Pentraxin-3 vs. C-reactive protein and other prognostic biomarkers in acute coronary syndromes: A substudy of the PLATelet inhibition and patients Outcomes (PLATO) trial.

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ABSTRACT

Aims: We investigated the dynamics, associations with patient characteristics, other biomarkers, and clinical outcomes of pentraxin 3 (PTX3) in acute coronary syndromes (ACS).

Methods and Results: In multivariate analyses, PTX3 measured in 5154 patients randomised in the PLATO trial (NCT00391872) were compared with leukocytes, hs-CRP, IL-6, cystatin C, NT-proBNP, hs-TnT and GDF-15 concerning prediction of clinical outcome.

PTX3 peaked earlier than hs-CRP and was more strongly correlated with NT-proBNP and hs-TnT than with hs-CRP. The frequency of cardiovascular (CV) death, spontaneous myocardial infarction (sMI) or stroke by quartiles of PTX3 at admission was 6.1%, 7.3%, 9.7% and 10.7%, respectively ($p < 0.0001$). The hazard ratio (HR) per 50% increase of PTX3 was 1.13 (95% confidence interval [CI]: 1.07-1.19), $p<0.0001$. This association remained significant after stepwise adjustments for leukocytes/ hs-CRP (1.09 [1.02-1.15]), $p=0.009$, IL-6 (1.07 [1.01-1.14]), $p=0.026$, and cystatin C (1.07 [1.00-1.13]), $p=0.044$, but not after adjustment for NT-proBNP, hs-TnT and GDF-15. Admission PTX3 was also associated with several of the individual endpoint components (CV death/sMI; $p=0.008$, CV death; $p=0.026$, and sMI; $p=0.017$), but not with stroke. PTX3 measured in the chronic phase (i.e. at 1 month) was still predictive of the composite endpoint in univariate analysis (1.12 [1.04-1.20] per 50% increase) $p=0.0024$, but not after adjustment for the other biomarkers.

Conclusion: Admission level of PTX3 is a modestly stronger predictor than hs-CRP and IL-6, but not than NT-proBNP or hs-TnT, concerning CV outcome in ACS. But, PTX3 is more strongly correlated with NT-proBNP and hs-TnT than with hs-CRP.

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INTRODUCTION

Biomarkers reflecting different facets of the underlying pathophysiological processes in coronary artery disease (CAD) are associated with the risk of new events both in the acute and chronic phase of this condition. C-reactive protein (CRP), the prototypic marker of inflammation, has been shown in several series to be closely linked to adverse outcome, as has interleukin-6 (IL-6), a potent inducer of CRP. Other well-established biomarkers for outcome prediction reflect myocardial necrosis (cardiac troponin) and dysfunction (N-terminal pro b-type natriuretic peptide [NT-proBNP]), and impaired renal function (estimated glomerular filtration rate [eGFR] and cystatin C). Cardiac and inflammatory biomarkers both peak in the acute phase of myocardial infarction and are mainly related to infarct size and thereby to mortality and development of heart failure, whereas the association of biomarkers to clinical outcome including risk of reinfarction in the chronic phase may be more closely linked to the progression of the underlying vascular disease.

The long pentraxin 3 (PTX3) is an evolutionarily preserved, multimeric acute phase protein from the same superfamily of inflammatory glycoproteins as CRP and is one of several *long* pentraxins recently identified, whereas CRP and human serum amyloid P component (SAP) are the two prototypic *short* human pentraxins.[1,2] PTX3 shares homology with CRP at the C-terminal, but has no homology to any known protein at its N-terminal.[3] Unlike CRP, PTX3 is produced at sites of inflammation including the vascular endothelium, and might add information on the role of inflammation in atherosclerotic disorders beyond that of CRP. Several earlier studies on PTX3 in patients with CAD were rather small and included narrow patient populations making the generalizability of the results difficult.

The objectives of the current study were to investigate, in a contemporary cohort of patients with acute coronary syndrome (ACS), the dynamics of PTX3 release in both the acute and chronic phases of CAD, to assess the associations between PTX3 levels with fatal and non-fatal cardiovascular outcomes in both disease stages, and to compare these dynamics and associations with those of CRP, IL-6, and cardiorenal biomarkers.

METHODS

Study population

The PLATO (PLATelet inhibition and patient Outcomes [NCT00391872]) trial was a multicentre, randomised trial that enrolled 18624 patients with the whole spectrum of ACS (i.e. unstable angina, non-ST-segment elevation myocardial infarction [NSTEMI], and ST-segment elevation myocardial infarction][4]. In- and exclusion criteria in the PLATO trial (identical for this substudy) are listed in the online supplement together with number of patients in each diagnosis category. All patients received optimal medical therapy, including aspirin, and were randomised to either clopidogrel or ticagrelor. The follow-up in the study was up to 12 months with a median of 9 months. Details of the study design have been previously published.[5]

The present substudy was part of a prespecified biomarker research project embedded in the extended PLATO trial program. All PLATO study patients at selected sites were invited on a consecutive basis to participate in this program which included venous blood sampling scheduled at randomisation, discharge, and after 1 and 6 months. Informed consent was obtained from all patients, and the trial complied with the Helsinki declaration. The research protocol was approved by the locally appointed ethics committees.

Study endpoints

The primary efficacy endpoint of this substudy was the composite of cardiovascular (CV) death, spontaneous myocardial infarction (sMI) or stroke within one year of follow-up. The individual components of the primary endpoint were also analysed separately. An independent, blinded clinical events adjudication committee assessed all efficacy and safety endpoints in the PLATO trial.[4] Efficacy endpoint definitions are listed in the online supplement.

Laboratory analyses

Venous blood samples were drawn at randomisation, but before study drug administration, and within 24 hours of start of chest pain, as well as at hospital discharge and 1- and 6-month follow-up. After centrifugation, the serum was stored in aliquots at -70ºC in a central biobank. PTX3 levels

were measured by enzyme immunoassay (Stillwater, MN, USA) with intra- and inter-assay coefficients of variation 2.5% and 8.7%, respectively, and a sensitivity of 0.031 μg/L. Repeated analyses both after sample storage at room temperature and up to 10 freeze-thaw cycles indicated that PTX3 is a robust protein with little degradation, and if anything more stable than CRP (suppl. tab. 1). Detailed information about laboratory methods concerning other biomarker analyses is given in the online supplement.

Statistical analyses

Crude event rates at 1 year by PTX3 quartiles at baseline were estimated as well as Kaplan Meier event rates. The associations of baseline PTX3 concentrations (ln-transformed) with the primary composite endpoint and its individual components were assessed both by multivariable Cox proportional hazards models, with the hazard based on a 50% increase in biomarker concentration as well as by PTX3 quartiles. Six models, with incremental addition of co-variates, were used. Model 0 included randomised treatment only (ticagrelor or clopidogrel). Model 1 added clinical baseline risk factors; age, gender, body mass index (BMI), diabetes mellitus (DM), chronic kidney disease (CKD), hypertension, smoking status, type of ACS, and history of congestive heart failure (CHF), MI, percutaneous coronary intervention (PCI), coronary artery by-pass grafting (CABG), stroke or peripheral artery disease (PAD). Model 2 included all variables from model 1 together with inflammatory biomarkers (2A: leukocyte count and hs-CRP; 2B: leukocyte count, hs-CRP and IL-6). Model 3 included all the previous mentioned co-variates, with the addition of cystatin C. Model 4 included hs-TnT and NT-proBNP to all variables in model 3, and model 5 contained GDF-15 in addition to all aforementioned variables.

Further details to the statistical methods are given in the online supplement.

RESULTS

PTX3 IN THE ACUTE PHASE

PTX3 levels in relation to clinical characteristics and other biomarkers

Acute phase levels of PTX3 were available for 5154 patients with a median value (IQR) of 1.89 ng/ml (1.22-2.97 µg/L). Baseline characteristics by PTX3 quartiles are presented in tab. 1. Age, female gender, diabetes, CHF, CKD, higher TIMI and GRACE risk scores, and the use of diuretics or proton pump inhibitors were positively correlated with PTX3 levels. Current smoking, ST-elevation MI, indicators of the metabolic syndrome (weight, BMI, or dyslipidaemia) and statin use were associated with lower PTX3 levels.

Table 1: Baseline characteristics and biomarker levels according to quartiles of acute phase (i.e. admission) PTX3 levels. *: P-values from chi-square test (categorical variables) or Kruskal-Wallis test (continuous variables).

		PTX3 (µg/L)				
Characteristic		**Q1 <1.2 n=1289**	**Q2 1.2 - 1.9 n=1289**	**Q3 1.9 - 3.0 n=1288**	**Q4 >3.0 n=1288**	**P-value***
Demographics	Age yrs median (Q1-Q3)	59 (52-67)	61 (54-69)	63 (54-71)	65 (56-73)	<.0001
	Female	339 (26.3%)	377 (29.2%)	396 (30.7%)	433 (33.6%)	0.0007
	Weight kg median (Q1-Q3)	84 (74-93)	80 (72-90)	80 (70-90)	78 (68-88)	<.0001
	BMI kg/m^2 median (Q1-Q3)	28.3 (25.8-31.1)	27.7 (25.1-30.9)	27.4 (24.9-30.4)	27.0 (24.2-29.8)	<.0001
Risk factor	Habitual smoker	551 (42.7%)	456 (35.4%)	476 (37.0%)	415 (32.2%)	<.0001
	Hypertension	834 (64.7%)	865 (67.1%)	856 (66.5%)	846 (65.7%)	0.6041
	Dyslipidaemia	590 (45.8%)	545 (42.3%)	540 (41.9%)	507 (39.4%)	0.0118
	Diabetes mellitus	264 (20.5%)	289 (22.4%)	272 (21.1%)	321 (24.9%)	0.0347
Medical history	Angina pectoris	606 (47.0%)	622 (48.3%)	606 (47.0%)	566 (43.9%)	0.1537
	Myocardial infarction	251 (19.5%)	255 (19.8%)	246 (19.1%)	257 (20.0%)	0.9511
	Congestive heart failure	64 (5.0%)	59 (4.6%)	74 (5.7%)	99 (7.7%)	0.0034
	PCI	180 (14.0%)	147 (11.4%)	164 (12.7%)	146 (11.3%)	0.1349
	CABG	63 (4.9%)	68 (5.3%)	54 (4.2%)	71 (5.5%)	0.4359
	TIA	23 (1.8%)	28 (2.2%)	30 (2.3%)	32 (2.5%)	0.6530
	Non-haemorrhagic stroke	34 (2.6%)	42 (3.3%)	53 (4.1%)	48 (3.7%)	0.1936
	Peripheral arterial disease	81 (6.3%)	89 (6.9%)	86 (6.7%)	93 (7.2%)	0.8131
	Chronic renal disease	30 (2.3%)	27 (2.1%)	56 (4.3%)	67 (5.2%)	<.0001
Type of ACS	ST-elevation MI	661 (51.3%)	568 (44.1%)	563 (43.7%)	569 (44.2%)	0.0001
Risk scores	TIMI risk score median (Q1-Q3)	3.0 (2.0-4.0)	4.0 (2.0-5.0)	4.0 (3.0-5.0)	4.0 (3.0-5.0)	<.0001
	GRACE risk score median (Q1-Q3)	127 (113-143)	133 (116-149)	136 (120-153)	142 (124-161)	<.0001
Anti-thrombotic treatment in hospital	Aspirin	1274 (98.8%)	1266 (98.2%)	1271 (98.7%)	1260 (97.8%)	0.1620
	Unfractioned heparin	673 (52.2%)	696 (54.0%)	696 (54.0%)	750 (58.2%)	0.0172
	LMW heparin	686 (53.2%)	692 (53.7%)	709 (55.0%)	696 (54.0%)	0.8167
	Fondaparinux	32 (2.5%)	19 (1.5%)	10 (0.8%)	12 (0.9%)	0.0009
	Bivalirudin	17 (1.3%)	27 (2.1%)	20 (1.6%)	12 (0.9%)	0.0982
	GP IIb/IIIa inhibitor	355 (27.5%)	311 (24.1%)	345 (26.8%)	359 (27.9%)	0.1264
Other medication in hospital	Beta-blockers	1141 (88.5%)	1123 (87.1%)	1126 (87.4%)	1095 (85.0%)	0.0618
	ACE-inhibition and/or ARB	1137 (88.2%)	1118 (86.7%)	1103 (85.6%)	1119 (86.9%)	0.2885
	Cholesterol lowering (statin)	1226 (95.1%)	1210 (93.9%)	1213 (94.2%)	1179 (91.5%)	0.0018

Characteristic		PTX3 (µg/L) Q1 <1.2 n=1289	Q2 1.2 - 1.9 n=1289	Q3 1.9 - 3.0 n=1288	Q4 >3.0 n=1288	P-value*
	Ca-inhibitor	268 (20.8%)	275 (21.3%)	268 (20.8%)	266 (20.7%)	0.9758
	Diuretic	415 (32.2%)	448 (34.8%)	479 (37.2%)	615 (47.7%)	<.0001
	Proton pump inhibitor	512 (39.7%)	522 (40.5%)	584 (45.3%)	650 (50.5%)	<.0001
Biomarkers	hs-Troponin T (ng/L) median (Q1-Q3)	65.1 (20.4-226.0)	123.0 (35.6-394.0)	207.0 (61.7-623.0)	376.0 (105.0-1163)	<.0001
	NT-proBNP (pmol/L) median (Q1-Q3)	205.0 (70.0-548.0)	345.0 (117.0-843.0)	461.0 (177.0-1147)	933.0 (305.0-2574)	<.0001
	Cystatin C (mg/L) median (Q1-Q3)	0.77 (0.64-0.93)	0.81 (0.67-0.97)	0.83 (0.67-1.02)	0.87 (0.70-1.11)	<.0001
	GDF-15 (ng/L) median (Q1-Q3)	1386 (1055-1869)	1463 (1111-1955)	1535 (1147-2140)	1871 (1308-2881)	<.0001
	hs-CRP (mg/L) median (Q1-Q3)	2.7 (1.3-5.4)	3.1 (1.5-7.0)	3.6 (1.6-9.8)	6.2 (2.2-20.0)	<.0001
	IL-6 (ng/L) median (Q1-Q3)	2.6 (1.7-4.3)	3.0 (1.7-5.7)	3.6 (2.1-7.3)	5.8 (2.6-12.0)	<.0001

Abbreviations: ACE: Angiotensin converting enzyme, ARB: Angiotensin receptor blocker, BMI: Body mass index, Ca: Calcium, CABG: Coronary artery bypass graft surgery, GDF-15: Growth differentiation factor 15, GP: Glycoprotein, GRACE: Global Registry of Acute Coronary Events, hs-CRP: High sensitive C-reactive protein, IL-6: Interleukin-6, LMW: Low molecular weight, MI: Myocardial infarction, NT-proBNP: N-terminal prohormone of brain natriuretic peptide, PCI: Percutaneous coronary intervention, TIA: Transitoric ischaemic attack, TIMI: Thrombolysis In Myocardial Infarction.

Acute phase levels of PTX3 correlated significantly with cardiac and renal (i.e. hs-TnT and NT-proBNP) as well as inflammatory (hs-CRP, IL-6, and leukocytes) biomarkers (p<.0001 for all). Strongest correlations were seen between PTX3 and biomarkers of myocardial damage and dysfunction (Spearman correlation coefficient for PTX3 vs. hs-TnT: 0.33, and PTX3 vs. NT-proBNP: 0.34), while weaker correlations were found with inflammatory biomarkers; hs-CRP: 0.24, IL-6: 0.29, and leukocyte count: 0.13 (suppl. tab. 2).

To assess the strength of association (F-value) of PTX3 to the other biomarkers, a multivariable general linear model containing biomarkers and clinical covariates was conducted with PTX3 level as dependent variable. The F statistics was used for analysis of variance, representing explained versus unexplained variance. The highest F-value (i.e. strongest association) was seen between PTX3 and NT-proBNP and hs-TnT, respectively (Fig. 1). Scatterplots for PTX3 vs. hs-TNT and NT-proBNP are shown in suppl. fig. 1a and b.

In multivariable linear regression analyses including clinical characteristics only, age (p < 0.0001), female gender (p = 0.0045), and chronic kidney disease (p = 0.0011) remained independently associated with higher PTX3 levels, whereas dyslipidaemia (p = 0.0013) and BMI ≥ 30 kg/m^2 (p < 0.0001) were the only factors still significantly associated with lower levels. In fully-adjusted multivariable regression analyses including both baseline characteristics and other biomarkers, higher PTX3 levels remained independently associated with the acute phase cardiac biomarkers hs-TNT and

NT-proBNP, and stronger so than with hs-CRP, leukocyte count and GDF-15, whereas the associations with IL-6 and cystatin C were no longer statistically significant (table 2).

Table 2: Association of acute (i.e. admission) and chronic phase (i.e. at 1 month post-ACS) PTX3 levels with other biomarkers in adjusted* multivariable linear regression analyses. The relative increase is the adjusted geometric mean ratio for 10% increase in biomarker levels.

Biomarker	Phase	Relative increase	95% CI	P-value
NT-proBNP	A	1.0090	1.0072 - 1.0109	<.0001
	C	1.0060	1.0032 - 1.0089	<.0001
hs-Troponin T	A	1.0066	1.0053 - 1.0080	<.0001
	C	1.0003	0.9961 - 1.0044	0.9065
GDF-15	A	1.0132	1.0086 - 1.0177	<.0001
	C	1.0138	1.0066 - 1.0210	0.0002
Cystatin-C	A	0.9945	0.9877 - 1.0014	0.1198
	C	1.0045	0.9935 - 1.0156	0.4255
hs-CRP	A	1.0041	1.0023 - 1.0058	<.0001
	C	1.0036	1.0009 - 1.0063	0.0098
WBC	A	1.0274	1.0206 - 1.0341	<.0001
	C	1.0068	0.9961 - 1.0176	0.2158
IL-6	A	1.0018	0.9990 - 1.0048	0.1958
	C	0.9946	0.9905 - 0.9987	0.0096

* Adjusted for age, gender, diabetes, CHF, type of ACS (i.e STEMI vs non-STEMI), in-hospital treatment approach, previous PCI/CABG/MI/PAD/NH-Stroke, chronic kidney disease, arterial hypertension, smoking status, dyslipidemia, BMI, and aspirin at entry.

Abbreviations: A: Acute phase, C: Chronic phase, CABG: Coronary artery by-pass grafting, CHF: Congestive heart failure, GDF-15: Growth differentiation factor 15, hs-CRP: High sensitive C-reactive protein, , IL-6: Interleukin 6, MI: Myocardial infarction, NH: Non-haemorrhagic, NT-proBNP: N-terminal prohormone of brain natriuretic peptide, STEMI: ST-elevation myocardial infarction, PAD: Peripheral artery disease, PCI: Percutaneous coronary intervention, WBC: White blood cell (leukocyte) count.

Association of PTX3 with clinical outcome

The primary composite endpoint was observed in 435 of 5154 patients (8.4%) with 193 were CV-deaths, 242 sMIs, and 62 strokes. The crude event rates of the primary endpoint were 6.1%, 7.3%, 9.7% and 10.7% per quartile of PTX3, respectively ($p<0.0001$). Kaplan-Meier estimates for the primary outcome are presented in fig. 2. The acute phase PTX3 level (with HR per 50% increase of PTX3 as continuous variable) was significantly associated with the primary composite endpoint, HR: 1.17 (95% CI: 1.11-1.23), $p<0.0001$.

In multivariable Cox regression analyses, the acute phase PTX3 level remained significantly associated with the primary outcome after adjustment for randomised treatment, clinical risk factors, leukocyte count and hs-CRP (1.09 [1.02-1.15]), $p=0.009$ (model 2A). This association remained statistically significant also after stepwise entering of IL-6 (1.07 [1.01-1.14]), $p=0.026$ (model 2B), and cystatin C (1.07 [1.00-1.13]), $p=0.044$ (model 3) into the analyses. PTX3 was furthermore significantly associated with the following individual components of the primary endpoint after adjustment for leukocyte count and hs-CRP (model 2A): CV death/sMI, CV death, sMI and with CV death/all MI/stroke, and remained statistically significant also after entering both IL-6 and cystatin C into the model (model 2B and 3) (tab. 3). Figure 3 shows, however, that the associations of PTX3 levels with the individual outcome measures were non-linear. There was no association between PTX3 and stroke alone.

Table 3: Association of acute phase (i.e. admission) PTX3 levels with the primary composite endpoint and its individual components. (Multivariable Cox regression analyses with HR per 50% increase of PTX3 as continuous variable. Stepwise adjustments for leukocytes / hs-CRP (model 2A), leukocytes / hs-CRP / IL-6 (model 2B), and leukocytes / hs-CRP / IL-6 / cystatin C (model 3).

Outcome	Leukocytes / hs-CRP HR (95% CI)	P-value	Leukocytes / hs-CRP / IL-6 HR (95% CI)	P-value	Leukocytes / hs-CRP / IL-6 / cystatin C HR (95% CI)	P-value

CV death / sMI / stroke	1.09 (1.02-1.15)	0.0085	1.07 (1.01-1.14)	0.0264	1.07 (1.00-1.13)	0.0438
CV death / sMI	1.11 (1.05-1.19)	0.0009	1.10 (1.03-1.18)	0.0042	1.09 (1.02-1.17)	0.0076
CV death	1.16 (1.06-1.27)	0.0012	1.13 (1.03-1.24)	0.0111	1.11 (1.01-1.23)	0.0262
sMI	1.10 (1.02-1.20)	0.0180	1.11(1.02-1.20)	0.0143	1.11 (1.02-1.20)	0.0166
CV death / all MI / stroke	1.09 (1.03-1.16)	0.0016	1.08 (1.02-1.15)	0.0063	1.08 (1.02-1.14)	0.0103
stroke	0.88 (0.75-1.03)	0.1124	0.88 (0.75-1.04)	0.1340	0.88 (0.75-1.03)	0.1092

Abbreviations: CV: Cardiovascular, hs-CRP: High sensitive C-reactive protein, IL-6: Interleukin 6, M: Myocardial infarction, sMI: Spontaneous myocardial infarction.

In fully-adjusted adjusted analyses (i.e. including NT-proBNP, hs-TnT and GDF-15), PTX3 was no longer significantly associated with the primary outcome measures or any of its individual components. The association with clinical outcome did not remain statistically significant when PTX3 was applied as categorical variable (suppl. tab. 3).

The PTX3 level at discharge was significantly lower in the clopidogrel vs. ticagrelor treatment group (geometric mean clopidogrel: 1.67, ticagrelor: 1.78, ratio of geometric means (95%CI): 1.07 (1.03-1.11), $p = 0.0002$), whereas no difference was seen in the PTX3 levels at admission, 1 and 6 months, respectively. No significant interaction was seen between the acute phase PTX3 levels and randomised treatment for the primary endpoint (suppl. fig. 2) or any of the other outcome measures (suppl. tab. 4). Corresponding analyses adjusted for NSTEMI vs. STEMI did not reveal any difference concerning outcome between these groups (suppl. tab. 5 and suppl. fig. 3).

PTX3 IN THE CHRONIC PHASE

Dynamics of PTX3 vs. CRP and IL-6 release

Serial PTX3 measurements during follow-up revealed an early peak at baseline with a gradual decrease during the acute phase and continuing through 6 months. Median PTX3 value (IQR) at entry was 1.89 (1.22-2.97) µg/L, at discharge 1.74 (1.13-2.65) µg/L ($p<0.0001$ vs. baseline, n=4584), 1 month: 1.23 (0.79-1.86) µg/L ($p<0.0001$ vs. baseline, n=4259), and 6 months: 0.99 (0.61-1.49) µg/L ($p<0.0001$ vs. baseline, n=3106), respectively. When comparing the dynamics of PTX3 release with

hs-CRP and IL-6, the very early maximum level was similar to IL-6 and more rapid than the maximum of hs-CRP seen several days later. The elevation of PTX3 still lasted longer than that of both hs-CRP and IL-6 (fig. 4).

Association of PTX3 with clinical characteristics and other biomarkers

After one month, independent positive associations remained between PTX3 levels and the following clinical characteristics: Female gender (p <0.0122) and dyslipidaemia (p =0.0058), whereas among those variables negatively correlated with PTX3 in the acute phase only current smoker remained significantly associated with PTX3 ($p < 0.0001$).

As opposed to the acute phase, there was no longer any association of PTX3 with hs-TnT at this stage, but the association with NT-proBNP remained statistically significant ($p < 0.0001$) as did the associations with GDF-15 ($p = 0.0002$) and hs-CRP ($p = 0.0098$). IL-6 was now negatively associated with PTX3 ($p = 0.0096$), and no significant association was present with leukocyte count or cystatin C (table 2).

Association of PTX3 with clinical outcome

Follow-up measurements at 1 month were available for 4233 patients without preceding CV events since their index event, of which 240 (5.7%) later suffered a primary composite endpoint. PTX3 was significantly associated with the composite primary outcome with HR (95%CI) per 50% increase: 1.12 (1.04-1.20), $p = 0.0024$. This association did not remain statistically significant when adjusted for any of the other biomarkers including (suppl. tabs. 6 and 7), a result that also applied to hs-CRP measured at this point in time (suppl. tab. 8). IL-6 remained, however, significantly associated with this outcome in models 2 and 3 applied as continuous variable (suppl. tab. 9).

Statistical analyses concerning the predictive value of PTX3 at 6 months was not performed due to both the fact that a substantial number of patients were followed up for only a few weeks thereafter, and a low number of events of the primary outcome measure occurred after that point (ticagrelor: 46, clopidogrel: 41).

DISCUSSION

We found that the acute phase (i.e. admission) level of PTX3 was a significant predictor of the composite of CV death, sMI and stroke within 12 months, independently of hs-CRP and IL-6, and of cystatin C. Furthermore, elevated PTX3 predicted increased risk for several of the individual components of the primary outcome. However, after adjustment for NT-proBNP and hs-TnT, with or without GDF-15, the association with outcome was no longer statistically significant. Our findings suggest that PTX3 may better reflect activation of the inflammatory pathways in ACS than hs-CRP and IL-6 but seems inferior to markers of myocardial damage and dysfunction in the prediction of clinical outcome.

Our study is by far the largest series in the literature to investigate PTX3 concerning clinical outcome in a broad ACS population. To the best of our knowledge, this study is also the first to include IL-6, a potent inducer of CRP, and cystatin C in the analyses, and is one of very few to include stroke in the outcome measures. Thus, PTX3 measured in the acute phase seems to be one of the strongest inflammatory predictors of clinical outcome in patients with ACS. However, PTX3 measured in the chronic phase was not predictive of clinical outcome.

Our results are concordant with a previous study showing that PTX3, but not hs-CRP, was significantly associated with fatal outcome within 3 months after acute ST-elevation MI.[6] Neither NT-proBNP nor TnT was predictive of mortality in that study, but there were marked differences to our trial in the management strategy since more than 60% received thrombolytic therapy and PCI was performed in only 4.3%. In contrast, thrombolytic therapy was an exclusion criterion for enrolment in the PLATO trial, and PCI was performed in 61% during initial hospitalisation. Thus, in our opinion, the present study more correctly reflects the predictive value of PTX3 concerning outcome in a contemporary ACS population.

In another study of patients admitted with suspected ACS, elevated admission levels of PTX3 and BNP, but not hs-CRP, were independently associated with fatal outcome within 24 months.[7] The combination of PTX3 and BNP enhanced the predictive value significantly over either biomarker alone. A 7-years follow-up report on this cohort revealed that PTX3 and BNP were still associated with late mortality, while hs-CRP and BNP were predictive of new MI.[8] However, this study included

up to 50% non-ACS patients. Furthermore, the prevalence of heart failure was much higher in the former study (27.4% vs 5.7%), and all-cause mortality was applied as efficacy endpoint as opposed to CV mortality in our study.

Another recent study found that PTX3, BNP and GRACE-score, but not hs-CRP, was associated with CV mortality at 5-year follow-up after acute MI in a single centre study.[9] That study differs markedly from ours by excluding patients with CV disease including previous MI, coronary revascularisation, heart or renal failure, and recent inflammatory or infectious diseases.

The observation that PTX3 measured at one month had no predictive value concerning CV outcome is in line with a substudy of a lipid lowering trial in which PTX3 was not associated with recurrent MI or coronary death within 5 years among stable coronary artery disease patients randomised to pravastatin or placebo.[10]

We found that acute phase PTX3 was more strongly correlated with hs-TnT and NT-proBNP, i.e. markers of myocardial necrosis and dysfunction, than with inflammatory biomarkers. To our knowledge, a similar finding has not been reported earlier. The mechanisms behind this remain to be further elucidated in new studies, but one can speculate whether this is because PTX3 may be more closely linked than hs-CRP to local pathophysiological processes in the vasculature and myocardium. An increasing body of data indicate diverse functions of PTX3 in human physiology and pathophysiology. Although PTX3 is upregulated in high-risk plaques in ST-elevation MI as well as in carotid stenosis,[11, 12] and is a stronger marker than hs-CRP of endothelial dysfunction,[13] PTX3/ApoE-double-knockout mice had increased vessel wall inflammation compared with ApoE-knockout controls.[14] Furthermore, PTX3-deficient mice showed increased area of myocardial damage after coronary artery ligation.[15] Thus, PTX3 and CRP may represent different inflammatory processes in the development of atherosclerosis. CRP is induced mainly in the liver as a systemic response to an inflammatory stimulus, primarily IL-6, but also IL-1 and IL-17. CRP accumulates in lipid-rich plaques where it co-localizes with T-lymphocytes. In contrast, PTX3 is produced by different cell types (vascular cells and innate immune cells), although not by hepatocytes upon stimulation by IL-1β, TNFα, and toll-like receptor agonists, but not by IL-6. It is expressed in advanced atherosclerotic plaques where it co-localizes with CD163-positive macrophages. PTX3 furthermore contributes to

thrombosis by induction of tissue factor expression in monocytes and endothelial cells, but may also dampen leucocyte recruitment by binding to P-selectin.

CRP may activate the complement system through both the classical and alternative pathways by binding to C1q and factor H, respectively. PTX3 has been shown to modulate all three complement pathways including the mannose-binding lectin (MBL) pathway, resulting in both pro- and anti-inflammatory effects. Thus, CRP clearly contributes to atherosclerosis progression, whereas PTX3 seems to exert both pro- and anti-inflammatory actions which regulate the immune-inflammatory response. The complete biological roles of these two pentraxins remain, however, still to be fully understood[16].

Both the results of this study and own experiences concerning the analytical performance and robustness of PTX3 as compared with hs-CRP, indicate that PTX3 outperforms the latter as a predictor of adverse clinical outcome in ACS. PTX3 can be analysed with readily available methods at a cost comparable with hs-CRP. However, with strong predictors like NT-proBNP and hs-TnT, only further studies can more clearly define the overall role of inflammatory biomarkers in this context.

Study limitations

Although the PLATO trial encompassed the whole spectrum of ACS including unstable angina as well as NSTEMI and STEMI. STEMI patients were eligible only if scheduled for primary PCI. Furthermore, need of dialysis or oral anticoagulation as well as recent significant bleeding were all exclusion criteria in the trial. As ticagrelor was associated with lower mortality, a survival bias in favour of this treatment group may have been present. However, interaction analyses revealed no significant association of randomised treatment with acute phase PTX3 levels concerning clinical outcome.

Conclusions

In a large, contemporary ACS population, admission levels of PTX3 predict adverse clinical outcome including a composite of cardiovascular death, spontaneous MI or stroke, and the individual components of cardiovascular death and spontaneous MI, but not stroke alone, independently of hs-

CRP, Il-6, and cystatin C. These findings suggest that PTX3 may better reflect activation of the inflammatory pathways in ACS than the established prototypical inflammatory marker hs-CRP. But, the predictive value of PTX3 concerning clinical outcome is not sustained in analyses adjusting for markers of myocardial damage and dysfunction, and GDF-15.

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CONFLICTS OF INTEREST

FK: consultancy fees/ honoraria for lectures, advisory board membership, and fee for research work from AstraZeneca; advisory board membership and consultancy fees from Merck & Co, all are considered modest.

TA, TU, AEM, PA: nothing to report.

AÅ: Institutional research grant and speakers fee from AstraZeneca; institutional research grant from Roche Diagnostics, all are considered significant.

TG, MB: Institutional research grants from AstraZeneca, Roche Diagnostics, considered significant.

RCB: Scientific advisory board member for Ionis Pharmaceuticals, AstraZeneca; safety review committee member for Portola and Akcea Therapeutics, all are considered modest.

AH: Reports being an employee of AstraZeneca, considered significant.

SKJ: Institutional research grant, honoraria and consultant/advisory board fee from AstraZeneca, considered significant; institutional research grant and consultant/advisory board fee from Medtronic, considered significant; institutional research grant from Roche Diagnostics, considered significant; institutional research grants and honoraria from The Medicines Company; consultant/advisory board fees from Janssen, Bayer, all are considered modest.

AS: Institutional research grants from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb/Pfizer and GlaxoSmithKline, Olink Proteomics, all are considered significant.

RFS: Institutional research grants, consultancy fees, and honoraria from AstraZeneca, considered significant; Institutional research grants and consultancy fees from PlaqueTec, considered significant; consultancy fees from Bayer, considered significant; consultancy fees from Actelion, Avacta, Bristol-Myers Squibb/Pfizer, Novartis, The Medicines Company and Idorsia, all are considered modest.

LW: Institutional research grants, consultancy fees, lecture fees, and travel support from AstraZeneca, Bristol-Myers Squibb/Pfizer, Boehringer Ingelheim, all are considered significant; institutional research grant, consulting fee, lecture fee, travel support, honoraria from GlaxoSmithKline, considered significant; institutional research grants from Merck & Co, Roche Diagnostics, considered significant; consultancy fees from Abbott, considered modest; holds two patents involving GDF-15 licensed to Roche Diagnostics (EP2047275B1 and US8951742B2).

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FIGURE LEGENDS

Figure 1: Strength of association of acute phase (i.e. admission) PTX3 level with biomarkers included in the multivariable analyses.

Figure 2: Kaplan-Meier estimated event rates of the primary outcome measures per quartile of the acute phase (i.e. admission) PTX3 level.

Figure 3: Restricted cubic spline of the interaction between the acute phase (i.e. admission) level of PTX3 and 12-months estimated event rates of the individual outcome measures in the whole study population.

Figure 4: Dynamic changes of PTX3, hs-CRP, and IL-6 from admission through 6 months follow-up.

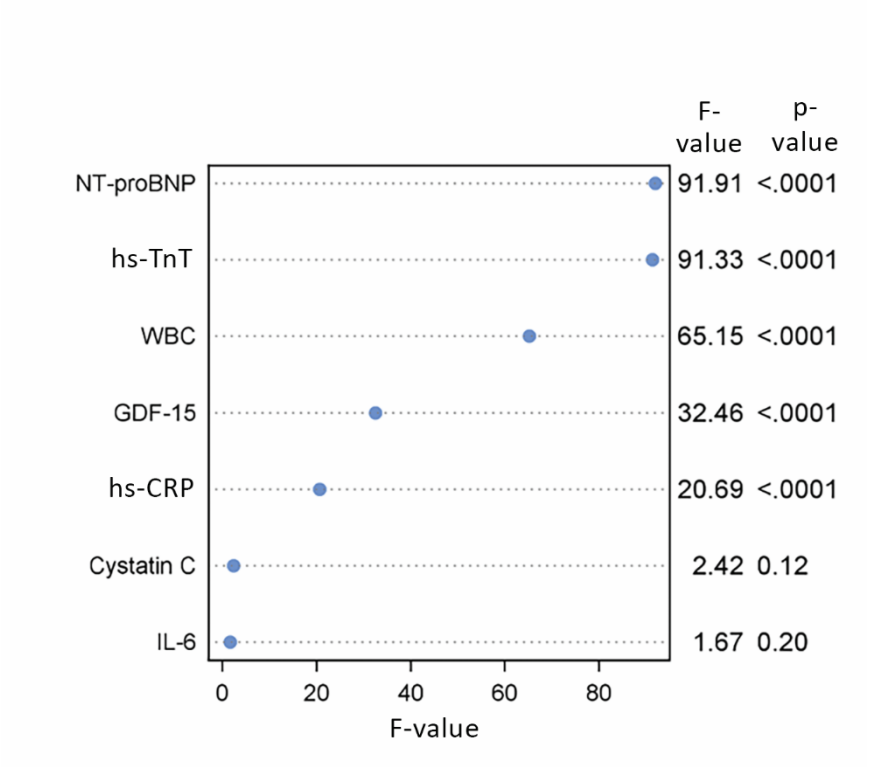
F-value
p-value
NT-proBNP
91.91
<.0001
hs-TnT
91.33
<.0001
WBC
65.15
<.0001
GDF-15
32.46
<.0001
hs-CRP
20.69
<.0001
Cystatin C
2.42
0.12
IL-6
1.67
0.20
0
20
40
60
80
F-value

Fig. 1

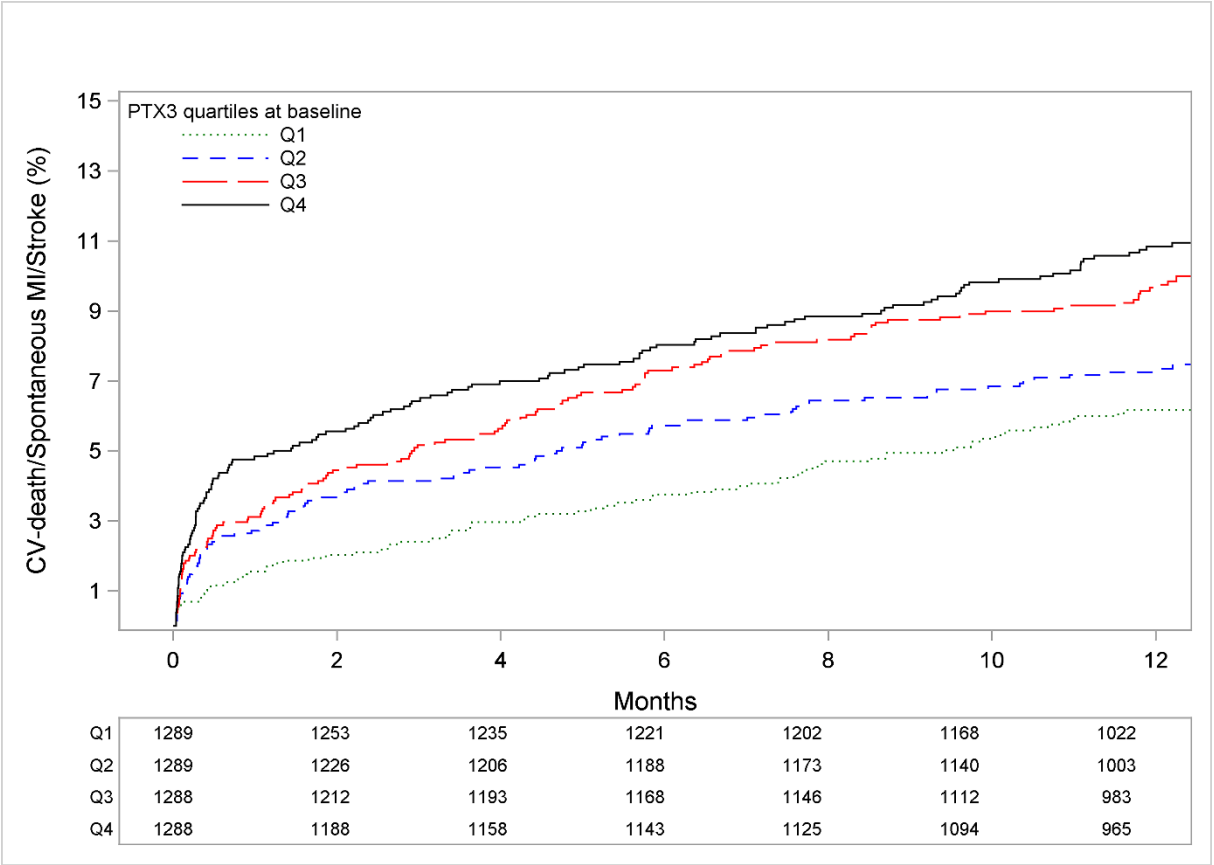
PTX3 quartiles at baseline
Q1
Q2
Q3
Q4
CV-death/Spontaneous MI/Stroke (%)
15
13
11
9
7
5
3
1
0
2
4
6
8
10
12
Months
Q1 1289 1253 1235 1221 1202 1168 1022
Q2 1289 1226 1206 1188 1173 1140 1003
Q3 1288 1212 1193 1168 1146 1112 983
Q4 1288 1188 1158 1143 1125 1094 965

Fig 2

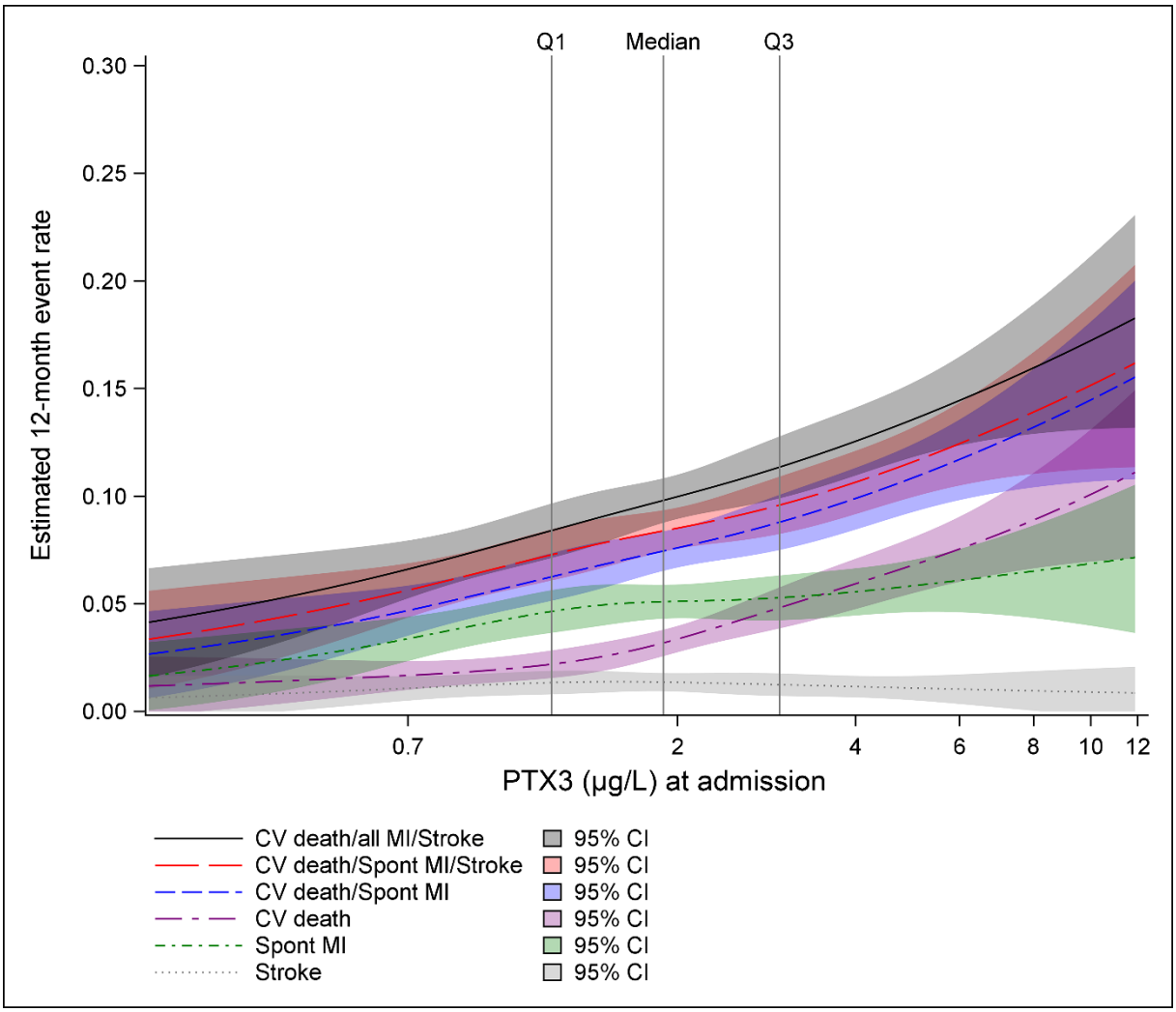
Q1
Median
Q3
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Estimated 12-month event rate
0.7
2
4
6
8
10
12
PTX3 (µg/L) at admission
CV death/all MI/Stroke
95% CI
CV death/Spont MI/Stroke
95% CI
CV death/Spont MI
95% CI
CV death
95% CI
Spont MI
95% CI
Stroke
95% CI

Fig 3

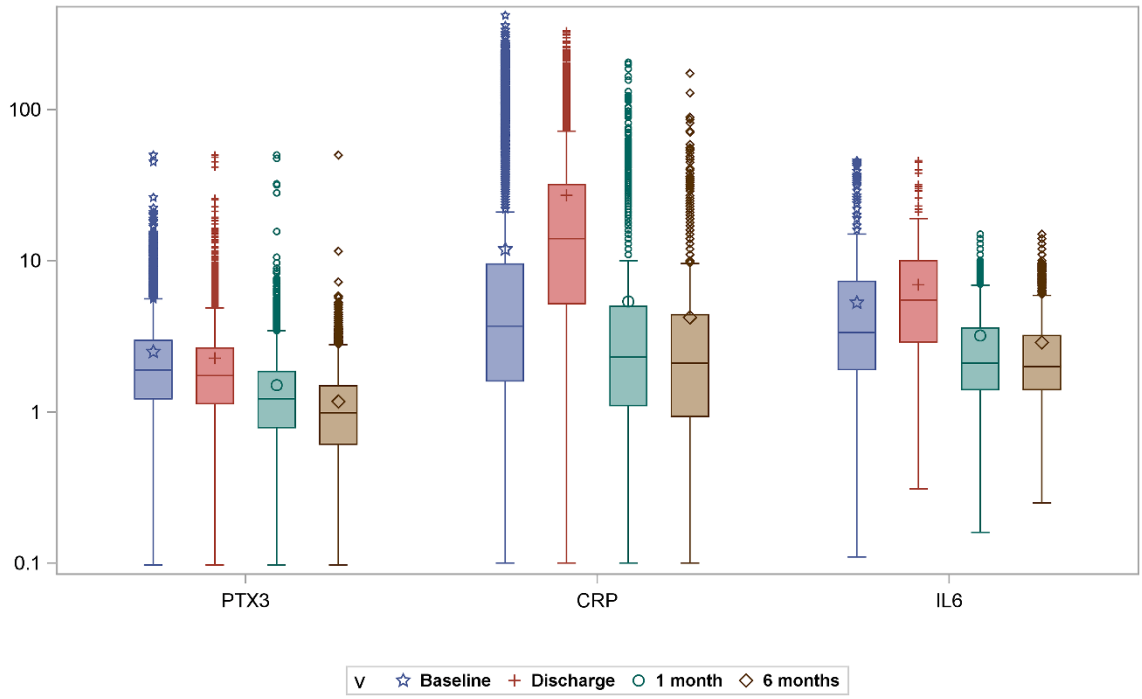

Fig 4