EDITORIAL

Circulating tissue factor in humans

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In this issue of the *Polish Archives of Internal Medicine* (*Pol Arch Intern Med*), Stankowska et al[1] reported that in patients with essential thrombocythemia (ET), history of thrombosis, and presence of the *JAK2 V617F* mutation, an increased risk of thrombosis could be a result of an increased tissue factor (TF) and decreased tissue factor pathway inhibitor (TFPI) activities.

TF is an integral transmembrane protein and is a component of the factor VIIa–TF complex enzyme. TF is essential for hemostasis and, under normal circumstances, cells in contact with blood do not express functional TF. When damage of the vascular wall occurs, subendothelial TF is expressed/exposed to blood flow, binds plasma factor VIIa, and forms the factor VIIa–TF complex that initiates blood coagulation by activating factor IX and factor X to their respective serine proteases, factor IXa and factor Xa. These serine proteases form complex enzymes with their nonenzymatic cofactors (factor VIIIa and factor Va, respectively) on the surface of cell/microvesicle membranes, robustly producing thrombin. Thrombin accelerates its own generation via several feedback reactions, cleaves fibrinogen, and activates factor XIII, ultimately leading to the formation of a cross-linked insoluble fibrin clot.[2]

Although TF is essential for normal hemostasis, an excessive expression/exposure of TF can result in pathological thrombosis in certain settings, such as cancer, sepsis, surgery, trauma, and others.[3] It was also established that the presence of functional TF in patients with cardiovascular and inflammatory diseases is associated with the severity of the disease and worse functional outcomes.[4]

TFPI, a Kunitz-type serine protease inhibitor, is an anticoagulant protein that blocks the initiation of coagulation by inhibiting the factor VIIa–TF complex and early forms of prothrombinase in a factor Xa-dependent manner.[5] Similarly to increased levels of TF, reduced levels of TFPI are associated with the development of thrombosis.[3]

TF is a relatively small (263 amino acids) and structurally simple protein. However, controversies related to its molecular weight, role of posttranslational modifications on function, specific activity, and its presence/absence in blood and on various cells are immensely bigger than the TF protein itself. A discrepancy between a real and "commonly accepted" molecular weight of TF could serve as an example for this statement. TF is a low-abundance protein; therefore, it is not surprising that its recombinant form has been used in laboratories worldwide as a substitute for the natural TF protein. Due to the absence of more precise analytical data, an approximately 45 kDa value for TF has been used in published studies, which was based on its mobility on the SDS-PAGE gel. Several years ago, a paper from our laboratory was published[6] that showed, based on the mass-spectrometry data, that the molecular weight of a natural human placental TF is 36.2 kDa. Nevertheless, the authors of the majority of more recent publications, including that by Stankowska et al,[1] still use SDS-PAGE-based values (45–47 kDa). This suggests, to some extent, that the authors of those publications do not do a search of original research publications on the subject of their research, but instead use old or not updated review papers to gain their knowledge.

Another subject related to TF controversy is platelet TF. The researchers working with platelets and writing about platelet TF could be divided into 3 categories: 1) believers; 2) nonbelievers; and 3) neutral. The first category of researchers who claim that there is platelet TF built their beliefs on the basis of research conducted using commercial reagents for the immunorecognition of TF.[7-9] The major problem of the commercially available immunoassays for TF is the use of antibodies that are not specific for TF and recognize anything but TF.[10-12] Based on references presented in the paper and on the text of the Discussion, it is quite obvious that Stankowska et al[1] could be assigned to the believers. However, despite their beliefs, they were supposed to acknowledge existing controversy on the subject of platelet TF. The nonbelievers base their conclusion that there is no platelet TF not only on immunoassays, but also on functional TF assays. For TF quantitation,

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both immunologically and by activity, nonbelievers use monoclonal TF-specific antibodies generated and characterized in-house in their studies.[13,14] However, not every laboratory, especially those in the field of biomedical research, can generate their own specific anti-TF antibodies. Additionally, commercially available TF assays and antibodies are quite scarce and do not provide enough choice for researchers. Thus, researchers working with TF face a dilemma: to use unreliable but commercially available assays (especially because others use them and have no difficulties publishing their research) or not to do such research at all. The third group comprises some of the researchers who do not directly investigate the subject of platelet TF, but use the knowledge of existing published studies. They usually are open to both possibilities, that is, the presence and the absence of platelet TF.[15]

Stankowska et al[1] quantitated both TF antigen and activity[1] and found that TF antigen is approximately 2-fold higher and activity is approximately 18-fold higher in the ET group than in the control group. Despite the potential shortcomings of the assays used, these results are interesting because they support an observation that specific TF activity is more dependent on properties of the surface (cell, microvesicle, and artificial membrane) where TF is located than on the concentration of the TF antigen.[16]

There are several more inconsistencies and shortcomings in the paper.[1] The control group, for example, does not match the study group by age (49 and 61 years, respectively). The authors also made no attempt to explain potential reasons for contradictions between their study and previously published studies. However, some data related to the activity of TF in patients with ET versus that in healthy controls and the data suggesting the influence of previous thrombosis and *JAK2 V617F* mutation are quite interesting.

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