Comparative diagnostic accuracy of serum levels of neutrophil activating peptide-2 and pentraxin-3 versus troponin-I in acute coronary syndrome

Akut koroner sendromda troponin-I ile nötrofil-etkinleştirici peptit-2 ve pentraksin-3'ün serum düzeylerinin karşılaştırmalı tanısal değerleri

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ABSTRACT

Objective: We measured the levels of neutrophil activating peptide-2 (NAP-2) and pentraxin-3 (PTX-3) in acute coronary syndromes (ACS) patients and compared their diagnostic accuracy with cardiac troponin I (cTnI).

Methods: We conducted a prospective cohort study to determine the diagnostic accuracy of PTX-3, NAP-2 and cTnI for the prediction of ACS. Consecutively eighty-three patients with sudden chest pain admitted to Dicle University Emergency Department within the first six hours of symptom onset were included in our study. Mean serum levels of PTX-3, NAP-2 and cTnI were compared between control and patient groups and ACS subgroups. Their sensitivities and specificities in early diagnosis of ACS were identified. Receiver operating characteristic (ROC) analysis was used to assess the diagnostic validity of the markers, and areas under the ROC curve (AUC) were compared.

Results: In the patient group, mean serum concentrations of NAP-2 (53.03+22.77 ng/ml) and PTX-3 (1.73+0.82 ng/ml) were considerably higher than those of the control group (24.54+9.50 and 0.50+0.39 ng/ml, respectively) ($p<0.01$). When compared with the control group, PTX-3 levels of all three ACS subtypes (unstable angina pectoris (USAP) - 1.62+0.41 ng/ml, non-ST elevation myocardial infarction (NSTEMI) -1.63+0.31 ng/ml and ST-elevation myocardial infarction (STEMI) - 1.75+0.89 ng/ml) were higher, whereas NAP-2 levels were higher in USAP (56.29+22.60 ng/ml) and STEMI (52.05+20.99 ng/ml) patients ($p<0.01$). For diagnosing ACS within the first six hours of presentation, PTX-3 sensitivity was 98.5% and specificity was 92.3%, and NAP-2 sensitivity - 98.1% and specificity - 41.3%. The ROC curve AUC values were: 0.962 for PTX-3 (95% CI 0.802 - 1.073), 0.840 for NAP-2 (95% CI 0.684 - 0.991), and 0.683 for cTnI (95% CI 0.610 - 0.940).

Conclusion: Pentraxin-3 is a sensitive and specific marker for ACS diagnosis when compared with cardiac markers in patients admitted to the emergency department (ED) within the first six hours of onset of chest pain. *(Anadolu Kardiyol Derg 2011; 11: 588-94)*

Key words: Neutrophil activating peptide-2, pentraxin-3, acute coronary syndromes, emergency department, sensitivity, specificity, diagnostic accuracy

ÖZET

Amaç: Akut koroner sendromlu hastalarda serum nötrofil aktive peptit 2 (NAP2) ve pentraksin 3 (PTX3) düzeylerini araştırmak ve bunların tanısal doğruluğu kardiyak troponin I (cTnI) ile karşılaştırılması amaçlandı.

Yöntemler: Dicle Üniversitesi Tıp Fakültesi, Acil Servisine ani başlayan göğüs ağrısı şikayetiyle, ağrının başlangıcından itibaren ilk 6 saatte başvuran ardışık 83 hasta çalışmaya alındı. Akut koroner sendrom (AKS) tanısı alanlar hasta grubu (n=70), kalp dışı bir nedene bağlı göğüs ağrısı tanısı alanlar kontrol grubu (n=13) olarak belirlendi. Hasta grubu da kendi arasında kararsız anjina pektoris (n=9), ST elevasyonu olmayan miyokart enfarktüsü (n=5) ve ST elevasyonu olan miyokart enfarktüsü (n=56) grubu olarak 3 gruba ayrıldı. Serum PTX3, NAP2 ve cTnI düzeylerinin ortalaması kontrol grubu ile hasta grubu ve hasta grubunun subtipleri arasında karşılaştırıldı. Markırların tanısal doğruluğunu belirlemek için ROC analizi kullanıldı ve eğri altında kalan alanlar (EAA) karşılaştırıldı.. NAP2 ve PTX3'ün AKS'deki duyarlılık ve özgüllük değerleri belirlendi.

Bulgular: Hasta grubunda NAP-2 (53.03+22.77 ng/ml) ve PTX-3 (1.73+0.82 ng/ml) ortalama serum konsantrasyonları kontrol grubuna göre önemli derecede yüksekti (sırası ile 24.54+9.50 ve 0.50+0.39 ng/ml) ($p<0.01$). Kontrol grubu ile kıyaslandığında PTX-3 seviyeleri tüm AKS subtiplerinde

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This study was presented in part at the 5th Turkish Emergency Medicine Congress (TATKON), October 29- November 1, 2009, Antalya, Turkey.

Accepted Date/Kabul Tarihi: 06.05.2011 **Available Online Date/Çevrimiçi Yayın Tarihi:** 12.09.2011

doi:10.5152/akd.2011.160

((kararsız anjina pektoris (USAP) - 1.62+0.41 ng/ml, ST- elevasyonsuz miyokart enfarktüsü - 1.63+0.31 ng/ml, ve ST- elevasyonlu miyokart enfarktüsü (STEMI) - 1.75+0.89 ng/ml)) olmak üzere yüksek ve NAP-2 seviyeleri USAP (56.29+22.60 ng/ml) ve STEMI (52.05+20.99 ng/ml) hastalarında daha yüksekti ($p<0.01$). AKS hastaları için ilk altı saatte PTX-3 duyarlılığı %98.5 ve özgüllüğü %92.3 ve NAP-2 duyarlılığı %98.1 ve %41.3 idi. ROC eğrisinin EAA değerleri: PTX-3 (%95 GA 0.802-1.073) için 0.962, NAP-2 (%95 GA 0.684-0.991) için 0.840 ve cTnI (%95 GA 0.610 -0.940) için 0.683 idi.
Sonuç: Bizim sonuçlarımıza göre acil servise göğüs ağrısı ile gelen hastalarda ilk 6 saat içinde kardiyak belirteçleri özgüllük ile duyarlılıkla birlikte değerlendirildiğinde acil servis hekimine AKS tanısında en faydalı olabilecek belirteç PTX3'dür.
(Anadolu Kardiyol Derg 2011; 11: 588-94)
Anahtar kelimeler: Nötrofil aktive peptit 2, pentraxin 3, akut koroner sendrom, acil servis, duyarlılık, özgüllük, tanısal değer

Introduction

Chest pain is the second the most common cause of admission to the Emergency Department (ED) (epigastric pain is the most common). Approximately 5 million patients with chest pain are admitted to the EDs in the USA annually. Of the patients with chest pain admitted to the ED, 30-50% have acute coronary syndrome (ACS) (1). About 1.5 million people suffer from ACS every year, two-thirds of whom are admitted into the coronary intensive care unit and treated (1).

Evaluating a patient with chest pain in the ED is a challenging task. Despite the clinical experience of the emergency care practitioners, the use of electrocardiography (ECG) and biochemical parameters, 2-5% of ACS patients are inappropriately discharged from ED (2). ACS diagnosis is based on evaluation of risk factors, careful and rapid evaluation of ECG, and measurement of cardiac enzymes. Measurement of biomarkers in the circulation has facilitated the early diagnosis and evaluation of risk in ACS. The cardiac troponins are routinely used in the diagnosis of ACS, but they do not elevate during the first hours of ACS, and their usefulness in early diagnosis of ACS is disputable. To prevent inappropriate discharge of patients with chest pain admitted to the ED and to stop unnecessary hospitalization, cardiac markers are needed to provide early and secure diagnosis of ACS.

Neutrophil activating peptide-2 (NAP-2) and pentraxin-3 (PTX-3) are thought to be biomarkers with a role in ACS pathophysiology. It has been stated that NAP-2 has a potential role because of increasing the inflammatory response in atherosclerotic plaques. Because of its effects in increasing the action of endothelial cells on leukocytes in the artery wall, the inflammation carried out by NAP-2 causes ACS and plaque rupture (3). Rupture of the atherosclerotic plaque at the beginning of this process is the most important reason for ACS, but platelet activity in the circulation is important for the development and formation of an intracoronary thrombus. Relation between the tissue factor and thrombocyte is provided due to the downfall of the endothelial continuity and the thrombocyte activation starts. Finally, the granular content changes and membrane proteins are expressed on the cell surface. One of the other thrombocyte metabolites in the granules that is oscillated during degranulation is NAP-2 (4). NAP-2 in platelets and monocytes obtained from thrombus material in plaque ruptures of patients with ST-elevation myocardial infarction (STEMI) was specified through immunohistochemical methods (5).

PTX-3 is a member of the pentraxin family called tumor necrosis factor (TNF) stimulated gene 14 (TSGF 14). It has a molecular weight of 45-50 kDa (6). PTX-3 was the first long pentraxin to be defined; it is coded by an interleukin (IL)-1 inducible gene in endothelial cells and by a TNF-α-inducible gene in fibroblasts (7, 8). Due to stimulation of primary inflammatory mediators such as lipopolysaccharide, IL-1, and TNF-α, PTX-3 is released from macrophages and other cell types (9). PTX-3 is also released from cardiomyocytes during cardiac ischemia. PTX-3 could be an independent indicator of myocyte. Elevated plasma levels of PTX-may reflect myocyte damage during the early period of acute myocardial infarction. Besides common cardiac markers used in patients with ACS, can be used PTX-3 and NAP-2 to clinicians for diagnosing of ACS, particularly in the first few hours.

We measured the levels of neutrophil activating peptide-2 and pentraxin-3 in ACS patients and compared their diagnostic accuracy with cardiac troponin I (cTnI).

Methods

Patients and control subjects

This prospective study was approved by the Board of Ethics of the Faculty of Medicine of Dicle University. All patients provided written informed consent.

Eighty-three patients admitted to Dicle University Emergency Department due to complaints of chest pain and who best fitted the acceptance criteria were the study population. Having sudden chest pain and admission within the first 6-hour from pain onset were inclusion criteria. Exclusion criteria were patients: diagnosed with renal failure, collagen tissue disease, infection, vasculitis, depression or somatization; who had undergone angioplasty, bypass surgery, or open heart surgery; admitted to the ED after the first 6-hour after pain onset.

Those admitted with chest pain and diagnosed with ACS were assigned to the patient group (n=70). Subjects diagnosed with chest pain related to a non-heart-cause (myalgia, pleuritis, gallstones, regurgitant esophagitis) comprised the control group (n=13).

The patient group was divided into three ACS subgroups: unstable angina pectoris (USAP) (n=9), non-ST-elevation myocardial infarction (NSTEMI) (n=5) and STEMI (n=56). The USAP group included patients: with typical response pain lasting >20 min; with a newly started chest pain within the last two months or increase in the intensity of any typical previous chest pain; without a typical increase in troponin. The NSTEMI group had patients: suffering

typical chest pain lasting >30 min; whose ST-segment depression and T-wave negativity was accompanied by elevation in troponin. The STEMI group was comprised of patients with: typical chest pain lasting >30 min; ST-segment elevation (2 mm in chest derivations, 1 mm in extremity derivations) in two or more neighboring derivations in electrocardiography together with elevation in troponin.

Sample collection and measurement of biochemical markers

Blood samples of patients with suspected ACS admitted within 6 h after onset of chest pain were taken at admission. Tests of cTnI (Ultimate® Germany, reference interval 0-1.0 ng/ml, with chemiluminescent method) was carried out at the emergency laboratory using the blood samples. Blood samples taken for NAP-2 and PTX-3 were centrifuged (10 min, 3500 rpm). The serum samples obtained were stored at -80°C until use. Researchers randomly studied the plasma cTnI, NAP-2 and PTX-3 levels.

Measurement of pentraxin-3

PTX-3 was measured with an enzyme-linked immunosorbent assay (ELISA) method. A PTX-3 kit (ALX-850-299-KI01, Alexis Biochemicals, Switzerland) prepared for ELISA was used. The 96-well ELISA plate (Nunc MaxiSorp 446612) was plated with 100 µL of monoclonal antibody (MNB4; lot number L 20136 for PTX-3, human) . The covering solution was formed of 15 Nm carbonate in 9.6 pH. After plating, the ELISA plate was incubated at 4°C overnight. After incubation, plates were washed thrice with phosphate-buffered solution and covered with 0.05% saline solution. Tween 20 and 300 mL of 5% powdered milk were added to the washing solution to block the coupling points. Plates were washed thrice after incubation at room temperature for 2-hour. To each of the wells in the plate was added 50 µL pure recombinant human PTX-3 standard (lot number L19763) standardized from 2.4 ng/mL to 75 pg/mL together with RPMI 1640 containing 2% bovine serum albumin (BSA). This was left to incubate at 37°C for 2-hour. Plates were then washed five times with washing solution. To each of the wells was added 100 µL of polyclonal antibody for biotinized human PTX-3 (lot number L 20653). Plates were left to incubate at 37°C for 1 h, and washed five times with 300 mL of washing solution. After the washing process, 100 µL of streptavidin-horseradish peroxidase (1: 4000) was added to each of the wells, and left to incubate for 1-hour. After washed in washing solution 5 times following incubation 100 µl of ABTS chromogen substrate was added. Finally, it was left to the reading process for 15 minutes in 405 mm wavelength in spectrophotometry (ASYS Expert plus, Austria) equipment. The cut-off value was 0.98 ng/mL for PTX-3.

Measurement of neutrophil activating peptide-2

Preparation of testing reagents

Item A: The 96-well ELISA plate was plated with anti-human NAP-2 (lot number L 176023).

Item B: Twenty-times-concentrated washing solution of 25 mL was prepared.

Item C: Standard human NAP-2 recombinant sample (lot number L19763) was taken.

Item D: Obtained by taking 30 mL of 0.09% saline used to dilute serum and plasma.

Item E: Prepared through concentrating the solution used to dilute standard cell cultures five times.

Item F: Biotinised (Lot no: L 20653) NAP-2 to sample used to define the NAP-2 antibody.

Item G: Horseradish peroxidase (HRP) prepared by taking the 8 µl HRP- streptavidin concentrate of 10.000 times concentrated HRP concentrate.

Item H: 12 mL of tetramethyl benzidine was used.

Item I: 8 mL of 2M sulfuric acid was the stop solution.

A NAP-2 kit (RayBio®Human NAP-2 ELISA kit, lot number 176023; RayBiotech, USA) prepared for the ELISA test was used. Reagents and samples were kept at room temperature before use. One-hundred microliters of standard solution was placed in each well. Samples were also placed in the wells. They were left to incubate at 4°C overnight, followed by washing (four times) with 200 µL of wash solution. One-hundred microliters of streptavidin concentrate was added to each well at room temperature, and the reaction allowed to proceed for 45 min. This was followed by washing (four times) with 200 µL of wash solution. Then 100 µL of Item H solution was added to each well. Item I (stop solution) was then added. Finally, it was left for a 15-minute reading process in 405 mm wavelength in spectrophotometry (ASYS Expert plus, Austria) equipment. The cut-off value was 38.09 ng/mL for NAP-2.

Statistical analysis

Statistical analyses were undertaken using SPSS version 14 (SPSS Inc., Chicago, IL, USA). Results are given as mean ± SD. Univariate statistical analysis was carried out using the Chi-square test for categorical variables and the Student t-test for continuous variables. Differences between ACS subtypes and control group were assessed using the Kruskal-Wallis test and as posttest Mann-Whitney U test. The cut-off values of NAP-2 and PTX-3 according to control group means were estimated, and their sensitivities and specificities in ACS were identified. Receiver operating characteristic (ROC) analysis was used to assess the diagnostic validity of the markers, and areas under the ROC curve (AUC) were compared. In intra-group comparisons, a $p<0.01$ value was considered significant; in other comparisons, a $p<0.05$ value was considered significant.

Results

Clinical and demographic features

Of eighty-three patients in the study, 70 (83.3%) had ACS (patient group), 13 (17.7%) were non-ACS chest pain (control group). Mean admission time was 4.01±1.55 hours (1-6 hours).

Fifty-nine members of the patient group (84.3%) were male and 11 (15.7%) were female. Mean age was 57.79±13.09 (range, 27-79 y).

In the control group, 8 cases (61.6%) were male and 5 (38.4%) were female. Mean age was 57.08±10.76 (range, 36-78 y). A statistically significant difference between the patient group and the control group with respect to age and sex was not observed.

When groups were classified according to ACS subtypes; 9 (12.9%) were in the USAP group, 5 (7.1%) were in the NSTEMI group, and 56 (80%) were in the STEMI group.

Only 4 patients (5.7%) died and all were in the STEMI group.

Serum concentrations of cTnI, NAP-2 and PTX-3 in control and patient groups

In the patient group, serum concentrations of cTnI, NAP-2 and PTX-3 were considerably higher than those of the control group (p<0.01) (Table 1). When values of PTX-3 and NAP-2 in the patient group were compared in both sexes, the mean PTX-3 value in males was 1.72±0.81 ng/mL, and NAP-2 was 51.47±25.51 ng/mL; in females the PTX-3 value was 1.75±0.87 ng/mL, and NAP-2 was 51.40±18.56 ng/mL. A statistically significant relationship between values of PTX-3 and NAP-2 and sex was not observed.

When the control group was compared with patient group subtypes on a one-to-one basis: serum values of NAP-2 (p<0.001) and PTX-3 (p<0.001) were significantly higher in the USAP group with respect to the control group, whereas the elevation in serum cTnI (p=0.020) was not significant. Serum levels of cTnI (p=0.002) and PTX-3 (p<0.001) in the NSTEMI group were considerably higher than those of the control group, but elevation in NAP-2 level (p=0.059) was not statistically significant. Levels of serum NAP-2 (p<0.001) and PTX-3 (p<0.001) in the STEMI group were considerably higher than those of the control group, but elevation in serum levels of cTnI (p=0.014) was not statistically significant (Table 2).

Table 1. Mean serum concentrations of troponin I, neutrophil activating peptide-2 and pentraxin-3 in the patient group and control group

Variables	Control group (n=13)	Patient group (n=70)	p*
Troponin I, ng/ml	0.24±0.20	7.72±18.76	0.001
Pentraxin-3, ng/ml	0.50±0.39	1.73±0.82	<0.001
Neutrophil activating peptide-2, ng/ml	24.54±9.50	53.03±22.77	<0.001

Data are presented as mean+SD
*Unpaired Student's t-test

Diagnostic validity of NAP-2 and PTX-3

In the control group, NAP-2 values range was 9.57-38.09 ng/mL, and PTX-3 values range was 0.05-0.98 ng/mL. Based on these values, the cut-off values were 38.09 ng/mL for NAP-2, and 0.98 ng/mL for PTX-3.

Sensitivities of PTX-3, NAP-2 and cTnI for ACS diagnosis according to admission time are given in Table 3; their specificities are given in Table 4.

Pentraxin-3 had a high sensitivity at all time points, but the sensitivity ratios of all four markers were quite similar (Table 3). The specificity ratios seen in Table 4 indicate the superiority of PTX-3 to other markers at all time points. After PTX-3 had the highest specificity ratio in the first 6-h period (78.5%), followed by NAP-2 and cTnI.

Figure 1 indicates the ROC curve analysis results within the first 3-hour interval after symptom onset. Figure 2 indicates the ROC curves analysis results 3-6-hour interval after symptom onset. Figure 3 indicates ROC curve analysis results within 6-hour interval after symptom onset. The AUC of PTX-3 was significantly larger than the areas of the other three markers within the first 3 hours and the first 6 hours after symptom onset (p<0.05). The AUC of PTX-3 on the 3rd hour and the 6th hour after symptom onset were equal to each other and larger than that of the others. Although the AUCs of all markers according to the time points showed a numerical difference, the alignment of these four markers with respect to AUC magnitude did not change. In the first six hours, The AUCs were 0.962 for PTX-3 (95% CI: 0.802 to 1.073), 0.840 for NAP-2 (95% CI: 0.684 to 0.991), and 0.683 for cTnI (95% CI: 0.610 to 0.940) (Fig. 3).

Discussion

The present study demonstrated that the serum concentrations of NAP-2 and PTX-3 could be identified directly after symp-

Table 2. Mean serum concentrations of troponin I, neutrophil activating peptide-2 and pentraxin-3 in the control group and patient group subtypes

Variables	Control (n=13)	Unstable angina pectoris (n=9)	Non-ST-elevation myocardial infarction (n=5)	ST-elevation myocardial infarction (n=56)	Chi-square	p*
Troponin I, ng/ml	0.24±0.20 (0.02-0.75)	4.84±12.64 (0.05-38.50)	8.08±9.42** (0.33-24.0)	8.15±20.25 (0.01-100.0)	10.046	0.004
Pentraxin-3, ng/ml	0.50±0.39 (0.05-0.98)	1.62±0.41** (1.03-2.37)	1.63±0.31** (1.35-2.16)	1.75±0.89** (1.01-5.14)	32.845	0.000
Neutrophil activating peptide-2, ng/ml	24.54±9.50 (9.57-38.09)	56.29±22.60** (29.56-93.67)	58.14±42.41 (26.62-125.4)	52.05±20.99** (8.93-140.13)	21.216	0.000

Data are presented as mean+SD and median (min-max) values
*Kruskal-Wallis test
**Mann-Whitney U test p<0.01as compared to control group

tom onset in patients with ACS by the ELISA method. This assay might be useful for the early diagnostic assessment of patients suspected of having ACS. We designed this study to assess the diagnostic validity of NAP-2 and PTX-3 as cardiac biochemical markers. During the first six hours after symptom onset, we examined the diagnostic validity, sensitivity and specificity of NAP-2 and PTX-3 in ACS patients, and compared them with the currently used cardiac marker cTnI.

Pentraxin-3 is a multifunctional protein with roles that are only now beginning to be unraveled (10, 11). PTX-3 binds apoptotic cells and is present in damaged myocardial tissues (12). Introna et al. (9) developed an ACS model by tying the coronary artery in rodents and observed the very high amount of PTX-3 excreted in the heart. Inoue et al. (13) showed considerably high levels of PTX-3 in the peripheral blood of patients with USAP compared with controls. We hypothesized that high levels of PTX-3 could be used as a marker for ACS. We specified various PTX-3 levels in different ACS subtypes. In all three ACS subtypes (USAP, STEMI, and NSTEMI) PTX-3 levels were considerably higher compared with the control group. Similarly, cTnI was significantly high only in the NSTEMI group. Due to the fact that nonsignificant elevation of cTnI during the first hours of ACS, we conclude that high levels of PTX-3 during the first hours of ACS would be helpful for the clinicians. If myocardial necrosis is not present (as in USAP), and if cTnI is insufficient for ACS diagnosis, elevation in PTX-3 levels could be used for ACS diagnosis.

Table 3. Sensitivity of pentraxin-3, neutrophil activating peptide-2 and troponin I in the group of patients with confirmed ACS within six hours after symptom onset

Admission time, hours	n	Sensitivity, %		
		Pentraxin-3	Neutrophil-activating peptide 2	Troponin I
0-3	30	100	100	100
3-6	40	97.5	96.9	96.4
0-6	70	98.5	98.1	96.8

The sensitivity was calculated as the percentage of patients with confirmed ACS whose results were at or above the cut-off level. The cut-off levels used for pentraxin-3, neutrophil activating peptide-2, and troponin I were 0.98 ng/mL, 38.09 ng/mL and 1.0 ng/ml, respectively
ACS - acute coronary syndrome

NAP-2 may have a potential role in increasing the inflammatory response in atherosclerotic plaques, and NAP-2-driven inflammation could promote plaque rupture and ACS (3). We have not encountered a study detailing serum measurements of NAP-2 to determine the role of NAP-2 in patients with chest pain and in which these measurement are compared with other cardiac markers. A tissue study introducing the presence of NAP-2 using an immunohistochemical method in platelets and monocytes obtained from the thrombus material in the plaque ruptures of patients with STEMI has been noted (5). We found that serum levels of NAP-2 in ACS patients were considerably higher than in the control group. This elevation was especially significant in USAP and STEMI groups. ECG differences observed in STEMI patients eliminated the need for cardiac markers for diagnosing ACS. If acute differences are not present in the electrocardiogram (as in USAP), and if cTnI type marker is insufficient to make the diagnosis in the patient group, new cardiac markers are needed to prevent inappropriate discharge of

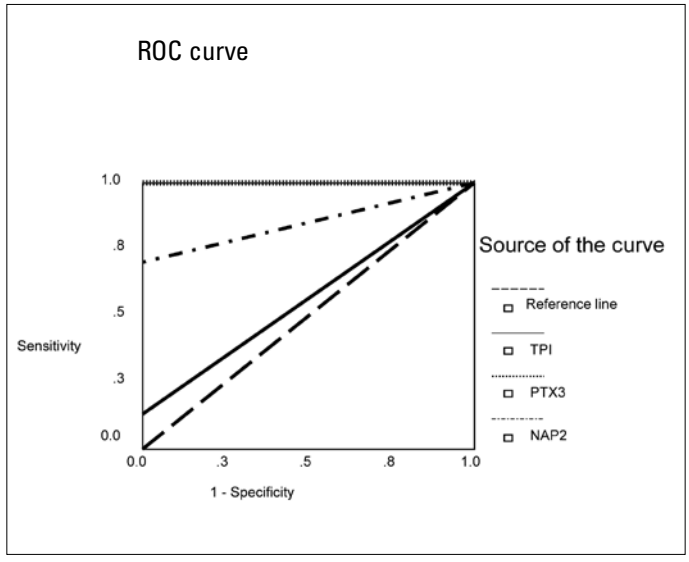

Figure 1. ROC curves of concentrations of PTX-3, NAP-2 and cTnI in the patient group and the control group within three hours after symptom onset. ROC curves were constructed by plotting the sensitivity within 3 h after symptom onset for the ACS group (n=30) on the y-axis and 1-specificity for the control group (n=5) on the x-axis. The AUCs are 1.000 for PTX-3, 0.850 for NAP-2 and 0.567 for cTnI
AUC - area under the curve, cTnI - cardiac troponin I, NAP-2 - neutrophil activating peptide-2, PTX-3 - pentraxin-3, ROC - receiver operator characteristics analysis

Table 4. Specificity of pentraxin-3, neutrophil activating peptide-2 and troponin I in the control group within six hours after symptom onset

Admission time, hours	n	Specificity, %					
		Pentraxin-3	Likelihood ratio	Neutrophil activating peptide-2	Likelihood ratio	Troponin I	Likelihood ratio
0-3	5	100	-1.01	35.7	-2.88	16.1	-6.62
3-6	8	87.5	-1.12	46.6	-2.12	35	-2.83
0-6	13	92.3	-1.07	41.3	-2.43	23.5	-4.30

The specificity was calculated as the percentage of patients in the control group whose results were below the cut-off level. The cut-off levels used for pentraxin-3, neutrophil activating peptide-2 and troponin I were 0.98 ng/mL, 38.09 ng/mL and 1.0 ng/ml, respectively

patients from the ED and to initiate early treatment. We propose PTX-3 and NAP-2 to be these new markers because both markers are elevated within the first six hours in this patient group.

The sensitivities of all four markers were similar, but PTX-3 was indisputably superior to the others within the first three hours after symptom onset. We, therefore suggest that PTX-3 may be more valuable and benefit in diagnosing of ACS rather than other cardiac markers, particularly within the first three hours after the onset of chest pain in patients admitted to the ED. In the present study, the most valuable serum markers was PTX-3 due to their sensitivity and specificity in a period of 3-6 h after symptom onset compared with cTnI. Elevated levels of PTX-3 are in favor of ACS for patients admitted in this time slot. According to our results, if the sensitivities and specificities of the cardiac markers of patients with chest pain admitted to ED within the first six hours were evaluated together, the most useful marker to the emergency physician in diagnosing ACS would be PTX-3. Despite the sensitivity of NAP-2 being higher than cTnI, which is commonly used cardiac marker in diagnosis of ACS at all time points, the low specificity of NAP-2 would reduce its reliability in diagnosis of ACS.

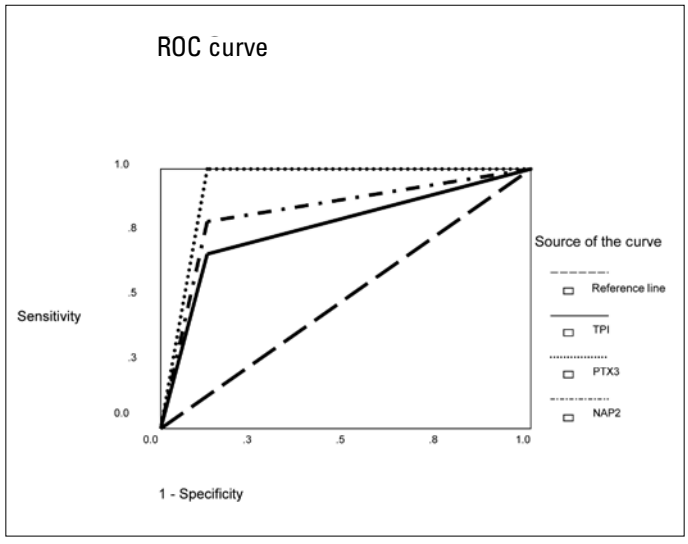

Figure 2. ROC curves of concentrations of PTX-3, NAP-2 and cTnI in the patient group and the control group within 3-6 h after symptom onset. ROC curves were constructed by plotting the sensitivity within 3-6 hours after symptom onset for the ACS group (n=40) on the y-axis and 1-specificity for the control group (n=8) on the x-axis. The AUCs are 0.938 for PTX-3, 0.838 for NAP-2 and 0.775 for cTnI

AUC - area under the curve, cTnI - cardiac troponin I, NAP-2 - neutrophil activating peptide-2, PTX-3- pentraxin-3, ROC - receiver operator characteristics analysis

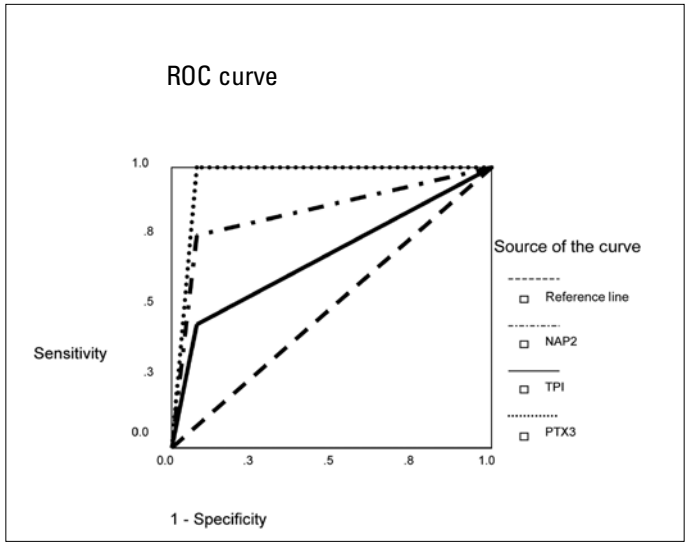

Figure 3. ROC curves of concentrations of PTX-3, NAP-2 and cTnI in the patient group and the control group within 6 h after symptom onset. ROC curves were constructed by plotting the sensitivity within 6 h after symptom onset for the ACS group (n=70) on the y-axis and 1-specificity for the control group (n=13) on the x-axis. The AUCs were 0.962 for PTX-3 (95% CI: 0.802 to 1.073), 0.840 for NAP-2 (95% CI: 0.684 to 0.991), and 0.683 for cTnI (95% CI: 0.610 to 0.940)

AUC - area under the curve, cTnI - cardiac troponin I, NAP-2 - neutrophil activating peptide-2, PTX-3- pentraxin-3, ROC - receiver operator characteristics analysis

Estimation of sensitivity and specificity are related to the cut-off value used in the study. To compare the diagnostic validity of PTX-3, NAP-2 and cTnI, we analyzed ROC curves. The AUC of PTX-3 was the largest during the first 3, 3-6 and 6-hour from symptom onset. This was followed by the AUCs of NAP-2 and cTnI. This shows that PTX-3 had diagnostic correctness after the first six hours (even in the first three hours) of the onset of chest pain.

Study limitations

The present study has the following limitations. The present study was performed in a single department, and we were able to provide data containing only one-time point measurement for each patient in the small sized o patient population. If verified in a larger multicenter study cohort, these findings could provide practical assistance to physicians regarding the best diagnostic options for ACS. Measurement of PTX-3 and NAP-2 was time-consuming and labor-intensive. A measurement method for PTX-3 and NAP-2 that can provide results in a shorter time should be developed. Despite troponin T remains to be the gold standard for the diagnosis of suspected ACS, we have investigated consecutive troponin I measurements because of it is solely available as a specific cardiac serum biomarker in our hospital's biochemistry laboratory.

Conclusion

Besides common cardiac markers used in patients with ACS, PTX-3 and possibly NAP-2 may be useful to clinicians for diagnosing of ACS, particularly in the first few hours. We introduce that PTX-3 and NAP-2 deserve further study for the purpose of confirmation as a novel cardiac markers.

Conflict of interest: None declared.

Acknowledgements

This study was carried out with the financial support of the Board of Scientific Research Projects and approval of the Directorate of the Board of Ethics of the Faculty of Medicine of Dicle University

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