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Comment on "Tissue factor expressed by microparticles is associated with mortality but not with thrombosis in cancer patients"

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The article by Hernandez and colleagues in the recent issue of Thrombosis and Hemostasis analyzes levels of tissue factor (TF) present in the plasma of cancer patients using various methods (1). The study concludes that TF present on microparticles (MPs) in patients with cancer is associated with mortality but not with thrombosis. We believe that the methods used to quantify circulating TF-positive MPs in this study are inaccurate and flawed.

First, circulating MP-associated TF antigen is measured using light-scatter flow cytometry of MPs (1). Unfortunately the MP gating strategy used in this study is incorrect. The MP size gate was set using an upper and lower forward scatter-established size limit of 0.5 and 3.0 μm, respectively (1). MPs are known to be between 0.1 and 1.0 μm in size (2). Further, the International Society of Hemostasis (ISTH) has established a standardized method for quantification of MPs by flow cytometry which uses the size calibrated fluorescent bead solution megamix (Biocytex, Marceille, France) to establish an upper size limit of 0.9 μm (3, 4). However, recent data indicates that even the use of a 0.9 μm polystyrene bead to establish an upper size limit for MP detection could correspond to a vesicle size that is larger than the actual MP size range due to the large difference in refractive index of polystyrene beads versus MPs (5). In addition, MP events detected by the current gating strategy could be due to MP aggregates resulting from the use of excessive MP wash steps. Moreover, Basavaraj and colleagues have found that the anti-human TF monoclonal antibody used in this publication, HTF-1, is not the best choice for the detection of TF on MPs in plasma by flow cytometry as this antibody does not bind to TF that it bound with FVII/VIIa (6). In support of this conclusion, a recent study found that the level of TF-positive MPs measured using a functional MP TF activity assay correlated with the development of venous thromboembolism in cancer patients, whereas no correlation was found by MP flow cytometry using the HTF-1 antibody (7). Another study found no association between MP-associated TF antigen and activity (8). Another significant concern is that the level of TF-

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positive MPs detected in this study is extremely low (1), meaning that it is difficult to conclude that the TF-signal observed is anything more than background noise of the flow cytometer. Finally, the level of TF-positive MPs in clinical samples is very low and flow cytometry is relatively insensitive. We failed to detect TF-positive MPs by flow cytometry in plasma samples from LPS treated whole blood that had high levels of MP TF activity (9).

Secondly, Hernandez and colleagues measured plasma and MP TF activity levels using the Actichrome TF activity assay kit (American Diagnostica, Stamford, CT, USA) (1). This assay attempts to measure TF activity in plasma by adding FVIIa and FX and then quantifying the amount of FXa generated using a chromogenic substrate. However, Bogdanov and colleagues have previously demonstrated a lack of specificity of the Antichrome TF kit and found that the endpoint of the assay is greatly influenced by the initial color of the plasma (10). Further, the Actichrome assay-determined plasma TF activity could not be inhibited by FVIIai, which is a control used to demonstrate the TF-dependence of identified procoagulant activity (10).

Thirdly, plasma TF antigen levels were measured in this study using the Imubind TF ELISA (American Diagnostica, Stamford, CT, USA) (1). While the Imubind TF ELISA has been used in multiple published studies to quantify plasma TF antigen, the specificity of this TF ELISA has been questioned (11). Parhami-Seren and colleagues measured plasma TF antigen levels in 8 patients using two different TF ELISAs (11). The Imubind ELISA identified 2 patients with high TF antigen levels that the second in-house ELISA did not (11). This finding was attributed to the cross-reactivity of the Imubind ELISA with non-TF proteins (11). Another study also failed to observe a correlation between MP-associated procoagulant activity and the Imubind TF ELISA (12).

There are other issues in this paper that impact the validity of the results. For instance, plasma was prepared by centrifugation at 4°C (1). Chilling whole blood results in platelet activation and will cause an artificial increase in platelet MP production. Further, there is also an error within the patient inclusion criteria as patients who were on heparin or warfarin thromboprophylaxis were included in the analysis of the association between TF and the development of thrombosis (1). While many of these patients were on thromboprophylaxis for non-cancer-related prothrombotic conditions, these agents have been shown to reduce the incidence of VTE in cancer patients (13, 14) and would therefore impact thrombosis rates in this study. Since thromboprophylaxis directly impacts thrombosis rates without changing TF activity it confounds the results of the aforementioned analysis, and likely impacts the accuracy of the conclusions.

Since all three of the techniques used to measure circulating TF in cancer patients in this study have major flaws, the conclusions of this study are questionable.

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