Optimization of Tissue Factor Measurement in Plasma

Nimra Shaik[1], Sierra J. Archibald[1], Ana T. A. Sachetto PhD[1], Nigel Mackman PhD[1]
[1] UNC Blood Research Center, Department of Medicine, UNC Chapel Hill

Abstract (250/250)

Introduction: Tissue factor (TF) contributes to hemostasis and thrombosis. Healthy individuals have undetectable levels of TF in blood. Several diseases can increase the levels of TF+ extracellular vesicles (EVs). Activity assays are more sensitive than antigen assays to measure TF in plasma. Previously, we showed that commercial ELISAs do not accurately measure TF antigen in plasma because of a high background signal that may be due to high abundance proteins. We determined the effect of depleting high abundance proteins on the measurement of TF antigen.
Methods: TF positive and negative plasmas were prepared from blood incubated or not with bacterial lipopolysaccharide. The top 14 high-abundance proteins in plasma were depleted using a resin column containing antibodies. Plasma, EV-free plasma and EVs were prepared. EV TF activity and protein concentration were measured. TF antigen was measured using a commercial ELISA.
Results: EV TF activity was detected in +LPS sample. Protein depleted samples had the expected decrease in protein concentration. Protein depletion reduced the background signal detected by ELISA. Although TF signal was lower in EV-free plasma compared with plasma, we could not detect TF antigen in EVs, probably due to the very low concentration of TF and limited sensitivity of the ELISA.
Conclusion: The depletion of high abundance proteins in plasma decreased the non-specific signal detected by a commercial TF ELISA. However, it is possible that the technique used to deplete high abundance proteins may also deplete EVs. More studies are needed to optimize the detection of TF antigen in plasma.