N-glycosylated IGFBP-4 could be less susceptible to PAPP-A mediated proteolysis than the non-glycosylated form

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Introduction
N- and C-terminal proteolytic fragments of IGFBP-4 (NT-IGFBP-4 and CT-IGFBP-4) are strong predictors of major adverse cardiac events in patients with myocardial ischemia and patients with type 1 diabetes [1, 2].

NT-IGFBP-4 and CT-IGFBP-4 are the products of PAPP-A dependent cleavage of full-length IGFBP-4. PAPP-A specifically cleaves IGFBP-4 between Met135 and Lys136. We have previously developed sandwich immunoassays based on monoclonal antibodies (mAbs) that are specific to proteolytic neo-epitopes on NT-IGFBP-4 and CT-IGFBP-4 and have less than 1% of cross-reactivity to the full-length IGFBP-4 [1].

It has been shown previously that a fraction of circulating IGFBP-4 contains glycosylated Asn104 that is located in the N-terminal part of protein. Asn104 is relatively close to the PAPP-A-specific cleavage site and to the epitope of the mAb utilized in NT-IGFBP-4 assay. Thus it can be supposed that measurements of some portion of NT-IGFBP-4 may be compromised by the glycosylation. Therefore, the aims of the study were:

a) To evaluate the influence of glycosylation on the immunodetection of NT-IGFBP-4;
b) To compare the susceptibilities of glycosylated and non-glycosylated IGFBP-4 to PAPP-A dependent proteolysis.

Methods
Affinity chromatography and concanavalin A chromatography were used for the extraction of glycosylated IGFBP-4 and NT-IGFBP-4 from acute coronary syndrome (ACS) patients’ plasma. Purified proteins were analyzed using enhanced chemi-luminescence Western blotting and sandwich ELISA immunoassays based on mAbs combinations IBPIB6-IBP100 and IBPIB6-IBP3, respectively (Fig. 1).

The composition of the IGFBP-4 glycan was studied using deglycosylases PNGase F and 2-3,5,6-neuraminidase with consequent urea electrophoresis and WB. To investigate the glycosylation level total fractions of IGFBP-4 and NT-IGFBP-4 were immunoprecipitated from twelve EDTA plasma samples of ACS patients and then analyzed using WB.

To study the influence of IGFBP-4 glycosylation on its PAPP-A dependent proteolysis, the increases in the concentrations of the proteolytic fragments of glycosylated and non-glycosylated IGFBP-4 during the incubation with recombinant PAPP-A were measured. Sandwich fluorimuno-

immunoassays (IBPIB6-IBP3) and IBPIB6-IBP3m were used for NT-IGFBP-4 and CT-IGFBP-4 measurement, respectively (Fig. 1).

Conclusions
1. For the first time, the presence of glycosylated NT-
IGFBP-4 in human plasma was shown. The ratio of glyco/total NT-IGFBP-4 in plasma is significantly lower than the ratio of glyco/total full-length IGFBP-4 (9.8-23.5% vs 47.2-61.7%).

PAPP-A dependent proteolysis of glycosylated IGFBP-4 is 3-4 times less efficient if compared with the proteolysis of non-glycosylated IGFBP-4.

The glycosylated NT-IGFBP-4 displays the same immunoactivity as non-glycosylated NT-IGFBP-4 in the fragmentation assay IBPIB0-IBP3m. This immunoassay can be used for the reliable measurement of total (glycosylated and non-
glycosylated) NT-IGFBP-4 in the patients’ blood.