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N-terminal and C-terminal fragments of IGFBP-4 as new markers for short-term risk assessment of major adverse cardiac events (MACE) in patients presenting with ischemia

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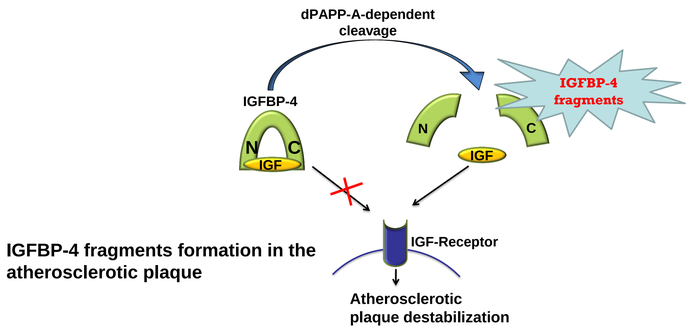
Introduction

Expression of the dimeric form of the Pregnancy Associated Plasma Protein A (dPAPP-A) is increased in vulnerable atherosclerotic plaques. Thus plasma dPAPP-A was suggested to be used as a marker for MACE prediction. However dPAPP-A measurements in patient's blood are complicated by low analyte concentration and heparin interference. dPAPP-A is known as a protease specifically cleaving IGF-binding protein 4 (IGFBP-4) to form its N-terminal and C-terminal fragments (NT- and CT-IGFBP-4) with molecular masses of 18 and 14 kDa, respectively. We supposed that IGFBP-4 fragments that are released from vulnerable atherosclerotic plaques in the circulation could be used for MACE risk assessment in patients with the symptoms of myocardial ischemia.



IGFBP-4 fragments formation in the atherosclerotic plaque

Materials and methods

Design of the immunoassays for NT- IGFBP-4, CT-IGFBP-4, and full-length IGFBP-4 measurement. Monoclonal antibodies (MAbs) were from HyTest Ltd. Mabs specific to NT-IGFBP-4 (MAb IBP3) and to CT-IGFBP-4 (MAb IBP163) recognized only novel epitopes raised by enzymatic cleavage of IGFBP-4 by dPAPP-A and having no cross-reaction with intact (full-length) IGFBP-4 molecule. Using one of these MAbs in pair with another MAb (MAbs IBP144, IBP182) recognizing corresponding fragment as well as intact IGFBP-4, we developed two sandwich immunoassays for NT-IGFBP-4 and CT-IGFBP-4 measurements (Fig.1).

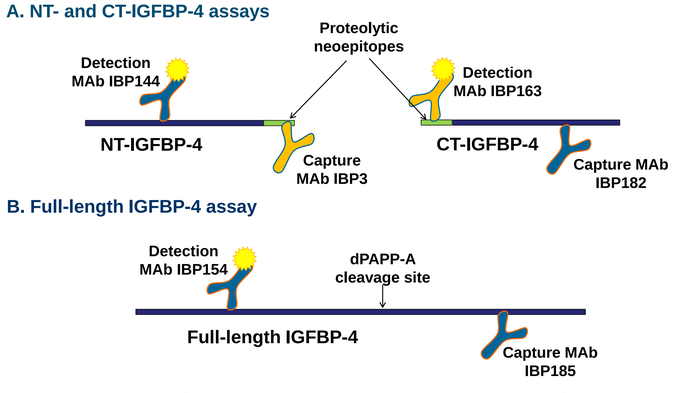


Figure 1. Schematic representation of NT- and CT-IGFBP-4 assays (A) and full-length IGFBP-4 assay (B) assays. NT- and CT-IGFBP-4 assays have no cross-reaction to full-length IGFBP-4.

Patients and study design. NT- , CT-IGFBP-4, and full-length IGFBP-4 were measured in EDTA-plasma of 180 patients admitted to emergency department with symptoms of myocardial ischemia but without ST-segment elevation. The incidence of MACE (nonfatal myocardial infarction, cardiac death, percutaneous coronary intervention) was measured during 6 months follow-up.

Statistical analysis. Cutoff values derived from the receiver operator curve (ROC) curves and were defined as the values that gave the best combination of sensitivity and specificity. We used the Cox proportional hazards model to estimate the hazard ratios (HR) of MACE. Cumulative event rates were estimated using the Kaplan–Meier method and were compared using the log-rank test. *P* values < 0.05 were considered to be statistically significant.

Results and discussion

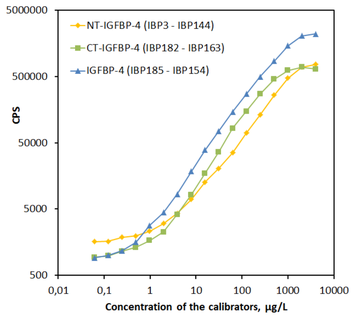


Figure 2. Calibration curves of NT-, CT-IGFBP-4, and full-length IGFBP-4 assays. Human recombinant NT-IGFBP-4 expressed in *E. coli*, CT-IGFBP-4 and full-length IGFBP-4 expressed in HEK293 cell line obtained from HyTest Ltd were used as calibrators.

Table 1. Baseline demographic and clinical characteristics as a function of MACE during 6 months of follow-up

Characteristic	Total Patients (180)	Patients without MACE (164)	Patients with MACE (16)	P Value
Demographics				
Female	84 (47%)	75 (46%)	9 (56%)	0.3810
Age, years	63 (12.7)	62 (12.5)	68 (12.9)	0.0686
Caucasian	117 (65%)	106 (65%)	11 (69%)	0.7290
African Americans	47 (26%)	45 (27%)	2 (13%)	0.2431
Other races	16 (9%)	13 (8%)	3 (19%)	0.1408
Past Medical History				
History of prior myocardial infarction	37 (21%)	29 (18%)	8 (50%)	0.0006
History of stenosis >50%	24 (13%)	19 (12%)	5 (31%)	0.0207
History of percutaneous coronary interventions including transluminal coronary angioplasty and stenting	17 (9%)	14 (9%)	3 (19%)	0.1748
History of coronary artery bypass grafting	10 (6%)	7 (4%)	3 (19%)	0.0204
History of heart failure	38 (21%)	33 (20%)	5 (31%)	0.2736
History of pulmonary disease	58 (32%)	54 (33%)	4 (25%)	0.5379
History of hypertension	158 (88%)	143 (87%)	15 (94%)	0.3083
History of diabetes	63 (35%)	58 (35%)	5 (31%)	0.7483
History of renal diseases	27 (15%)	20 (12%)	7 (44%)	0.0003
Presentation Data				
Body mass index (BMI), kg/m²*	32.2 (9.9)	32.9 (9.9)	25.4 (6.8)	0.0073
Discharge Diagnosis				
Acute, sub-acute or chronic ischemic heart disease	8 (4%)	6 (4%)	2 (13%)	0.1121

*** Variable missing >10% of responses.**

Immunoassays specific for NT- and CT-IGFBP-4 had 0.4% and 1.4% cross-reaction to the full-length IGFBP-4 molecule, respectively. Calibration curves are shown on Fig. 2. Within-assay imprecision and total imprecision of all three assays were not exceeded 3.7 and 8.9%, respectively. Recovery of all three assays were not less than 78%.

The baseline characteristics of the study population are shown in Table 1. At the end of the follow-up, 16 patients (8.9%) met the endpoint [10 (5.6%) nonfatal MI, 5 cardiac deaths (2.8%), and one revascularization (PCI) after myocardial infarction (0.6%)].

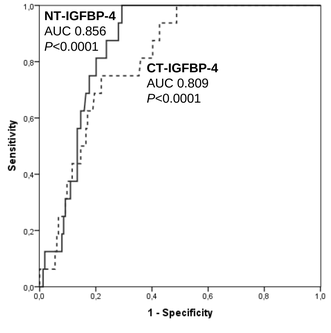


Figure 3. ROC curve analysis of MACE prediction at 6-months on the basis of NT- and CT-IGFBP-4 measurements.

Table 2. Adjusted HR (95% CI) for MACE within 6-months follow-up by NT- and CT-IGFBP-4 levels

	n (%)	n MACE (% of total MACE cases)	Unadjusted Model	*P* Value	Adjusted Model*	*P* Value
NT-IGFBP-4, < 214 µg/L	133 (73.9)	3 (18.8)	Reference		Reference	
≥ 214 µg/L	47 (26.1)	13 (81.3)	**15.51** (4.41 - 54.51)	<0.0001	**13.79** (3.43 - 55.48)	0.0002
CT-IGFBP-4, < 124 µg/L	127 (70.6)	4 (25.0)	Reference		Reference	
≥ 124 µg/L	53 (29.4)	12 (75.0)	**8.52** (2.75 - 26.34)	0.0002	**7.93** (2.20 - 28.61)	0.0016

*** Model adjusted for age, sex, prior myocardial infarctions, a history of stenosis > 50%, hypertension, diabetes, a history of renal diseases, and a history of ischemic heart disease.**

The median NT-, CT-IGFBP-4 and full-length IGFBP-4 concentrations (interquartile range) of the study cohort were 121 (76 - 215), 88 (62 - 138), and 822 (652 - 1013) µg/L, respectively.

The ability of NT-, CT-IGFBP-4, and full-length IGFBP-4 and their ratios to predict the MACE at 6 months was investigated by ROC analysis. Areas under ROC curves (AUC) were calculated (Fig. 3).

NT- and CT-IGFBP-4 were strong predictors of MACE [AUC 0.856 (95% CI 0.798 - 0.915), P< 0.0001 and AUC 0.809 (95% CI 0.726 - 0.892), *P* < 0.0001], respectively. Measurements of full-length IGFBP-4 were not predictive for MACE. Ratios of NT- and CT-IGFBP-4 to full-length IGFBP-4 did not strengthen the prediction ability of the fragments themselves. NT-IGFBP-4 concentrations ≥ 214 µg/L were 81% sensitive and 79% specific for MACE prediction. CT-IGFBP-4 concentrations ≥ 124 µg/L were 75% sensitive and 75% specific for MACE prediction.

Table 2 presents HRs for MACE within 6 months by applying predefined cutoff values of circulating NT- and CT-IGFBP-4 concentrations. After adjustment for different cardiovascular risk factors the HRs were somewhat attenuated but remained statistically significant.

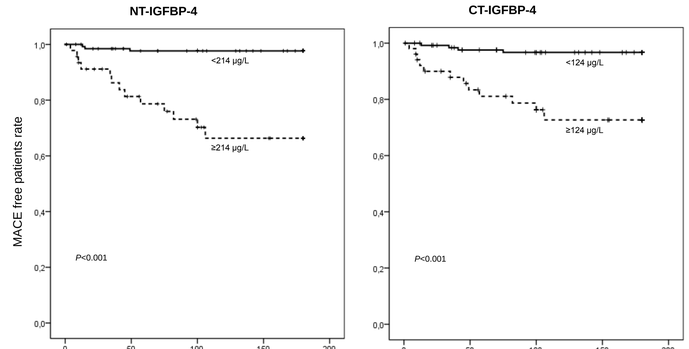


Fig. 4 Kaplan–Meier survival curves for MACE stratified by NT-IGFBP-4 and CT-IGFBP-4 cutoff values.

In the final stepwise multivariable Cox regression model, NT-IGFBP-4 remained the only significant predictor of MACE, whereas CT-IGFBP-4 was dropped as non-significant. Pearson tests for correlation have shown that circulating NT-IGFBP-4 levels were strongly related to CT-IGFBP-4 (r = 0.815; P < 0.0001) and had not correlated with full-length IGFBP-4 (r = 0.009; P = 0.906). Additionally, CT-IGFBP-4 had not correlated with full-length IGFBP-4 (r = 0.000; P = 0.998). Thus, NT- and CT-IGFBP-4 elevation in patients blood is not associated with full-length IGFBP-4 levels changes.

Figure 4 illustrates Kaplan–Meier survival curves for MACE using the predefined cutoff values of NT-IGFBP-4 (214 µg/L) and CT-IGFBP-4 (124 µg/L). In most cases with the following endpoints, the concentration of IGFBP-4 fragments was elevated. The major part of the endpoints of the study occurred within the first 3 months. Thus, IGFBP-4 fragments could be considered as specific short-term predictors of MACE.

Conclusions

For the first time, we report here that IGFBP-4 fragments can be utilized as markers for MACE prediction in patients with suspected acute coronary syndrome. New clinical trials on larger cohorts of patients with a larger number of endpoints are needed to confirm the value of IGFBP-4 proteolytic fragments as markers for risk assessment of MACE.

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This abstract has received
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