RESEARCH ARTICLE

Pentraxin-3 concentrations in stable coronary artery disease depend on the clinical presentation

Bartosz Hudzik[1,a], Aleksander Danikiewicz[2,a], Janusz Szkodzinski[1], Lech Polonski[1], Barbara Zubelewicz-Szkodzinska[2,3]

[1] Third Department of Cardiology, Silesian Center for Heart Disease, Medical University of Silesia, Zabrze, Poland
[2] Department of Nutrition-Associated Disease Prevention, Faculty of Public Health, Medical University of Silesia, Bytom, Poland
[3] Division of Endocrinology, County Hospital, Piekary Slaskie, Poland

Correspondence: Bartosz Hudzik, MD PhD. 3rd Department of Cardiology, Silesian Center for Heart Disease, Curie-Sklodowska 9, 41-800 Zabrze, Poland
<bartekh@mp.pl>

ABSTRACT. *Introduction:* **Pentraxin-3 (PTX3) is an acute-phase reactant that shares structural and functional homology with C-reactive protein (CRP). However, unlike CRP, which is synthesized mainly in the liver, PTX3 is produced at the site of inflammation. It has been suggested that PTX3 plays the same role in the periphery that CRP does in circulation. PTX3 may represent a rapid marker of local inflammation.** *Methods:* **Fifty-one patients with stable coronary artery disease (CAD) were enrolled. Blood samples were collected on admission. Plasma concentration of PTX3 and high-sensitivity CRP (hsCRP) were determined.** *Results:* **Median PTX3 concentration was 0.92 µmol/L (0.58–1.40). Median hsCRP concentration was 0.90 mg/L (0.75–1.10). There was a positive correlation between PTX3 and total cholesterol ($R = 0.34$; $P = 0.01$), PTX3 and LDL cholesterol ($R = 0.35$; $P = 0.01$), and PTX3 and hsCRP ($R = 0.46$; $P = 0.0005$). We found no correlation between hsCRP and all laboratory parameters. We found higher PTX3 concentrations in patients with Canadian Cardiovascular Society (CCS) functional class 3 (compared to CCS functional class 2) and in patients taking nitrates. Lower PTX3 concentrations were reported in patients taking calcium channel blockers (amlodipine). hsCRP concentrations remained similar among these subgroups of patients.** *Conclusions:* **PTX3 is a marker of clinically more advanced CAD (CCS2 vs CCS3; nitrates vs no nitrates). PTX3 is also associated with other cardiovascular risk factors (total cholesterol, LDL cholesterol, and hsCRP). PTX3 may be a potential early marker of cardiovascular risk before the increase of systemic markers like hsCRP.**

Key words: pentraxin-3, C-reactive protein, atherosclerosis, coronary artery disease, inflammation

Coronary artery disease (CAD) is closely associated with the inflammatory process ongoing within arterial walls, which leads to a cascade of events beginning with endothelial damage, through the formation of the atherosclerotic plaque and coronary artery stenosis, ending with coronary artery occlusion (clinically presenting as an acute coronary syndrome) [1]. Persisted inflammation in the coronary arteries is mediated by various ligands: C-reactive protein (CRP), cytokines, chemokines, growth factors, and adhesion molecules [2, 3]. CRP is a marker of inflammation widely used in everyday clinical practice [3, 4]. Moreover, it is of good prognostic value in assessing future adverse events. It belongs to the family of short pentraxins (PTXs). PTX3 is an acute-phase reactant that shares structural and functional homology with CRP. However, unlike CRP, which is synthesized mainly in the liver, PTX-3 is produced at the site of inflammation. It has been suggested that PTX-3 plays the same role in the periphery that CRP does in circulation. PTX-3 is secreted by endothelial cells,

smooth muscle cells, macrophages, monocytes and fibroblasts [5, 6]. Therefore, PTX-3 concentration reflects local rather than systemic inflammatory response to arterial wall injury and that is why it is possible to treat it as a more sensitive marker of inflammation within arterial wall [7]. Meanwhile, there are few reports concerning PTX-3 role in endothelial dysfunction in patients with stable CAD.

The aim of the study was to estimate inflammatory response measured by PTX-3 concentration in patients with stable CAD and its association with clinical manifestation of the disease.

PATIENTS AND METHODS

Patients with stable CAD [Canadian Cardiovascular Society (CCS) functional class 2 or 3] were enrolled. The study conforms to the Declaration of Helsinki. Exclusion criteria were previous PCI, previous coronary artery bypass graft surgery, coexisting autoimmune disorders, acute infectious disease, chronic inflammatory disease, chronic kidney disease-stage 3 or higher (baseline estimated glomerular

---

[a]Equal contribution to the paper.

filtration rate <60 mL/min/1.73 m^2), known malignant diseases, decompensated diabetes mellitus, hepatitis (including viral hepatitis, cholestatic jaundice with bilirubin concentration >1.5 mg/dL and/or alkaline phosphatase at least twice the upper limit of normal), severe trauma or burn during 12 months before admission, ischemic or hemorrhagic stroke during 12 months before admission, glucocorticoids and/or androgens therapy, psychiatric disorders. Fifty-one patients entered the study and were divided into two groups according to the clinical presentation of the disease: group 1 – patients in functional CCS class 2 and group 2 – patients in functional CCS class 3.

Venous blood samples were collected on admission separated by centrifugation at 4 °C, 1800 × g for 15 minutes. Samples were then stored at -80 °C until further analysis. Plasma PTX3 measurements were done using commercial kit enzyme-linked immunosorbent assays (ELISA) (R&D Systems, USA) in duplicates. Measurements for each patient were made with the same kit to avoid inter-kit variability. For the PTX3 measurements, the intra-assay coefficient of variation (%CV) was 3.2%, the inter-assay coefficient of variation (%CV) was 5.8%, and the sensitivity of the ELISA assay was 0.007-0.116 ng/mL.

Plasma hsCRP was determined with a high-sensitivity, automated, microparticle-enhanced latex turbidimetric immunoassay (COBAS Integra; Roche Diagnostics, Indianapolis, IN). The lower limit of detection was 0.2 mg/L, with an intra-assay and interassay coefficients of variation (%CV) were <5%.

Statistical analysis

Quantitative data are presented as medians and interquartile ranges (lower and upper quartiles). Qualitative data are presented as frequencies. The Shapiro-Wilk test was used to determine whether a random sample was normally-distributed. Mann-Whitney U-test was used to evaluate differences between the two groups. The Chi-square test with Yates' correction and Fisher's exact test were used to compare categorical variables. The relationship between hsCRP and PTX3 was evaluated by Spearman's rank correlation coefficient.

RESULTS

Baseline clinical characteristics are presented in *table 1*. Demographics were similar in both groups. The prevalence of hypertension, diabetes mellitus, and hyperlipidemia was comparable. Patients in functional CCS class 3 more often received nitrates. Laboratory findings are shown in *table 2*. There was a positive correlation between PTX3 and total cholesterol (R = 0.34 ; P = 0.01), PTX3 and LDL cholesterol (R = 0.35; P = 0.01), and PTX3 and hsCRP (R = 0.46; P = 0.0005). There was no correlation between hsCRP and all laboratory parameters. We observed higher PTX3 concentrations in patients with CCS functional class 3 (figure 1) and in patients taking nitrates [1.2 ng/mL (1.0-1.3) vs 0.9 ng/mL (0.6-1.0) P = 0.02]. hsCRP concentration was similar in both groups (*figure 2*). Lower PTX3 concentrations were reported in patients taking calcium channel blockers (amlodipine) [0.8 ng/mL (0.6-0.95) vs 1.15 ng/mL (0.98-1.24) P = 0.04]. hsCRP concentrations remained similar among these subgroups of patients [0.9 mg/L (0.8-1.0) vs 0.9 (0.7-1.0) P = 0.9 for nitrates and no nitrates respectively; and 0.8 mg/L (0.66-0.9) vs 0.9 mg/L (0.7-1.0) P = 0.8 for amlodipine and no amlodipine respectively]. Neither PTX3 nor hsCRP correlated with the duration of CAD (R = 0.2; P = 0.5 and R = 0.25; P = 0.7 respectively).

DISCUSSION

A strong relationship between inflammation and atherosclerosis has been reported in the literature. Prior studies have noted the importance of inflammatory

Table 1
Baseline clinical characteristics. Values are presented as medians (interquartile range) or N (percent).

	Group 1 (CCS 2) N = 31	Group 2 (CCS 3) N = 20	p
Age, years	62 (58-71)	62 (59-65)	0.8
Gender, males	20 (64.5)	12 (60.0)	0.6
Systemic hypertension	23 (74.1)	15 (75.0)	0.8
Hyperlipidemia	23 (74.1)	16 (80.0)	0.4
Diabetes mellitus	14 (45.1)	12 (60.0)	0.1
Smoking	12 (38.7)	8 (40.0)	0.8
BMI	25 (22-29)	28 (23-31)	0.4
LVEF (%)	45 (40-53)	45 (39-54)	0.3
Aspirin n (%)	31 (100)	20 (100)	1.0
Beta-blockers n (%)	30 (96.8)	20 (100)	0.6
ACE inhibitors n (%)	23 (74.2)	15 (75.0)	0.8
Statins n (%)	26 (83.9)	17 (85.0)	0.9
Nitrates n (%)	1 (3.2)	4 (20.0)	0.045
Calcium channel blockers n (%)	3 (9.7)	3 (15.0)	0.5

CAD – coronary artery disease, CCS – Canadian Cardiovascular Society, LAD – left anterior descending artery, Cx – circumflex artery, RCA – right coronary artery, BMI – body-mass index, LVEF – left ventricular ejection fraction, ACE – angiotensin converting enzyme

Table 2
Laboratory findings. Values are presented as medians (interquartile range).

|	Group 1 (CCS 2) N = 33	Group 2 (CCS 3) N = 20	P
Leucocytes (10^3/mm^3)	7.4 (6.5-8.5)	6.9 (5.8-8.1)	0.2
Erythrocytes (10^6/mm^3)	4.4 (4.2-4.7)	4.6 (4.2-5.0)	0.1
Hemoglobin (mmol/L)	8.4 (8.1-9.0)	8.7 (8.0-9.2)	0.3
Hematocrit (%)	41 (38-43)	41 (40-45)	0.6
Platelets (10^3/mm^3)	236 (205-263)	214 (186-292)	0.8
Total cholesterol (mmol/L)	4.8 (4.0-5.0)	4.7 (4.3-5.3)	0.6
HDL cholesterol (mmol/L)	1.1 (1.0-1.3)	1.0 (0.8-1.3)	0.2
LDL cholesterol (mmol/L)	2.9 (2.2-3.2)	2.9 (2.3-3.4)	0.8
Triglycerides (mmol/L)	1.3 (1.1-1.9)	1.6 (1.2-2.2)	0.1
Serum creatinine (μmol/L)	79 (70-96)	84 (64-94)	0.3
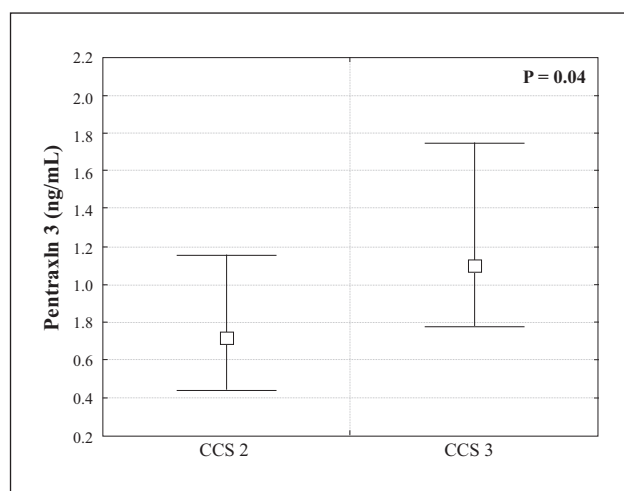
Figure 1
Median concentration (with interquartile range) of pentraxin-3 in the study groups.
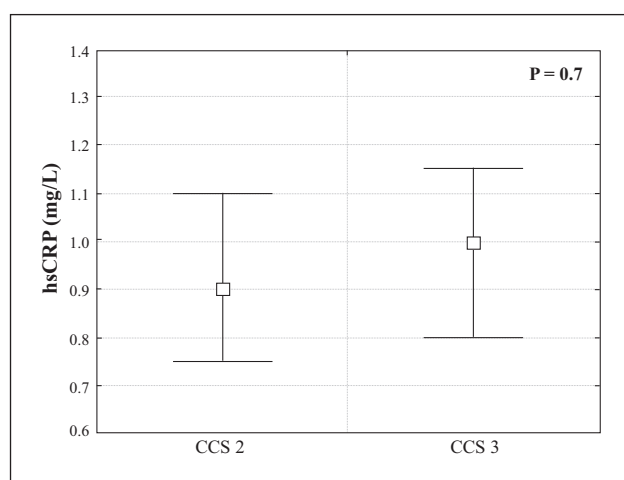

Figure 2
Median concentration (with interquartile range) of high-sensitivity C-reactive protein in the study groups.

markers such as hsCRP and interleukin-6. We investigated inflammatory response measured by PTX-3 concentration and its association with clinical manifestation in a population of patients with stable CAD. There are three key findings of our study. First, PTX3 was a marker of clinically more advanced CAD as defined by the functional CCS class and nitrate use. Second, PTX3 was associated with other cardiovascular risk factors (total cholesterol, LDL cholesterol, and hsCRP). Third, hsCRP concentrations were similar in patients in functional CCS class 2 and 3, thus undermining its significance in stable CAD.

In recent years, there has been an increasing interest in PTX3. Basic research studies have indicated that it is produced abundantly by various cells in atherosclerotic lesions and within vascular walls, including monocytes and macrophages, endothelial cells, vascular smooth muscle cells, fibroblasts, dendritic cells, and adipocytes, while CRP is mainly produced in the liver [8]. These findings suggest that plasma PTX3 concentration reflects local inflammation at the site of the atherosclerotic lesion itself [9]. Studies have shown elevated PTX3 expression in neutrophils and macrophages within advanced atherosclerotic plaques [10, 11]. In contrast to the latter, PTX3 levels were not associated with early stage atherosclerosis [12, 13].

Clinical studies demonstrated that patients with acute myocardial infarction and unstable angina had elevated plasma PTX3 concentrations [14, 15], thus supporting the previously suggested notion that plasma PTX3 concentrations reflect coronary plaque vulnerability. In stable settings, Haybar *et al.* reported PTX3 to confer diagnostic value for angiographically-detected CAD, independent of and in addition to traditional CAD risk factors [16]. Risk classification improved in intermediate-risk individuals, mainly through the identification of those unlikely to have CAD. Dubin *et al.* evaluated the associations of baseline PTX3 levels with all-cause mortality, cardiovascular (CV) events (myocardial infarction, stroke, or CAD death), and incident heart failure (HF) during 37 months among ambulatory persons with stable CHD participating in the Heart and Soul Study [17]. Among patients with stable CAD, higher PTX3 concentrations were associated with increased risk for all cause mortality, CV events, and incident HF independently of systemic inflammation.

Elevated plasma PTX3 concentrations has been also reported in heart failure [14, 18], following stent implantation [19, 20], and has been linked to an increased risk of restenosis [19]. PTX3 concentrations increased with

the number of vascular beds involved in the atherosclerotic process [21]. Preliminary data suggest PTX3 to be superior to hsCRP in predicting prevalent cardiovascular disease [20-22].

The current study found that PTX3 was a marker of clinically more advanced CAD (functional CCS class, nitrate use). Moreover, PTX3 was also associated with other cardiovascular risk factors (total cholesterol, LDL cholesterol, and hsCRP). Hence, the results of our study agree with those of Haybar *et al.* and Dubin *et al.* [16, 17].

Future perspective

The pathogenetic role of PTX3 in cardiovascular disease remains, however, unclear. In contrast to the aforementioned studies, some researchers speculate that PTX3 may exert cardioprotective effect. In light of the findings indicating PTX3 deficiency to be associated with increased heart damage after myocardial infarction [23], increased PTX3 concentrations have been linked to a protective physiological response that is correlated with the severity of disease [24]. Recent observations support the possibility that PTX3 may act as a molecule at the crossroads between pro-inflammatory and anti-inflammatory stimuli, perhaps balancing the overactivation of a proinflammatory, proatherogenic cascade [23, 25]. Therefore, the increased levels of PTX3 in cardiovascular disease could reflect a protective physiological response. Interestingly, PTX3 is modulated both by proinflammatory proatherogenic molecules [5] and anti-inflammatory atheroprotective [23, 24, 26] molecules.

These findings would suggest that PTX3 could be either an innocent bystander resulting from and witnessing the action of pro-inflammatory cytokines in damaged tissues or a molecule directly involved in the extent and outcome of the inflammatory response [7, 16]. The exact role of long pentraxins, with PTX3 being their foremost representative, is yet to be determined.

Study limitations

The study findings should be interpreted in light of their limitations. The small number of patients in our cohort could have rendered some differences insignificant. Blood samples were taken from venous blood, which do not accurately assess the cardiac PTX3 concentrations in comparison to the measurements performed in the coronary sinus, which drains blood from the coronary arteries and the myocardium. The patients did not underwent coronary angiography, hence we do not really know the extent of coronary artery disease. And lastly, the sample patients came from a major academic cardiology center. Whether our results could be extrapolated to the primary care settings needs to be further evaluated.

REFERENCES

1. Drakopoulou M, Toutouzas K, Michelongona A, Tousoulis D, Stefanadis C. Vulnerable plaque and inflammation: potential clinical strategies. *Curr Pharm Des* 2011; 17: 4190.

2. Libby P. Inflammation in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2012; 32: 2045.

3. Libby P, Ridker PM. Inflammation and atherosclerosis: role of C-reactive protein in risk assessment. *Am J Med* 2004; 116(Suppl 6A): 9S.

4. Nakou ES, Liberopoulos EN, Milionis HJ, Elisaf MS. The role of C-reactive protein in atherosclerotic cardiovascular disease: an overview. *Curr Vasc Pharmacol* 2008; 6: 258.

5. Mantovani A, Garlanda C, Bottazzi B, *et al.* The long pentraxin PTX3 in vascular pathology. *Vascul Pharmacol* 2006; 45: 326.

6. Abderrahim-Ferkoune A, Bezy O, Chiellini C, *et al.* Characterization of the long pentraxin PTX3 as a TNFalpha-induced secreted protein of adipose cells. *J Lipid Res* 2003; 44: 994.

7. Norata GD, Garlanda C, Catapano AL. The long pentraxin PTX3: a modulator of the immunoinflammatory response in atherosclerosis and cardiovascular diseases. *Trends Cardiovasc Med* 2010; 20: 35.

8. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol* 2005; 23: 337.

9. Soeki T, Niki T, Kusunose K, *et al.* Elevated concentrations of pentraxin 3 are associated with coronary plaque vulnerability. *J Cardiol* 2011; 58: 151.

10. Rolph MS, Zimmer S, Bottazzi B, Garlanda C, Mantovani A, Hansson GK. Production of the long pentraxin PTX3 in advanced atherosclerotic plaques. *Arterioscler Thromb Vasc Biol* 2002; 22: e10.

11. Savchenko A, Imamura M, Ohashi R, *et al.* Expression of pentraxin 3 (PTX3) in human atherosclerotic lesions. *J Pathol* 2008; 215: 48.

12. Knoflach M, Kiechl S, Kind M, *et al.* Cardiovascular risk factors and atherosclerosis in young males: ARMY study (Atherosclerosis Risk-Factors in Male Youngsters). *Circulation* 2003; 108: 1064.

13. Knoflach M, Kiechl S, Penz D, *et al.* Cardiovascular risk factors and atherosclerosis in young women: atherosclerosis risk factors in female youngsters (ARFY study). *Stroke* 2009; 40: 1063.

14. Peri G, Introna M, Corradi D, *et al.* PTX3, A prototypical long pentraxin, is an early indicator of acute myocardial infarction in humans. *Circulation* 2000; 102: 636.

15. Inoue K, Sugiyama A, Reid PC, *et al.* Establishment of a high sensitivity plasma assay for human pentraxin3 as a marker for unstable angina pectoris. *Arterioscler Thromb Vasc Biol* 2007; 27: 161.

16. Haybar H, Assareh A, Ghotbi Y, Torabizadeh M, Bozorgmanesh M. Incremental diagnostic value of circulating pentraxin in patients with intermediate risk of coronary artery disease. *Heart* 2013; 99: 640.

17. Dubin R, Li Y, Ix JH, Shlipak MG, Whooley M, Peralta CA. Associations of pentraxin-3 with cardiovascular events, incident heart failure, and mortality among persons with coronary heart disease: data from the Heart and Soul Study. *Am Heart J* 2012; 163: 274.

18. Kotooka N, Inoue T, Aoki S, Anan M, Komoda H, Node K. Prognostic value of pentraxin 3 in patients with chronic heart failure. *Int J Cardiol* 2008; 130: 19.

19. Kotooka N, Inoue T, Fujimatsu D, *et al.* Pentraxin3 is a novel marker for stent-induced inflammation and neointimal thickening. *Atherosclerosis* 2008; 197: 368.

20. Hudzik B, Szkodzinski J, Pietka-Rzycka A, *et al.* Plasma pentraxin 3 may be a more sensitive marker of inflammatory response than high-sensitivity C-reactive protein after bare-metal stent compared

to drug-eluting stent implantation. *J Interferon Cytokine Res* 2013; 33: 280.

21. Knoflach M, Kiechl S, Mantovani A, *et al*. Pentraxin-3 as a marker of advanced atherosclerosis results from the Bruneck, ARMY and ARFY Studies. *PLoS One* 2012; 7: e31474.

22. Matsui S, Ishii J, Kitagawa F, *et al*. Pentraxin 3 in unstable angina and non-ST-segment elevation myocardial infarction. *Atherosclerosis* 2010; 210: 220.

23. Norata GD, Marchesi P, Pulakazhi Venu VK, *et al*. Deficiency of the long pentraxin PTX3 promotes vascular inflammation and atherosclerosis. *Circulation* 2009; 120: 699.

24. Norata GD, Marchesi P, Pirillo A, *et al*. Long pentraxin 3, a key component of innate immunity, is modulated by high-density lipoproteins in endothelial cells. *Arterioscler Thromb Vasc Biol* 2008; 28: 925.

25. Salio M, Chimenti S, De Angelis N, *et al*. Cardioprotective function of the long pentraxin PTX3 in acute myocardial infarction. *Circulation* 2008; 117: 1055.

26. Doni A, Michela M, Bottazzi B, *et al*. Regulation of PTX3, a key component of humoral innate immunity in human dendritic cells: stimulation by IL-10 and inhibition by IFN-gamma. *J Leukoc Biol* 2006; 79: 797.