Optimization of Tissue Factor Measurement in Plasma

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Abstract

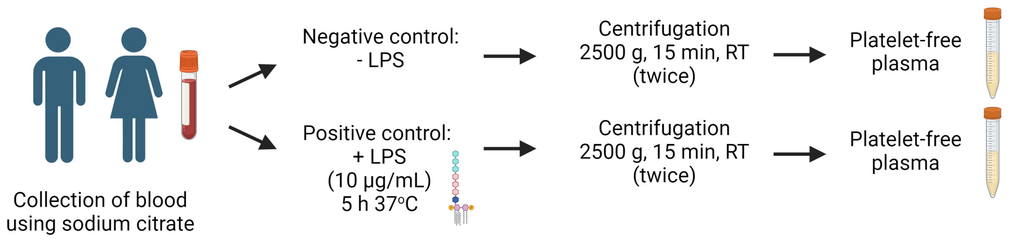
Tissue factor (TF) is the primary initiator of blood coagulation. Healthy individuals have undetectable levels of TF in blood. However, several diseases can increase the levels of TF+ extracellular vesicles. Levels of TF in plasma can be analyzed using activity assays. However, the measurement of TF antigen is difficult. Previously, we showed that commercial ELISAs do not accurately measure TF in plasma samples because of high background signal, presumably from high abundance proteins. We aimed to optimize the measurement of TF in plasma using ELISAs by depleting high abundance proteins in plasma. The establishment of a protocol for depletion of high abundance proteins was successful. The protein depleted samples showed a lower unspecific background signal using a TF ELISA compared with not depleted samples. Future work will focus on evaluating if a higher concentration of samples can improve the detection of TF using ELISAs and determining if TF is still active after the protein depletion.

Introduction

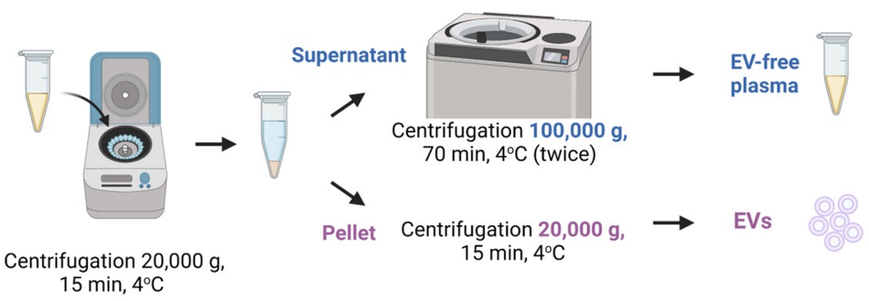
Tissue factor (TF) is the primary initiator of blood coagulation. TF is a transmembrane protein, and it is present in cells and extracellular vesicles (EVs). TF is essential for hemostasis but also contributes to thrombosis. Healthy individuals have undetectable levels of TF in blood. However, several diseases, such as cancer, bacterial and viral infections can increase the levels of TF+ EVs. Levels of TF in plasma can be analyzed using well established and specific activity assays. However, the measurement of TF antigen is difficult. There are 3 main challenges: TF is present in small quantities in plasma, plasma is a complex sample, and high background due to unspecific signals. Previously we showed that commercial ELISAs do not accurately measure TF in plasma samples. We aimed to optimize the measurement of TF in plasma using ELISAs.

Methods

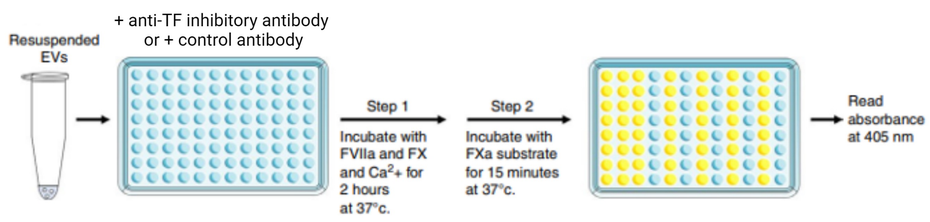
1 Preparation of TF positive and negative controls



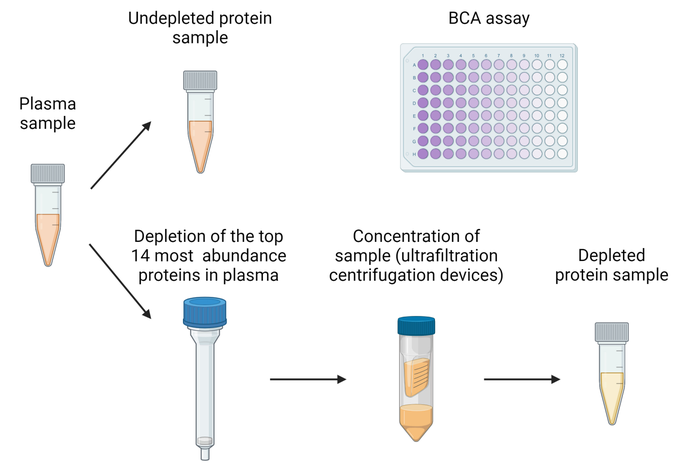
Isolation of EVs



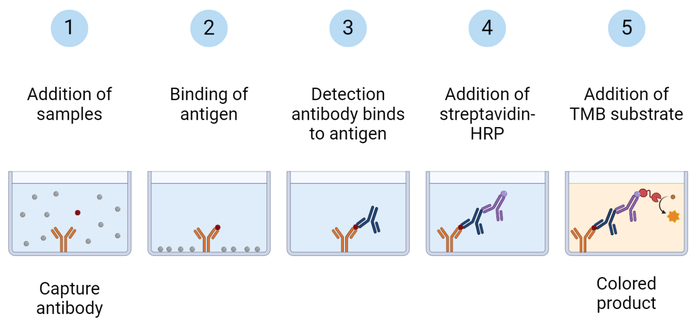
Measurement of TF using an EV TF activity assay



2 Depletion of high abundance proteins in plasma, concentration of samples and measurement of protein

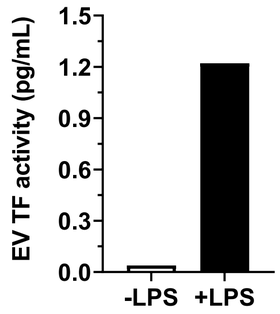


3 Measurement of TF using a commercial ELISA



Results and Discussion

1 TF is present in EVs from +LPS plasma

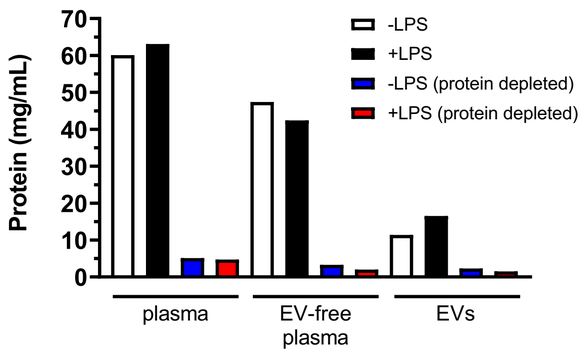


LPS induces blood cells (mainly monocytes) to induce TF expression and release TF+ EVs that have procoagulant activity.

The EV TF activity assay is sensitive and specific for the measurement of TF.

Figure 1. Tissue factor (TF) activity of extracellular vesicles (EVs) from negative and positive control plasma samples. LPS was absent in negative control samples (-LPS) and present in positive control samples (+LPS). As expected, TF activity was present in +LPS samples and not in -LPS samples.

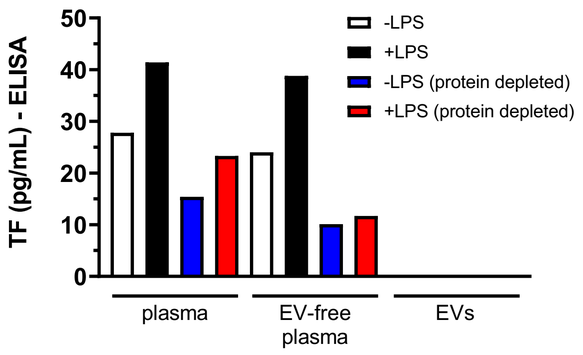
2 The establishment of a protocol for depletion of high abundance proteins in plasma was successful



Protein depleted samples had the expected decrease in protein concentration.

Figure 2. Measurement of protein concentration in plasma, EV-free plasma and EV samples with and without LPS (+LPS and -LPS respectively).

3 Protein depleted samples showed a lower background using a TF ELISA



This strategy was successful in decreasing the non-specific signal in plasma samples.

The lack of detection of TF in EVs may be due to the very low concentration of TF and the limited sensitivity of the ELISA.

Figure 3. Measurement of tissue factor (TF) antigen in in plasma, EV-free plasma and EV samples using an ELISA assay.

Conclusion

The depletion of high abundance proteins in plasma decreased the non-specific signal detected by a commercial TF ELISA. Future work will focus on evaluating if a higher concentration of samples can improve the detection of TF using ELISAs and determining if TF is still active after the protein depletion.

References

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(2) Hisada Y, Mackman N. Measurement of tissue factor activity in extracellular vesicles from human plasma samples. RPTH 2019;3(1):44–8.
(3) Sachetto ATA, et al. Evaluation of four commercial ELISAs to measure tissue factor in human plasma. RPTH 2023;7(3):100133.

Acknowledgments

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