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The multiple faces of Tissue Factor measured with laboratory assays

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Aim:

Measurement of Tissue Factor (TF) in plasma is a growing interest in various pathological states (tumor growth and metastasis, atherosclerosis, inflammation). TF can be in 3 different forms: anchored on cell membranes (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 FVIIa, Factor X, Ca⁺⁺ and the Factor Xa specific substrate CS 11(65). Calibration uses relipidated TF anchored to syntetic 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/mg. Incubation of human blood with LPS increased FL-TF, asTF and MP-TF.

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Introduction

Determination of Tissue Factor (TF) in plasma is of growing interest in various pathologies including cancer, atherosclerosis, diabetes, multiple myeloma, and inflammation¹.

"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 (asTF, 1-206) that lacks the transmembrane domain, and includes a unique N-terminal peptide².

There are discrepancies concerning Normal TF concentrations in plasma from one study to another, and according to the commercial assay used³.

Materials and Methods

Zymutest Full length TF (FL-TF):

Capture: Murine MoAb specific for an extracellular TF epitope, unexposed on asTF. **Revelation:** Murine MoAb reactive with an extra cytoplasmic TF epitope, present in the N-terminal region. Tested specimen are in a diluent which enhances the assay reactivity and suppresses non specific interactions (heterophilic antibodies).

Zymutest Total TF (T-TF):

Capture: Murine MoAb specific for an extracellular TF epitope, also exposed on truncated TF. **Revelation:** polyclonal antibody (PAb) reactive with all TF forms.

Zymuphen MP-TF:

Capture: same as Zymutest Total TF
Revelation: R1: recombinant human Factor VIIa (NovoSeven®), R2: highly purified human Factor X, (Hyphen BioMed), R3: Factor Xa specific substrate CS 11-(65) (Hyphen-Biomed).

Calibration and controls: All concentrations are expressed as FL-TF equivalent, in pg/ml. Full length recombinant TF (1-263) (ADI) has been relipidated (Zymuphen MP-TF) with synthetic liposomes (HBM) with a phospholipids/FL-TF ratio of 0,1nM PS / 1pg TF, or directly diluted, in the presence of stabilizers, (Zymutest Full length or Total TF) and lyophilized.

Plasma: Citrated normal or pathological plasmas.

LPS induction: Whole heparinized blood is incubated with Lipopolysaccharides (LPS; O111:B4 from Sigma) and plasmas are prepared by double centrifugation (15 min. at 1500g and 2 min. at 13.000g) at room temperature. Controls are the same plasmas prepared from untreated blood.

Full length TF (1,263), human, recombinant: ADI.

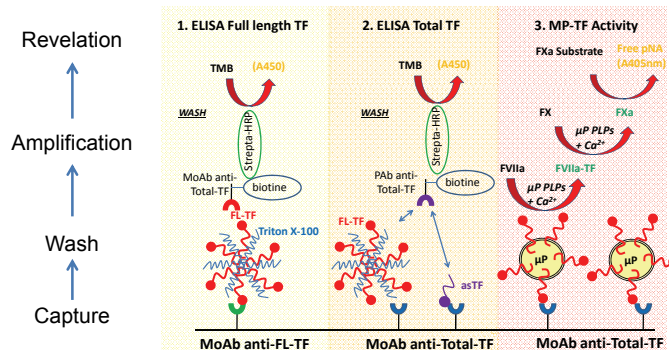
Truncated TF (1,219), human, recombinant: Nova-sep.

Aim

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

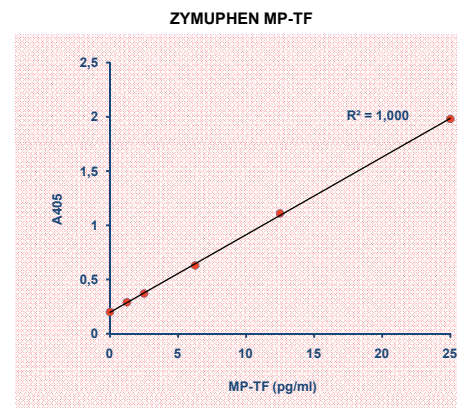
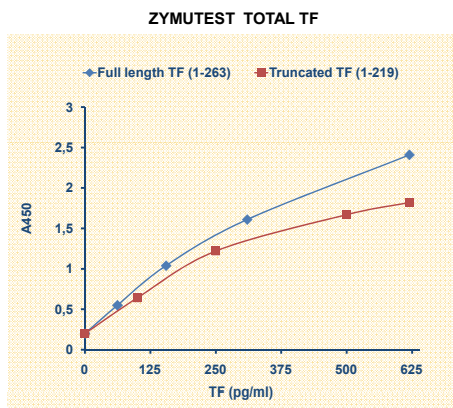
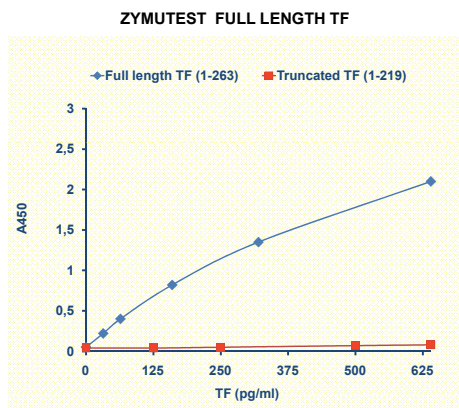
- 2 ELISA kits, Zymutest Full length TF and Zymutest Total TF, to quantify respectively, full length TF antigen or Total TF antigen,
- 1 Bio-immunoassay, Zymuphen MP-TF, that permits the determination of the procoagulant activity of Microparticles exposing Tissue Factor (MP-TF).

Assay principles

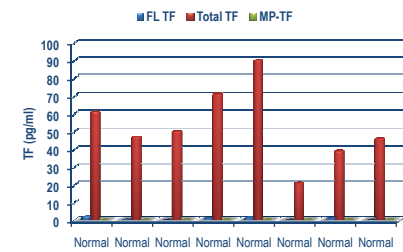


Results

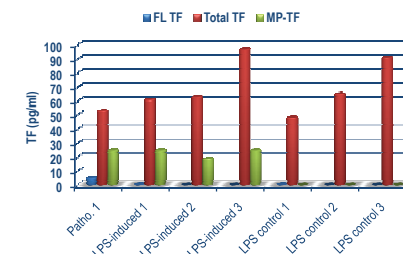
CALIBRATION CURVES



MEASUREMENTS ON NORMAL PLASMAS



MEASUREMENTS ON PATHOLOGICAL PLASMAS AND FOLLOWING LPS STIMULATION



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. asTF, using Zymutest Total TF.
- ➔ Incubation of human blood with LPS increased MP-TF significantly, whereas Total TF and Full length TF remains unchanged.

References

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3. Parhami-seren B., Butenas S. et al. "Immunologic quantitation of tissue factors". *J Thromb Haemost*, 4:1747-1755, 2006.