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Levels of osteopontin, suppression of tumorgenicity 2 and myeloperoxidase activity in acute coronary syndrome patients with fragmented QRS

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Abstract

In recent years, studies have focused on new markers to predict the risk of cardiovascular diseases (CVD). There are several studies analyzing biomarkers such as myeloperoxidase (MPO), suppression of tumorgenicity 2 (sST2), and osteopontin (OPN) in CVD, however; there are no studies that show their relationship in patients who have fragmented QRS (fQRS). It aimed to investigate the levels of OPN, sST2, and MPO activity in patients who were diagnosed with acute coronary syndrome (ACS) in the present study. Sixty ACS patients and 26 healthy individuals were included in the study. Patients diagnosed with ACS were divided into two groups; (+)fQRS (n=30) and (-)fQRS (n=30). Levels of OPN and sST2 were measured by ELISA, and MPO activity was measured by colorimetric methods. In ACS patients, serum activity of MPO (33.7 U/L), and levels of OPN (103.29 ng/mL) and sST2 (495.4 pg/mL) were found to be significantly higher than those in the control (23.14 U/L, 42.65 ng/mL, 344.11 pg/mL, respectively; $p<0.01$, $p<0.01$, $p<0.05$). However, there were no significant differences between the activity of MPO, levels of OPN, and sST2 in patients with (+)fQRS (respectively, 32.74 U/L, 101.89 ng/mL and 451.97 pg/mL) and (-)fQRS (respectively, 34.67 U/L, 104.69 ng/mL, 535.73 pg/mL). There were also positive correlations between MPO activity and platelet ($r=0.376$, $p<0.05$) levels in the (+)fQRS group, and sST2 and triglyceride levels in (-)fQRS group. When the diagnostic performances for ACS were examined, the sensitivity of CK-MB, troponin-I, MPO, OPN, and sST2 were 83%, 80%, 65%, 85%, 58%, and specificity was 96%, 100%, 72%, 96%, 80%, respectively. In conclusion, MPO, OPN, and sST2 may be included in the factors that may contribute to the diagnosis and development of ACS. In addition, the discovery of the high diagnostic sensitivity and specificity of OPN in acute coronary syndrome is a new finding obtained in this study.

Keywords: acute coronary syndrome, osteopontin, sST2, myeleporoxidase

1. Introduction

The term “acute coronary syndrome” (ACS) is a general term used for conditions that occur because of an interruption of blood flow to the heart. All clinical symptoms that develop due to acute myocardial ischemia are called ACS. The determination of the clinical type of ACS is based on electrocardiography (ECG) findings. According to ECG findings, there are three different types of ACS: unstable angina pectoris (UAP), non-ST-segment elevation myocardial infarction (NSTEMI), and ST-segment elevation myocardial infarction (STEMI) (1).

Fragmented QRS (fQRS) is a depolarization abnormality that can be easily detected from 12-lead ECG. fQRS detected in the routine ECG recordings of individuals with coronary artery disease or individuals with suspected coronary artery disease is an independent marker for mortality due to myocardial scar, arrhythmic events, and coronary artery diseases (2).

ST2 is an interleukin-33 (IL-33) receptor secreted by most living cells in response to cell damage, and it means "suppression of tumorgenicity 2". It has been revealed that it is secreted by cardiac cells in response to myocardial stress (3). ST2 has two main isoforms: transmembrane (ST2L) and soluble (sST2) forms. IL-33 acts directly as a transcriptional regulator and shows its effects by binding to the transmembrane receptor ST2L. Studies have demonstrated that the IL-33/ST2L interaction is cardioprotective and reduces myocardial fibrosis, cardiomyocyte hypertrophy, and apoptosis, as well as improving myocardial function (4,5). The levels of sST2, which is the other isoform of ST2, increase in inflammatory conditions, and studies have shown that high sST2 levels are consistently related to the mortality risk in acute/chronic cardiovascular conditions (6).

Osteopontin (OPN), an extracellular matrix protein, is released from osteoblasts and osteoclasts that are effective in

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bone formation in bone tissue. Several studies have been conducted to define the relationship between OPN and atherosclerotic cardiovascular diseases (7). OPN was first detected in the artery tissue by Giachelli et al. (8) and its relationship with cardiovascular diseases has not been elucidated yet. OPN, which is a multifunctional protein, has been shown to have important functions in cardiovascular disease, cancer, diabetes, renal lithiasis, infection, biomineralization, cell viability, and wound healing (9).

Myeloperoxidase (MPO) is a myeloid-based enzyme with strong antibacterial properties, and it is largely synthesized by neutrophils. MPO, a product of systemic inflammation, plays an important role in both the oxidative stress and inflammation process and takes part in the oxidation process of lipoproteins. Moreover, MPO contributes to the development of atherosclerotic plaques by disrupting endothelial function (10), LDL oxidation (11), generation of a thrombogenic environment (12), and thinning of the plaque fibrous cap (13).

The main approach to preventing the negative consequences of acute coronary syndromes is to detect high-risk individuals long before the development of disease-related complications. In this study, we aimed to investigate the activity of myeloperoxidase (MPO), osteopontin (OPN), and soluble ST2 (sST2) levels in acute coronary syndrome (ACS) patients with and without fQRS. We also aimed to investigate whether these biomarkers would be useful in the diagnosis of coronary diseases.

2. Materials and Methods

2.1. Study design and participants

This study concerned a total of 60 ACS patients who were admitted to the emergency department of Ordu University Training and Research Hospital with chest pain and then taken into the intensive care unit. After the approval of the Ordu University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 15/11/2018 and the number of ethical committee decisions is 2018/228. The patient population was divided into two groups: patients with fQRS (+fQRS) and patients with no fQRS (-fQRS). The control group consisted of 26 healthy individuals who are compatible with ACS patients due to age and gender.

A 12-lead ECG was obtained from all patients during their resting state. Exclusion criteria were as follows: patients with LV EF (left ventricular ejection fraction)<50%, presence of advanced valvular disease, patients with bundle branch block on ECG, patients with known cardiomyopathy and permanent pacemakers, patients with severe electrolyte imbalance, those with acute and chronic bacterial or viral inflammation, those with severe liver and kidney failure, patients with rheumatological and orthopedic diseases, patients receiving anti-inflammatory (other than aspirin) medications, hormones, cytokines or growth factors were excluded from the study. Fasting blood samples of patients were collected between the 12^{th} and 36^{th} hours considering the time at which the pain began. After waiting for 30 minutes, the samples were centrifuged in 1800 xg for 10 minutes and the serum was stored at -80℃ until analysis.

2.2. The Biochemical Analysis

Serum sST2 and OPN levels were measured by commercially available Enzyme-linked Immunosorbent Assay (ELISA) Kits (respectively, Thermo Fisher Scientific BMS2066/BMS2066TEN, USA; Elabscience Biotechnology Co., Ltd. E-EL- H1615, USA). Results were read at 450 nm wavelength on the ELISA reader (BioTek ELX800 reader, BioTek ELX50 washer, Winooski, Vermont, United States). Serum MPO activity was measured via methods developed by Bradley et al. (14). The principle of the method that O-dianicide, known as a peroxidase substrate, is oxidized by MPO to form a yellow-orange product in the presence of hydrogen peroxide (H_2O_2). This oxidation reaction was monitored at 460 nm with an increase in optical density depending on time.

Other parameters included in our study included fasting glucose, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol, blood urea nitrogen (BUN), lactate dehydrogenase (LDH), C-reactive protein (CRP) and creatine kinase (CK) were measured by the commercial kit and the auto-analyzer (Hitachi Cobas-c 501) produced by Roche Diagnostics Ltd., Japan. Troponin-I and mass CK-MB (creatine kinase-myocardial isoenzyme) were measured by the commercial kit and the autoanalyzer (Hitachi Cobas-e 601) produced by Roche Diagnostics. Hemoglobin, leukocyte, neutrophil, and platelet levels were measured by the commercial kit and the autoanalyzer (Sysmex XN-1000) produced by Sysmex Corporation Ltd., Japan.

The diagnosis of dyslipidemia was defined according to the National Cholesterol Education Program (NCEP, ATP III) final report (15).

2.3. Statistical Analysis

In this study, the primary variable was OPN for fQRS. According to previous studies, effect size (d) was assumed to be 0.96, and a Z value of 1.96 was used for the 0.05 types I error rate. It calculated that a 99% power value at d = 0.96 effect size and a 95% confidence level can be reached with at least 26 subjects (6).

The normal distribution control of the data was checked with the Kolmogorov-Smirnov test. The homogeneity of the group variances was checked by the Levene test. A comparison of the two groups was made using the Mann-Whitney U test, and the comparison of the three groups was made by one-way analysis of variance. After variance analysis, the Tukey HSD test was used as a multiple comparison test. The dependency between categorical variables was examined by the two-way chi-square test. Relationships between normally distributed variables were examined with the Pearson correlation coefficient and the relationships between non-normally

distributed variables were examined with the Spearman rank correlation coefficient. The results are presented as the mean± standard error (SE). Before performing multiple logistic regression analysis to determine the risk factors for acute coronary syndrome, LR was used to identify variables that significantly contributed to the model. Then, a multiple logistic regression analysis was performed with these variables. All calculations were made with the SPSS v25 (IBM Corp, Chicago, IL, USA) statistical package program. The statistical significance of all variables was established at $p<0.05$.

3. Results

The demographic and descriptive characteristics of the groups are shown in Tables 1 and 2. The comparison of sST2, OPN, MPO, and other biochemical parameters in groups are given in Table 3. There were no significant differences in terms of levels of sST2, OPN, and MPO activity between (+)fQRS and (-)fQRS groups ($p>0.05$). When the (+)fQRS and (-)fQRS groups were compared to the controls, OPN levels and MPO activity were found to be higher (p 0.01). In addition, the sST2 level increased significantly in the (-)fQRS group as compared to the controls ($p=0.03$).

Table 1. Demographic characteristics of the groups included in the study

Parameters	Control (X±SE)	(+)fQRS (X±SE)	(-)fQRS (X± SE)	p
Age	59.62±1.55	63.90±2.14	64.33±2.76	0.289
Male	15(%57)	22(%73)	16(%53)	0.249
Female	11(%43)	8(%27)	14(%47)	

(+)fQRS: ACS patients with fQRS ; (-)fQRS: ACS patients without fQRS

Table 2. Descriptive characteristics of the groups diagnosed with ACS

Parameters	(+)fQRS (X±SE)	(-)fQRS (X±SE)	p
Diabetes Mellitus	12(%40)	8(%27)	0.224
MI	25(%83.3)	27(%90)	0.448
Hypertension	16(%55.2)	13(%44.8)	0.438
Dyslipidemia	19(%55.9)	15(%44.1)	0.297
SBP (mm Hg)	121.46±2.85	118.1±4.78	0.547
DBP (mm Hg)	76.56±1.84	75.36±3.08	0.741
Heart Rate (pulse/min)	77.56±2.1	71.43±3.45	0.135

(+)fQRS: ACS patients with fQRS ; (-)fQRS: ACS patients without fQRS; MI: a history of myocardial infarction; SBP: systolic blood pressure; DBP: diastolic blood pressure

The diagnostic performance levels of OPN, sST2, and activity of MPO were calculated in ROC analyses to differentiate the diagnosis of fQRS (as shown in Fig. 1). Lower and upper limit values for the area under the curve (AUC), sensitivity, specificity, and 95% confidence interval for MPO, OPN, and sST2 are also presented in Fig.1. There was no diagnostic value of MPO, OPN, and sST2 in the diagnosis of fQRS.

Table 3. Comparison of biochemical parameters among the groups

Parameters	Group	n	Mean	SE	p value
MPO (U/L)	Control	26	23.14	1.12	
	(+)fQRS	30	32.74 ^{a*}	3.14	0.007
	(-)fQRS	30	34.67 ^{a**}	2.84	
OPN (ng/mL)	Control	26	42.65	4.14	
	(+)fQRS	30	101.89 ^{a***}	6.18	0.000
	(-)fQRS	30	104.69 ^{a***}	4.66	
sST2 (pg/mL)	Control	26	344.11	49.06	
	(+)fQRS	30	451.97	49.81	0.03
	(-)fQRS	30	535.73 ^{a*}	45.60	
Glucose (mg/dL)	Control	26	98.00	1.2	0.002
	(+)fQRS	30	137.9^{a**}	8.92	
	(-)fQRS	30	126.33^{a**}	8.97	
BUN (mg/dL)	Control	26	13.47	1.03	0.049
	(+)fQRS	30	18.06^{a*}	1.28	
	(-)fQRS	30	18.24^{a*}	1.92	
TG (mg/dL)	Control	26	125.46	7.69	0.071
	(+)fQRS	27	152.56	11.5	
	(-)fQRS	29	175.86	21.4	
TC (mg/dL)	Control	26	185.54	6.15	0.6
	(+)fQRS	27	180.89	5.74	
	(-)fQRS	29	190.14	7.39	
HDL-C (mg/dL)	Control	26	51.77	2.26	0.001
	(+)fQRS	27	42.41^{a**}	2.31	
	(-)fQRS	29	41.45^{a**}	1.77	
LDL-C (mg/dL)	Control	26	110.72	5.62	0.113
	(+)fQRS	26	109.05	5.06	
	(-)fQRS	28	134.24	5.67	
CRP (mg/dL)	Control	26	0.33	0.04	0.009
	(+)fQRS	30	3.28^{a**}	1.0	
	(-)fQRS	30	1.56	0.4	
LDH (U/L)	Control	26	173.5	4.51	0.001
	(+)fQRS	30	399.03^{a**}	47.9	
	(-)fQRS	28	382.43^{a**}	54.59	
CK (U/L)	Control	26	80.18	6.49	0.002
	(+)fQRS	30	736.13^{a**}	183.2	
	(-)fQRS	30	416.43	76.12	
CK-MB (ng/mL)	Control	26	1.64	0.1	0.001
	(+)fQRS	29	56.68^{a**b*}	14.8	
	(-)fQRS	29	24.03	6.4	

*; $p<0.05$, **; $p<0.01$, *** ; $p<0.001$, a; when compared with control, b; when compared with (-)fQRS MPO; myleperoxidase, OPN; osteopontin, sST2; suppression of tumorgenicity 2, BUN; Blood urea nitrogen, TG; Triglycerides, TC; Total Cholesterol HDL-C, High density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; CRP; C-reactive protein, LDH; Lactate dehydrogenase, CK; creatinine kinase, CK-MB; creatinine kinase MB.

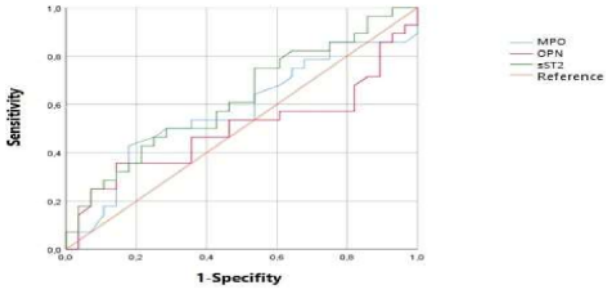

Fig 1. Diagnostic value of MPO, OPN ans ST2 for fORS

The sensitivity and specificity levels of Troponin-I (TnI), CK-MB, OPN, sST2, and, MPO activity were also calculated in ROC analyses (as shown in Figure 2). The results related to the diagnostic performance of Troponin-I, CK-MB, MPO, OPN, and sST2 are also given in Figure 2. In the ACS diagnostic performance analysis, AUC was 0.925 for OPN. When 73.64 ng/mL was taken as the cut-off value for the diagnosis, the sensitivity of OPN was 85% and the specificity was 96% (p=0.021; 95% CI, 0.534-0.792).

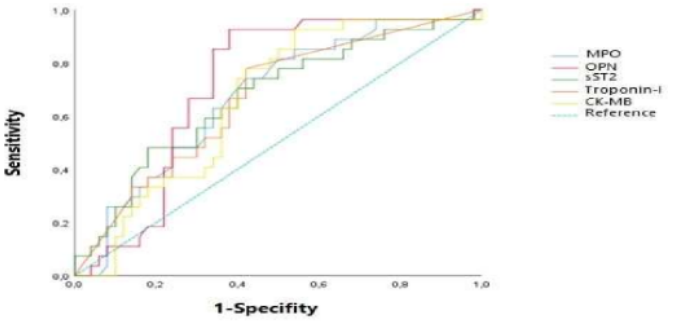

Fig 2. Diagnostic value of Troponin-I, CK-MB, MPO, OPN and sST2 for ACS

Table 4. Logistic regression analysis of the impact of various prognostic factors on acute coronary syndrome

Parameters	β	SE	Wald' s $\chi 2$	df	p	Odds Ratio (OR)
Neutrophil	1,169	0,552	4,485	1	0,034	3,217
Osteopontin	0,104	0,031	11,161	1	0,001	1,110
HDL-C	-0,222	0,087	6,539	1	0,011	0,801
Constant	-0,903	3,393	,071	1	0,790	0,405

Classification Table[a]			
	Predicted		
Observed	**Control**	**ACS**	**Percentage Correct**
Control	24	2	92,3
ACS	3	53	94,6
Overall Percentage (%)			93,9
Cox & Snell R Square = %62.4; Nagelkerke R Square= %87.6			

a: the cut value is 0.500, ACS; acute coronary syndrome.

Multiple logistic regression analysis was performed with variables (neutrophil, HDL-C, and OPN) that significantly contributed to the model, and the analysis results are summarized in Table 4. The odds ratio for a 1 unit increase in neutrophil level was 3.217 (95% CI: 1.09-9.48). Similarly, OR for OPN was found to be 1.11 (95% CI: 1.04-1.18). On the other hand, since the coefficient value of HDL-C is negative and OR is lower than one, a 1 unit decrease in HDL-C level increases the risk of getting ACS (1 / 0.8 = 1.25) 1.25 times.

4. Discussion

This study investigated the diagnostic importance of sST2, OPN, and MPO in patients with ACS. The principal findings of our study are as follows: (a); It was revealed that MPO, OPN, and sST2 may be included in the factors that may contribute to the diagnosis and development of ACS, but these parameters were not associated with the presence of fQRS. (b); OPN has higher diagnostic sensitivity than troponin I, CK-MB, MPO, and sST2 in patients with ACS. (c); Finally, we think this study is important in that it is the first study to exhibit the relationship between MPO, OPN, and sST2 and fQRS development.

fQRS is a non-invasive, easy-to-interpret, and cost-effective ECG parameter for clinicians. Extant studies have focused on the relationship of fQRS with myocardial ischemia and scars. Some studies have shown that the presence of fQRS on ECG is associated with myocardial ischemia (16). Further, some studies have shown a relationship between the presence of fQRS and the prognosis of cardiac events in patients with CAD. In a study conducted by Das et al. (17), the percentage of all-cause mortality and adverse cardiac events was found to be higher in patients with fQRS than in the group without fQRS.

sST2 levels increase in inflammatory diseases and heart diseases, therefore, the sST2 molecule is considered a valuable prognostic marker in both cases. In previous studies, a high sST2 level has been associated with the prognosis of acute coronary syndrome, pulmonary artery hypertension, and acute/chronic heart failure (18). As a result of our study, no significant difference was found between sST2 levels of (+)fQRS and (-)fQRS groups. Most patients with UA have coronary artery stenosis, and the main pathophysiological mechanisms that increase the degree of ischemia in the progressive process are plaque cracking, thrombus formation, lumen narrowing, and vasoconstriction. Demyanets et al. (19) have shown that patients diagnosed with ACS have higher levels of sST2 than individuals with stable coronary artery disease. The low sensitivity and specificity of sST2 can be considered the diagnostic value of sST2 insufficient for ACS. Marino et al. (20) compared sST2 and hs-cTnI concentrations and tested the short-term prognostic value of these two biomarkers in their study consisting of patients admitted to the emergency department with chest pain. As a result of their study, sST2 and hs-cTnI concentrations of ACS patients were found to be significantly higher than those in patients without ACS. Considering all ACS subclasses, sST2 showed a higher relative risk estimation than hs-cTnI. The authors concluded

that sST2 was found to have a greater prognostic value than hs-cTnI for cardiac mortality 30 days after discharge (20). Because of the results of our and previous studies (20,21), it is predicted that sST2 may be a more important parameter in estimating negative cardiac events of ACS and risk classification than having a diagnostic value for ACS.

Under basal conditions, OPN is found in very small amounts in the heart tissue (22). However, OPN expression in the heart tissue increases significantly under several pathological conditions (23). Mazzone et al. (24) reported that basal OPN levels were associated with rapid coronary plaque progression and OPN levels were also found in higher concentrations in ACS patients than in other groups. In the present study, while patients with ACS had higher OPN levels than the control group, there was no statistically significant difference in OPN levels between ACS patients with and without fQRS. Cardiac studies have been performed up to now and have focused on investigating the importance of OPN in the diagnosis of acute coronary syndrome. Yu et al. (25) reported that OPN levels were significantly higher in ACS patients as compared to controls, and also increased OPN levels were associated with an increase in the severity of ACS and the onset time of ACS symptoms. Our results have extended previous studies by demonstrating that increased OPN level is associated with an increased risk of ACS. Few studies have been found in the literature about the diagnostic value of OPN. While the diagnostic specificity of OPN was found at the same level as CK-MB, its sensitivity was found to be higher than CK-MB. In a study dealing with the diagnostic value of OPN in ACS, AUC was 0.897, the specificity value was 99.29% and the sensitivity value was 82.5% for OPN. Furthermore, in this study, the diagnostic accuracy of OPN in distinguishing ACS patients from controls was found to be 88.34% (26).

MPO is an inflammatory biomarker secreted by neutrophils, monocytes, and tissue-bound macrophages. It has been reported that MPO activity is increasing in AMI cases (27, 28). MPO activity reaches its peak level in a short time after AMI and, there is also a decrease in activity over time (29). In patients diagnosed with AMI it was determined that serum MPO activity increased 2 hours after the onset of chest pain (28). In the present study, although the MPO activity of ACS patients was higher than that of the control group, no significant difference was found between the ACS clinical subtypes and between the groups with and without fQRS. MPO levels were found to be significantly higher in ACS patients than in controls, and these results are compatible with the literature. When 48 U/mL is taken as the cut-off value in a study conducted with samples collected between the 4th and 6th hours after the onset of symptoms from patients by Gururajan et al. (30), it was observed that MPO had higher specificity (96%) and sensitivity (95%) values compared to TnI and CK-MB. Elevation of troponin levels takes 4-6 hours following myocardial injury. However, MPO, even in patients who are TnI negative, initially rises within 2 hours after symptom onset and peaks at the 4th hour (30). Therefore, in our study, it is an expected result that the diagnostic value of MPO is lower than the diagnostic value of TnI and CK-MB in the blood samples collected between the 12th and 36th hours. These findings suggest that increased MPO levels may be useful for the diagnosis of ACS and may be a marker for unstable angina before myocardial necrosis.

This study has certain limitations. First, our study population is small. Hence, with larger groups, more significant logistic regression analysis could be done. Second, we examined biomarkers associated with inflammation that might cause fQRS formation. However, we could not attain the results that would reveal this relationship. This suggests that these biomolecules may also be elevated due to causes independent of atrial fibrillation or scar tissue. Third, parameters related to inflammation were evaluated before starting any medical treatment. Since our study was performed in patients who are in the acute period of the ACS, it is recommended to examine patients with fQRS for long-term mortality and to investigate whether these parameters will change in the chronic period.

In conclusion, our study suggests that MPO, OPN, and sST2 may be included among the factors that may contribute to the diagnosis and development of ACS but they are not related to fQRS. High ACS diagnostic sensitivity and specificity of OPN is a new finding in this study. Also, this study is the first study that demonstrates the relationship between MPO, OPN, sST2, and fQRS development. Therefore, we believe that more comprehensive studies are needed to clarify this relationship and to develop new diagnoses and treatment methods for high-risk patients with elevated MPO, OPN, and sST2 levels.

Ethical Statement

Approval was obtained from Ordu University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 15/11/2018 and the number of ethical committee decisions is 2018/228.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: S.S.M., T.N., O.B., Design: S.S.M., T.N., Data Collection or Processing: S.S.M., T.N., O.B., Analysis or Interpretation: S.S.M., T.N., O.B., Literature Search: S.S.M., T.N., Writing: S.S.M., T.N.,

References

1. Kumar A, Cannon CP. Acute coronary syndromes: diagnosis and management, part I. Mayo Clin Proc. 2009; 84(10):917-938. doi:10.1016/S0025-6196(11)60509-0.
2. Take Y, Morita H. Fragmented QRS: What is the meaning? Indian Pacing Electrophysiol J. 2012; 12(5):213-25. doi: 10.1016/s0972-6292(16)30544-7.
3. Pascual-Figal DA, Januzzi JL. The biology of ST2: The international ST2 consensus panel. Am J Cardiol. 2015; 115(7 Suppl):3B-7B. doi: 10.1016/j.amjcard.2015.01.034.
4. Binas D, Daniel H, Richter A, Ruppert V, Schlüter KD, Schieffer B et al. The prognostic value of sST2 and galectin-3 considering different aetiologies in non-ischemic heart failure. Open Heart. 2018; 26;5(1): e000750. doi: 10.1136/openhrt-2017-000750.
5. You H, Jiang W, Jiao M, Wang X, Jia L, You S, et al. Association of soluble ST2 serum levels with outcomes in pediatric dilated cardiomyopathy. Can J Cardiol. 2019; 35(6):727-735. doi: 10.1016/j.cjca.2019.02.016.
6. Aimo A, Migliorini P, Vergaro G, Franzini M, Passino C, Maisel A et al. The IL-33/ST2 pathway, inflammation and atherosclerosis: Trigger and target? Int J Cardiol. 2018; 267:188-192. doi: 10.1016/j.ijcard.2018.05.056.
7. Mohamadpour AH, Abdolrahmani L, Mirzaei H, Sahebkar A, Moohebati M, Ghorbani M, et al. Serum osteopontin concentrations in relation to coronary artery disease. Arch Med Res. 2015; 46(2):112-7. doi: 10.1016/j.arcmed.2015.02.005.
8. Giachelli CM, Bae N, Almeida M, Denhardt DT, Alpers CE, Schwartz SM. Osteopontin is elevated during neointima formation in rat arteries and is a novel component of human atherosclerotic plaques. J Clin Invest. 1993; 92(4):1686-96. doi: 10.1172/JCI116755.
9. Icer MA, Gezmen-Karadağ M. The multiple functions and mechanisms of osteopontin. Clin Biochem. 2018; 59, 17-24. doi: 10.1016/j.clinbiochem.2018.07.003.
10. Van der Veen BS, de Winther MP, Heeringa P. Myeloperoxidase: molecular mechanisms of action and their relevance to human health and disease. Antioxid Redox Signal. 2009; 11(11):2899-937. doi: 10.1089/ars.2009.2538.
11. Stocker R, Huang A, Jeranian E, Hou JY, Wu TT, Thomas SR, et al. Hypochlorous acid impairs endothelium-derived nitric oxide bioactivity through a superoxide-dependent mechanism. Arterioscler Thromb Vasc Biol. 2004; 24(11):2028-33. doi: 10.1161/01.ATV.0000143388.20994.fa.
12. Sugiyama S, Kugiyama K, Aikawa M, Nakamura S, Ogawa H, Libby P. Hypochlorous acid, a macrophage product, induces endothelial apoptosis and tissue factor expression: involvement of myeloperoxidase-mediated oxidant in plaque erosion and thrombogenesis. Arterioscler Thromb Vasc Biol. 2004; 24(7):1309-14. doi: 10.1161/01.ATV.0000131784.50633.4f.
13. Ehrenfeld P, Matus CE, Pavicic F, Toledo C, Nualart F, Gonzalez CB, et al. Kinin B1 receptor activation turns on exocytosis of matrix metalloprotease-9 and myeloperoxidase in human neutrophils: involvement of mitogen-activated protein kinase family. J Leukoc Biol. 2009 ;86(5):1179-89. doi: 10.1189/jlb.0109012.
14. Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol. 1982;78(3):206-9. doi: 10.1111/1523-1747.ep12506462.
15. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106(25):3143-421.
16. Caliskan B, Korkmaz AN, Erdem F. Contribution of fragmented QRS on myocardial perfusion imaging in the assessment of functionally significant coronary artery stenoses. Eur Rev Med Pharmacol Sci. 2016; 20(8):1575-81.
17. Das MK, Saha C, El Masry H, Peng J, Dandamudi G, Mahenthiran J et al. Fragmented QRS on a 12-lead ECG: a predictor of mortality and cardiac events in patients with coronary artery disease. Heart Rhythm. 2007; 4(11):1385-92. doi: 10.1016/j.hrthm.2007.06.024.
18. Maisel A, Xue Y, Shah K, Mueller C, Nowak R, Peacock WF, et al. Increased 90-day mortality in patients with acute heart failure with elevated copeptin: secondary results from the Biomarkers in Acute Heart Failure (BACH) study. Circ Heart Fail. 2011;4(5):613-20. doi: 10.1161/CIRCHEARTFAILURE.110.960096.
19. Demyanets S, Speidl WS, Tentzeris I, Jarai R, Katsaros KM, Farhan S, et al. Soluble ST2 and interleukin-33 levels in coronary artery disease: relation to disease activity and adverse outcome. PLoS One. 2014; 9(4):e95055. doi: 10.1371/journal.pone.0095055.
20. Marino R, Magrini L, Orsini F, Russo V, Cardelli P, Salerno G, et al. Comparison between soluble ST2 and high-sensitivity troponin I in predicting short-term mortality for patients presenting to the emergency department with chest pain. Ann Lab Med. 2017; 37(2):137-146. doi: 10.3343/alm.2017.37.2.137. PMID: 28029000.
21. Aleksova A, Paldino A, Beltrami AP, Padoan L, Iacoviello M, Sinagra G, et al. Cardiac biomarkers in the emergency department: the role of soluble ST2 (sST2) in acute heart failure and acute coronary syndrome-there is meat on the bone. J Clin Med. 2019; 8(2):270. doi: 10.3390/jcm8020270.
22. Trueblood NA, Xie Z, Communal C, Sam F, Ngoy S, Liaw L, et al. Exaggerated left ventricular dilation and reduced collagen deposition after myocardial infarction in mice lacking osteopontin. Circ Res. 2001; 88(10):1080-7. doi: 10.1161/hh1001.090842.
23. Szalay G, Sauter M, Haberland M, Zuegel U, Steinmeyer A, Kandolf R, et al. Osteopontin: a fibrosis-related marker molecule in cardiac remodeling of enterovirus myocarditis in the susceptible host. Circ Res. 2009 Apr 10;104(7):851-9. doi: 10.1161/CIRCRESAHA.109.193805.
24. Mazzone A, Parri MS, Giannessi D, Ravani M, Vaghetti M, Altieri P, et al. Osteopontin plasma levels and accelerated atherosclerosis in patients with CAD undergoing PCI: a prospective clinical study. Coron Artery Dis. 2011; 22(3):179-87. doi: 10.1097/MCA.0b013e3283441d0b.
25. Yu K, Yang B, Jiang H, Li J, Yan K, Liu X, et al. A multi-stage association study of plasma cytokines identifies osteopontin as a biomarker for acute coronary syndrome risk and severity. Sci Rep. 2019; 9(1):5121. doi: 10.1038/s41598-019-41577-4.

26. Rasool Hussein AA, Issa AH, Al-Saffar AF. Role of serum osteopontin and osteoprotegerin levels in the diagnosis of the acute coronary syndrome and its subtypes. Indian Journal of Public Health Research & Development 2019;10(5):371-76.

27. Omran MM, Zahran FM, Kadry M, Belal AA, Emran TM. Role of myeloperoxidase in early diagnosis of acute myocardial infarction in patients admitted with chest pain. J Immunoassay Immunochem. 2018; 39(3), 337-347.doi: 10.1080/15321819.2018.1492423.

28. Goldmann BU, Rudolph V, Rudolph TK, Holle AK, Hillebrandt M, Meinertz T, et al. Neutrophil activation precedes myocardial injury in patients with acute myocardial infarction. Free Radic Biol Med. 2009; 47(1):79-83. doi:10.1016/j.freeradbiomed.2009.04.004.

29. Sim DS, Ahn Y. Novel inflammatory biomarkers in acute coronary syndrome. Korean J Intern Med. 2013; 28(2), 156.doi: 10.3904/kjim.2013.28.2.156.

30. Gururajan P, Gurumurthy P, Nayar P, Babu S, Sarasabharati A, Victor D, et al. Serum myeloperoxidase: a novel biomarker for evaluation of patients with acute coronary syndrome. Heart Asia. 2009;1(1):41-6. doi: 10.1136/ha.2009.000778.