Prognostic Significance of the Long Pentraxin PTX3 in Acute Myocardial Infarction

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Background—Inflammation has a pathogenetic role in acute myocardial infarction (MI). Pentraxin-3 (PTX3), a long pentraxin produced in response to inflammatory stimuli and highly expressed in the heart, was shown to peak in plasma 7 hours after MI. The aim of this study was to assess the prognostic value of PTX3 in MI compared with the best-known and clinically relevant biological markers.

Methods and Results—In 724 patients with MI and ST elevation, PTX3, C-reactive protein (CRP), creatine kinase (CK), troponin T (TnT), and N-terminal pro-brain natriuretic peptide (NT-proBNP) were assayed at entry, a median of 3 hours, and the following morning, a median of 22 hours from symptom onset. With respect to outcome events occurring over 3 months after the index event, median PTX3 values were 7.08 ng/mL in event-free patients, 16.12 ng/mL in patients who died, 9.12 ng/mL in patients with nonfatal heart failure, and 6.88 ng/mL in patients with nonfatal residual ischemia (overall \( P<0.0001 \)). Multivariate analysis including CRP, CK, TnT, and NT-proBNP showed that only age ≥70 years (OR, 2.11; 95% CI, 1.04 to 4.31), Killip class ≥1 at entry (OR, 2.20; 95% CI, 1.14 to 4.25), and PTX3 (≥10.73 ng/mL) (OR, 3.55; 95% CI, 1.43 to 8.83) independently predicted 3-month mortality. Biomarkers predicting the combined end point of death and heart failure in survivors were the highest tertile of PTX3 and of NT-proBNP and a CK ratio >6.

Conclusions—In a representative contemporary sample of patients with MI with ST elevation, the acute-phase protein PTX3 but not the liver-derived short pentraxin CRP or other cardiac biomarkers (NT-proBNP, TnT, CK) predicted 3-month mortality after adjustment for major risk factors and other acute-phase prognostic markers. (Circulation. 2004;110:2349-2354.)

Key Words: C-reactive protein ■ myocardial infarction ■ natriuretic peptide, brain ■ troponin

The importance of circulating markers of cardiac damage in acute myocardial infarction (MI) for early diagnosis and prognostic stratification has expanded from markers of cardiac myocyte necrosis such as creatine kinase1-2 and troponins3-4 to markers of inflammation such as the short pentraxin C-reactive protein (CRP) and serum amyloid A protein.5-7

Pentraxin-3 (PTX3) is a prototypic long pentraxin produced mainly by dendritic cells, macrophages, and endothelial cells in response to primary inflammatory stimuli.8-10 In rodents, after systemic administration of microbial products and inflammatory cytokines or ligation of the left coronary artery to model acute MI, PTX3 is expressed at very high levels in the heart.11 Moreover, PTX3 is present in atherosclerotic lesions and in small-vessel vasculitis in humans,12-13 and it is induced by oxidized LDL in smooth muscle cells.14 PTX3 was shown to peak in plasma 7 hours after the onset of symptoms in patients with MI and to decrease thereafter toward baseline in a few days.15 In the same context, plasma CRP increased, but it peaked much later, between 24 and 48 hours after symptom onset.

Because the short pentraxin CRP is produced mainly by the liver in response to interleukin (IL)-6 and PTX3 by the heart and vasculature in response to primary inflammatory stimuli, we hypothesized that PTX3 could be an acute-phase reactant more closely related than CRP to cardiac injuries such as MI and therefore could be a sensitive and specific prognostic indicator in this context. The present study was designed to
evaluate the prognostic role of PTX3 in a prospective cohort of patients admitted to coronary care unit (CCU) within 12 hours from symptom onset of MI with ST elevation and to compare it to other accepted outcome indicators such as creatine kinase (CK) and troponin T (TnT), markers of myocardial necrosis; N-terminal pro-brain natriuretic peptide (NT-proBNP), a marker of left ventricular dysfunction and dilatation; and CRP, a classic marker of inflammation.

Methods

Patients
The study population was part of a prospective observational study (Lipid Assessment Trial Italian Network [LATIN]) of 1864 patients with acute coronary syndromes with or without ST elevation that was aimed at assessing the time course of plasma cholesterol levels. Patients were eligible for the present analysis if (1) they showed in the 12 hours preceding admission to CCU a typical episode of chest pain lasting >20 minutes, were not responsive to nitrates, and had persistent ST-segment elevation and (2) the maximal value of CK exceeded twice the upper limit of reference range during the first hours after the index event. Patients were excluded if they (1) had been admitted to hospital for any reason in the 2 months preceding the index event, (2) had received current treatment with a lipid-lowering drug, or (3) had inflammatory or infectious diseases within 1 month before the index event.

Ethics committees or institutional review boards in all participating centers approved the study. All patients gave written informed consent.

Outcome Events
Comparative predictivity of PTX3 and of 4 other known biomarkers was assessed with respect to the occurrence of major outcome events over 3 months: all-cause death, episodes of heart failure (HF), cardiac residual ischemia, and the combination of death and HF.

Episode of HF was defined during hospital stay as an episode of symptomatic HF or cardiogenic shock and from discharge to 3 months as the need to be rehospitalized for HF. Residual cardiac ischemia was defined during hospital stay as ECG-documented postinfarction angina, or reinfarction or induced ischemia at the provocative tests. From discharge to 3 months, residual ischemia was defined as reinfarction or the need to be hospitalized in a CCU for angina. The combined end point was obtained by adding the number of deaths and the number of patients alive with an episode of HF.

The cohort included in the analysis was constructed by adding to all patients with at least 1 outcome event over 3 months an equal number of randomly selected patients without the event who were matched by sex and age (±5 years). The study population was made up of 724 patients (362 with and 362 without events) with all 4 biomarkers analyzable.

Laboratory Assays
Blood was drawn after a median time of 3 hours (range, 1 to 12 hours) and 22 hours (range, 8 to 47 hours) from the onset of symptoms in EDTA vacuum containers. Plasma was stored at −80°C except for CK, which was assayed locally in each of the 59 participating CCUs as a routine marker of infarct size. Peak CK concentration was defined as the maximal value over a prespecified sampling scheme: at entry; 3, 6, 9, 12, 15, 18, 21, and 24 hours; and 2, 3, and 4 days after admission to CCU. To correct for between-laboratory difference, peak CK concentrations were expressed as the ratio between the actual measured value and the upper limit of the normal range in each laboratory. PTX3 was assayed with a noncommercial ELISA based on the monoclonal antibody PEN-3. Reference concentration of PTX3 assayed in 100 healthy blood donors was 1.34±0.91 ng/mL (median, 1.13 ng/mL; range, 0.91 to 1.69 ng/mL). CRP was determined by high-sensitivity immunonephelometric method (NLatex CRP Mono, Dade Behring) on the BN II Nephelometer Analyzer (Dade Behring). TnT and NT-proBNP were measured by ECLIA (ElectroChemiluminescence/immunoassay) running on MODULAR PE SWA (Roche Diagnostics, Italy).

Statistical Analysis
Plasma concentrations of biomarkers were divided into tertiles. Main analyses were conducted on the maximal value between the available measurements (at entry and the following morning or at entry only when the outcome event occurred on day 0 to 1).

Differences in proportions were evaluated by the χ² test. Because of the nonnormal distribution of all biomarkers (Kolmogorov-Smirnov test), their values are presented as medians and 25th and 75th percentiles. Continuous variables were compared by nonparametric (Wilcoxon or Kruskal-Wallis) tests.

Multivariate logistic analysis was used to calculate adjusted ORs and 95% CIs for each end point after 3 months in relation to TnT, NT-proBNP, CRP, and PTX3. ORs were calculated for the middle and upper tertiles with the lowest tertile used as reference. All clinical and laboratory variables associated with outcome in univariate analysis at a value of P<0.05, as well as sex and smoking status, were included in the model: age >70 years, treated hypertension, diabetes, Killip class at entry >1, heart rate at entry ≥100 bpm, systolic blood pressure at entry ≤100 mm Hg, anterior MI site, and CK >6-fold the upper limit. The same analysis was performed with CK, TnT, NT-proBNP, CRP, and PTX3 as continuous variables to check for possible bias resulting from the use of tertiles. Age was also entered as a continuous variable to correct for poor matching of age between patients with and patients without events (Table 1). All analyses were performed with SAS software.

Results
Baseline characteristics of the whole population and their distribution by patients with and without 3-month outcome events are reported in Table 1. Even after matching for age, patients with events were older and more frequently had signs or symptoms of heart failure (ie, Killip class at entry >1) and higher heart rate. Treatments initiated within the first day...
after admission to CCU were highly comparable in the 2 subpopulations and indicated an intensive strategy of care: thrombolysis (62.7%), aspirin (88.7%), ACE inhibitors (38.4%), nitrates (79.8%), and primary or rescue percutaneous coronary intervention (4.3%) (Table 1). Patients were discharged after a median hospital stay of 9 days (range, 1 to 38 days). Most of the events occurred in hospital: 59.3% of deaths, 87.0% of episodes of HF, and 60.9% of episodes of residual ischemia.

Maximal concentrations of the 5 biomarkers are reported in Table 2 compared with the levels observed in the 362 event-free patients. Plasma concentrations of all biomarkers were 1.2 to 1.9 times higher in the 54 patients who died and were 1.2 to 1.9 times higher in the 116 who had at least 1 episode of nonfatal HF but did not show any increase in the 192 patients with at least 1 episode of nonfatal residual cardiac ischemia.

The correlations between the concentrations of PTX3 and the other 3 markers, CRP, NT-proBNP, and TnT, were statistically significant but substantially weak (r from 0.15 to 0.33).

Multivariate logistic analysis showed PTX3 >10.73 ng/mL (eg, the lower limit of the upper tertile) to be the strongest independent predictor of all-cause 3-month mortality (OR, 3.55; 95% CI, 1.43 to 8.83 versus the lower tertile, PTX3 <5.50 ng/mL). No other biomarker independently predicted 3-month mortality (Figure). When mortality was combined with nonfatal HF, PTX3 was still a significant predictor (Figure).

After Killip >1 at entry and CK >6-fold the upper limit, high NT-proBNP appeared to be the strongest independent predictor of nonfatal HF (Figure) and consequently of the occurrence of the combined end point of death or HF (Figure). Increased risk of residual ischemia in survivors was predicted by the intermediate tertile of NT-proBNP but not by the upper tertile (Figure). Unexpectedly, the upper tertile of TnT predicted a lower risk for residual ischemia. Other variables significantly associated with increased risk of death were, as expected, age >70 years (OR, 2.11; 95% CI, 1.04 to 4.31) and Killip >1 at entry (OR, 2.20; 95% CI, 1.14 to 4.25). When concentrations at entry of TnT, NT-proBNP, CRP, and PTX3 were included in the logistic multivariate analysis,
PTX3 still was a significant \((P=0.0016)\) independent predictor of 3-month all-cause mortality (OR, 1.023; 95% CI, 1.009 to 1.038). The use of CK ratio, TnT, NT-proBNP, CRP, and PTX3 concentrations as continuous variables confirmed in all cases their independent relations with outcome events, found by comparing risks of highest versus lowest tertile (Figure). In fact, PTX3 was the only biomarker as a continuous variable that significantly \((P<0.0001)\) predicted death (OR, 1.023; 95% CI, 1.012 to 1.035). When age also was entered as a continuous variable, the OR for continuous PTX3 was 1.022 (95% CI, 1.011 to 1.033), which still is statistically significant \((P<0.0001)\).

**Discussion**

Compared with other biomarkers considered reliable predictors of mortality in patients with MI, PTX3 has been shown to be an earlier\(^{15}\) and stronger prognostic marker of death in a cohort of patients with MI prospectively followed up for 3 months. In particular, in the present study, patients with PTX3 the first day after symptom onset in the upper tertile \((>10.73 \text{ ng/mL})\) had a greater than 3-fold increase in risk of dying within 3 months than patients with PTX3 \(\leq 5.49 \text{ ng/mL}\) (the lower tertile). The risk of patients in the middle tertile was \(\approx 1.5\). When the maximal concentration of PTX3 was used with all other biomarkers as continuous variables, the risk of dying after MI increased by 2.3\% with every 1-ng/mL increase in PTX3. The statistically robust significance of the findings is corroborated by the consistency with the biological background of this long pentraxin with respect to the other markers.

Microbial products and inflammatory cytokines rapidly induce high levels of PTX3 expression in the heart, most prominently in heart endothelial cells.\(^{11}\) PTX3 is rapidly induced in mouse and rat models of MI and is present in atherosclerotic lesions.\(^{12}\) In vitro, this prototypic long pentraxin is induced in endothelial cells, smooth muscle cells, and mononuclear phagocytes by primary inflammatory cytokines (IL-1, tumor necrosis factor), oxidized LDL,\(^{14}\) and microbial products.\(^{9,11,22}\) Moreover, PTX3 induction is rapid, consistent with its discovery as an immediate to early gene.\(^{8,9}\)

Therefore, although the short pentraxin CRP is produced by the liver and represents a systemic response to local inflammation, the long pentraxin PTX3 is rapidly induced in damaged tissues and may more directly reflect the tissue inflammatory response, particularly the involvement of the vascular bed.

PTX3 is a multifunctional protein with in vivo roles that only now are beginning to be unraveled.\(^{8,10}\) PTX3 binds apoptotic cells\(^{23}\) and is present in areas of damaged myocardium (Vago et al, unpublished data). PTX3 binds C1q and activates the classic pathway of complement activation.\(^{24,25}\) Therefore, as suggested for CRP,\(^{26–29}\) locally produced PTX3 may cause and amplify tissue damage. Moreover, amplification of endothelial cell procoagulant activity by PTX3 has been demonstrated in vitro.\(^{30}\) Finally, recent results indicate that PTX3 binds and inactivates fibroblast growth factor-2.\(^{31}\)

Therefore, amplification of damage and inhibition of angiogenesis and repair may underlie the association between high levels of PTX3 and death from MI, even if the actual mechanisms responsible for the specificity of PTX3 in predicting all-cause death (90\% occurring as expected within the first 7 days after MI) cannot be explained easily.

Individually, all biomarkers considered here, except PTX3, which has not been tested before, were found to predict morbidity and mortality after MI.\(^{16,32–34}\) CRP has been shown to predict, with variable strength, outcome in patients after acute cardiovascular events and in apparently healthy individuals.\(^{34–36}\) CRP is produced by the liver in response to IL-6 and is a classic marker of inflammation. In addition, CRP may also be involved in the pathogenesis of lesions by favoring thrombosis and plaque rupture in affected vessels.\(^{19,20,37,38}\) TnT is a more sensitive and specific marker of myocyte necrosis than CK. In fact, TnT adds to the prognostic information given by usual clinical risk factors and CK alone in acute coronary syndromes.\(^{4}\) NT-proBNP and BNP are considered reliable predictors of outcome not only in heart failure but also in acute coronary syndromes.\(^{17,30}\) Natriuretic peptides in the first day after MI may reflect not only the severity of ischemic injury but also left ventricular dysfunction acutely ensuing from MI. All these biomarkers have been shown to provide complementary information in risk stratification of patients with acute coronary syndromes,\(^{40}\) consistent with the pathophysiology of the disease. Although not fully defined, the biological profile of PTX3\(^{30,11}\) further adds to the strength of considering this molecule a significant advancement in the knowledge of risk stratification in MI.

The characteristics of the population being studied provide support for the findings. Patients included in the analysis belong to a prospective cohort carefully diagnosed and followed up in the framework of a project designed to explore other issues but foresaw exclusion criteria that appear to be protective also for the new hypothesis about the role of PTX3, which was clearly defined a priori, with biochemical analyses performed in conditions of complete blindness with respect to the study protocol and sample origins.

The only puzzling finding of the study, which has little to do with its main focus, is that no single marker reliably predicted nonfatal residual ischemia, defined as post-MI residual angina, induced ischemia, or reinfarction. As expected, post-MI angina represented the vast majority \((\approx 80\%)\) of all cases of residual ischemia. The observed inverse relationship between TnT levels and residual ischemia occurrence might be explained by the fact that higher TnT levels are generally considered a marker of a more extensive cardiac damage; thus, less myocardial tissue is exposed to the risk of new ischemic events.

In conclusion, the contribution of PTX3 to the understanding and possible management of the clinical course of MI has been documented on top of and independently from the best-documented biomarkers of the acute phase. The early rise of PTX3 within 12 hours from onset of symptoms of MI confers to this molecule an extra potential advantage over other markers such as CRP, peaking later.\(^{15}\) The clinical usefulness of the findings can now be tested, with appropriate experimental designs, in contexts in which different intervention strategies could be dictated by earlier knowledge of reliable prognostic information.
Appendix

LATIN Study
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Application for the use of PTX3 as a diagnostic, as affiliates of the
University of Milan, Italy. Drs Latini and Peri have filed a patent
application for the use of PTX3 as a diagnostic, as an affiliate of ANMCO Research Center of Heart Care
Dr Maggioni has filed a patent application for the use of PTX3 as a diagnostic, as an affiliate of the
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Acknowledgments
This work was supported by the European Commission, MIUR, and
FIRB. Roche Diagnostics donated reagents for assays of TnI and
NT-proBNP. Giampietro Orsini and Marco Gorini were entirely
responsible for data management. Renato Urso helped in statistics.
FIRB. Roche Diagnostics donated reagents for assays of TnT and
molecular serum amyloid P component.

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Circulation. 2004;110:2349-2354; originally published online October 11, 2004; doi: 10.1161/01.CIR.0000145167.30987.2E

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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