PON2, and deficient cells had increased reactive oxygen species, increased volume, and increased binding of annexin V. In vitro studies with primary lung ECs showed increased basal levels of a variety of inflammatory mediators and a decrease in the anticoagulant TF pathway inhibitor. Surprisingly, the expression of other markers of EC activation, such as ICAM-1, E-selectin, or VCAM-1, were not increased. Primary lung PON2<sup>-/-</sup> ECs expressed TF messenger RNA and protein at similar levels to those in wild-type cells but had increased levels of TF activity (see figure panel B).

Because PON2 is expressed by macrophages, vascular smooth muscle cells, and ECs, it was important to determine which cell types contributed to the different phenotypes in PON2<sup>-/-</sup> mice. Importantly, an anti-mouse TF antibody normalized the prothrombotic phenotype, which indicates that the prothrombotic phenotype was due to TF. Bone marrow transplantation experiments showed that the prothrombotic phenotype tracked with PON2 deficiency in nonhematopoietic cells. However, there was significant



(A) TF is constitutively expressed by vascular smooth muscle cells (VSMCs) and activated monocytes and ECs. PON2 is expressed by macrophages, VSMCs, and ECs. (B) Loss of PON2 leads to increased levels of reactive oxygen species (ROS) in ECs and de-encryption of TF. In addition, increased levels of ROS may induce TF gene expression. Increased levels of EC TF activity are proposed to mediate the prothrombotic state in PON2<sup>-/-</sup> mice, which was assessed by measuring plasma FVIII, FIX, and FXI activities, prothrombin time (PT), activated partial thromboplastin time (aPTT), and tail bleeding. The antioxidant *N*-acetylcysteine (NAC) and an anti-mouse antibody (Ab) normalize the prothrombotic state in PON2<sup>-/-</sup> mice. KO, knockout.