The multiple faces of tissue factor measured with laboratory assays

Laroche M.¹, Peyrafitte M.¹, Vissac A.M.¹, Amiral J.²

¹HYPHEN BioMed, Research, Neuville sur Oise, ²HYPHEN BioMed, Neuville sur Oise, France
The multiple faces of tissue factor measured with laboratory assays

Laroche M.¹, Peyrafitte M.¹, Vissac A.M.¹, Amiral J.²
¹HYPHEN BioMed, Research, Neville sur Oise, France
²HYPHEN BioMed, Neville sur Oise, France

Aim: Measurement of Tissue Factor (TF) in plasma is of growing interest in various pathological states (tumor growth and metastasis, atherosclerosis, inflammation). TF can be in 3 different forms: anchored on cell membrane (monocytes, endothelial cells), in a cryptic or activated state; on microparticles’ surface (MP-TF); as an alternatively spliced form (asTF), soluble in plasma. We have developed various assays for these different TF forms: 2 ELISA assays for the measurement of full length TF:Ag (FL-TF) and “Total” TF:Ag (FL-TF and asTF); a bio-immunoassay for quantitating MP-TF activity.

Methods: FL-TF ELISA uses a capture murine MoAb specific for an extracellular TF epitope, unexposed on asTF and a second murine MoAb reactive with an extra cytoplasmic TF epitope. Tested specimen are in a diluent which enhances the assay reactivity and suppress non specific interactions (heterophilic antibodies). Total TF ELISA is a similar assay but the capture MoAb targets an extracytoplasmic epitope available on all TF forms. Assay ranges are from 0 to 500 pg/ml. This same MoAb, which does not inhibit TF activity, is used for capture in the MP-TF assay, which is revealed with Factor VIIa, Factor X, Ca++ and the Factor Xa specific substrate CS 11(65). Calibration uses relipidated TF anchored to synthetic phospholipids (0 to 5 pg/ml of TF and 0.1 nM PS/1 pg TF).

Results: In normal plasmas: FL-TF is below the detection limit (< 10 pg/ml); Total TF has a mean value of 50 pg/ml. Truncated recombinant human TF (1-219) has 1 % reactivity in the FL-TF (1-263) Elisa and 60 % in the Total-TF. Normals were < 0.2 pg/ml (TF equivalent) with the MP-TF assay but some patients were tested > 5 pg/ml. Incubation of human blood with LPS increased FL-TF, asTF and MP-TF.
The multiple faces of tissue factor measured with laboratory assays
Laroche M., Peyrafitte M., Vissac A.M., Amiral J.
HYPHEN BioMed, Research, Neuville sur Oise, France

Introduction
Determination of Tissue Factor (TF) in plasma is of growing interest in various pathologies including cancer, atherosclerosis, diabetes, multiple myeloma, and inflammation.

Blood-borne TF is present under 2 major forms in plasma: either as full length TF (1-263), incorporated into microparticles through its transmembrane domain, or as soluble alternatively spliced TF (aTF, 1-206) that lacks the transmembrane domain, and includes a unique N-terminal peptide.

There are discrepancies concerning TF concentrations in plasma from one study to another, and according to the commercial assay used.

Materials and Methods
Zymutest Full length TF (FL-TF)
Capture: MoAb MoAb specific, for an extracellular TF epitope, expressed on aFL-TF. Revelation: MoAb MoAb reacts with a FL-TF.
Zymutest Total TF (T-TF)
Capture: MoAb MoAb specific, for an extracellular TF epitope, also exposed on truncated TF. Revelation: polyclonal antibody (PAb) reactive with all TF forms.
Zymutest MP-TF
Capture: same as Zymutest Total TF

Calibration and controls: All concentrations are expressed as FL-TF equivalent, in pg/ml. Full length recombinant TF (1-263) (ALD) has been used as Zymutest MP-TF (synthetic lipopeptide (ZLP), with a phospholipid-L/T-FL ratio of 0.145 (1 pg TF, or directly diluted, in the presence of stabilizers. Zymutest Full length or Total TF is lyophilized.

Plasma: Citrated normal or pathological plasmas.
LPS induction: Whole heparinized blood is incubated with Lipopolysaccharides (LPS; 0.111/84 from Sigma), and plasmas are prepared by double centrifugation (15 min at 1500 g and 2 min at 13 000 g) at room temperature. Controls by the same plasmatic preparation from untreated blood.

Full length TF (1-263), human, recombinant: ADI.
Truncated TF (1-219), human, recombinant: Nova-sap.

Assay principles

Aim
We developed 3 complementary assays for measuring the various TF presentations in plasma:

- 2 ELISA kits, Zymutest Full length TF and Zymutest Total TF, to quantify respectively, full length TF antigen or Total TF antigen,
- 1 Bio-immunoassay, Zymuphen MP-TF, that permits the determination of the procoagulant activity of Microparticles exposing Tissue Factor (MP-TF).

Results

ZYMUTEST FULL LENGTH TF
ZYMUTEST TOTAL TF
ZYMUPHEN MP-TF

Conclusions

- Zymutest Full length TF specifically measures FL-TF, while Zymutest Total TF is sensitive for both forms of TF, with a reactivity of truncated compared to FL-TF of about 60%.
- Normal plasmas do not contain detectable MP-TF nor FL-TF. Mean Total TF is assayed at about 60 pg/ml (N=27, Mean=62pg/ml, SD=15pg/ml), expressed as FL-TF equivalent.
- Altogether, those results suggest that in normal plasmas only the truncated form of TF is assayed, i.e. aTF, using Zymutest Total TF.
- Incubation of human blood with LPS increased MP-TF significantly, whereas Total TF and Full length TF remains unchanged.

References