Combined Assessments of Multi-panel Biomarkers for Diagnostic Performance in Coronary Artery Disease: Case-Control Analysis

Amir Al-Mumin[1], Hayder Abdul-Amir Makki Al-Hindy[2*], Mazin Jaafar Mousa[3]

[1]Ass. Prof., Interventional Cardiologist, Hammurabi Medical College, University of Babylon, Iraq
Email: dr.amir.saheb@gmail.com
[*2]Ass. Prof., Ph.D., Medical Physiology, College of Pharmacy, University of Babylon, Iraq
Email: phar.hayder.abdul@uobabylon.edu.iq
[3]Ass. Prof., Histopathologist. College of Pharmacy, University of Babylon
E-mail: mazin.mousa@gmail.com
Author Correspondence: Hayder Abdul-Amir Makki Al-Hindy

ABSTRACT

Background: Understanding the etiopathology of coronary artery diseases (CAD) has directed studies towards the evaluation of novel serum biomarkers as potential diagnostic tools for the clinical setting. In addition to the current gold standard cardiac troponin-I (TnI), several other biomarkers had been studied for diagnosis, prediction, and prognosis of CAD. Of these biomarkers, N-terminal-proatrial natriuretic peptide (NT-ProANP), Cystatin-C (CyC), and highly-sensitive C-reactive protein (HSCRP).

Aim of Study: Evaluation of the diagnostic performance of combined multiple biomarkers in patients with CAD

Subjects and methods: the study included 136 patients diagnosed as CAD by cardiologists and 44 healthy group. All participants underwent echocardiographic examinations and LVEF % < 40 selected as a cutoff value for expressing LV-systolic dysfunction. Hence, CAD subjects were classified into two subgroups: LVEF ≤ 40% versus > 40%. Moreover, hematological tests for creatinine, urea, total lipid profile, TnI, NT-ProANP, CyC, and HSCRP within the first 24 h of admission were done. An angiographic study was completed by expert interventional cardiologists. Biostatistical scrutiny was finalized with SPSS Version-25, and GraphPad Prism 8.0.2. ROC study performed for all four markers to evaluate their, diagnostic accuracy for CAD. Pairwise assessment of ROC for the analytic ability of the multi-biomarkers was also performed using MedCalc 19.3.1 software.

Results: There were no significant differences regarding serum creatinine, urea nitrogen, and BMI between two study groups. The incidence of diabetes, hypertension, lipid profile components, and smoking were significantly higher in CAD patients. There was a significant difference between patients and control groups concerning mean LVEF%, however, only 1/4th of the CAD patients had an echocardiographic finding of LVSD. There were significantly higher concentrations of all four markers in CAD than in controls. ROC curves revealed that AUCs of TnI, NT-ProANP, CyC and HSCRP were, respectively, 0.93 (p-0.001), 0.69 (p-0.001), 0.82 (p-0.4) and 0.69 (p-0.001); the highest was for TnI. There was no statistically significant difference between the diagnostic performance power (differences in AUC) of NT-ProANP and HSCRP. Nevertheless, TnI has the highest AUC followed by CyC. Pairwise comparison of the combined biomarkers of TnI and HSCRP appeared to perform best in predicting CAD (P-0.0001). The next best performing couple is the addition of TnI and NT-ProANP (P-0.001). Using HSCRP with TnI and NT-ProANP in a three-markers panel, or a four-markers panel with CyC yielded no further diagnostic value (results not shown). Combining either CyC or HSCRP and NT-ProANP, provided no extra information in the diagnosis of CAD than it would if either were studied uncoupled

Keywords: Coronary artery disease, biomarkers, NT-ProANP, HSCRP, Cystatin-C and troponin-I

Correspondence:
Hayder Abdul-Amir Makki Al-Hindy
Ass. Prof., Ph.D., Medical Physiology, College of Pharmacy
University of Babylon
Iraq
E-mail: phar.hayder.abdul@uobabylon.edu.iq
DOI: 10.31838/srp.2020.6.99

INTRODUCTION

As a worldwide most common cause of debility and mortality (1), early diagnosis is critical in the management of patients with suspected or established coronary artery diseases (CAD) (2, 3). In the current era, a profound understanding of the etiopathology of atherosclerosis as the underlying mechanism of CAD has directed studies towards the evaluation of novel serum biomarkers as potential diagnostic tools for the clinical setting (4). Estimation of serum troponin-I (TnI) is considered the "gold standards" indicator for diagnosis of cardiac necrosis owing to its high cardiomyocytic expression rendering TnI as extremely sensitive/specific for cardiac necrosis (5). Additionally, several biomarkers have been introduced as sensitive biomarkers for minimal cardiac injury like N-terminal pro-Atrial natriuretic peptide (NT-ProANP) (6), cystatin-C (CyC) (3), highly sensitive CRP (HSCRP) (7), and others. Consequently, numerous analyses have confirmed the independent diagnostic value of elevated TnI, NT-ProANP, CyC, and HSCRP at admission in patients with CAD (6-10). However, the combination of these biomarkers for the diagnosis of CAD earlier post-admission has not been evaluated analytically (11). There is still a substantial argument about which biomarkers, in which combinations, should be designated. To address these topics, we measured the levels of multi-panel biomarkers at the time of presentation to the emergency department (ED).

AIMS OF THE STUDY

The present study aims to assess the value of combined multi-panel diagnostic accuracy of TnI, NT-ProANP, CyC, and HSCRP in patients with CAD at the time of admission.

MATERIALS AND METHODS

Study design and population

Of an overall 723 inpatients diagnosed as CAD by cardiologists and being admitted to Shahidul-Mihrab Cardiac Center, between September 1st - October 30th, 2019 with a diagnosis of CAD, we investigated 136 patients. All CAD patients were managed consistent with the standard protocols and undertook primary PCI. The diagnostic regimen included history, 12 leads ECG, blood assays, and a 2D echo-exam. The time between the onset of ischemic symptoms until the venous sample to evaluate blood markers was less than 24 hours. The residual 587 patients were excepted for a couple of reasons: > 24 hours elapsing prior

venous sampling, or if at least one of the four biomarkers had not been measured. The healthy control group (44 subjects) were selected sex and age-matched being free from any cardiac illnesses. Both echocardiographic and coronary angiographic examinations were completed by expert blinded operatives and interventionalists through standardized procedures. Biomarkers were measured routinely using available diagnostic kits in the markets: CALBIOTECH® ELISA kit (for both TnI and HSCRP) and Elabscience® Human ELISA kit (for both ProANP and CyC). The accepted normal value of TnI was 0.5 ng/ml, measurements ≥0.5ng/ml on admission was used as a serum marker for myocardial ischemia.

Plasma Analytes
All the studied participants had been inspected for the following blood investigations: creatinine, urea, TnI, NT-ProANP, CyC, HSCRP, triglycerides, total cholesterol, low-density, very low-density, and high-density lipoproteins. The entire biochemical assays were accomplished as detailed by the manufacturer conventions and available techniques.

Echocardiographic Examination
The systematized two-D chest echocardiography was achieved by two separate echographers using an ultrasound system (GE-Medical Systems, Vivid®-3). Left ventricular ejection fraction (LVEF%) was assessed by "modified Simpson's method". LVEF % < 40 selected as a cutoff- value for expressing LV-systolic dysfunction. Hence, CAD subjects had been classified into two subgroups: LVEF ≤ 40% versus > 40% (12).

Ethical Approval
Informed written permission at the beginning was obtained from each patient (or family member) & control groups separately, and the study was approved by the local committee for research ethics at the Babylon Health Directorate.

Biostatistical Analysis
Biostatistical scrutiny was finalized with SPSS Version-25 and GraphPad Prism 8.0.2. Comparisons of continuous data (given as mean±SD) were completed using *t*-tests for independent variables whereas categorical data as a percentage and compared by *chi*-square exam. A *p*-value of < 0.05 indicates statistica1 significance. ROC study performed for all four markers to evaluate their, specificity, sensitivity, and diagnostic accuracy for CAD. The resultant cutoff from ROC assay provided a proper critical point for each biomarker as a confident choice for evaluation if higher diagnostic value generated from using two combined markers. Pairwise assessment of ROC for analytic ability of the multiple biomarkers were also performed using MedCalc 19.3.1 software.

RESULTS
Patient Characteristics
The mean age of studied patients was 56.8 years (29 - 89 years), females represent 50 (27.8%) of them, while the mean BMI was 27.2 kg/m2, with no significant differences between CAD patients and controls. Sites of the ischemic cardiac segment of ACS patients were as follows: 39% was inferior, 39 % anterior, while 22 % were either inferolateral or unclassified sites. There were no significant differences regarding serum creatinine, urea nitrogen, and BMI between two study groups. The incidence of diabetes, hypertension, and smoking was significantly higher in CAD patients.

Echocardiographic Assessment
Although there was a significant difference between patients and control groups concerning mean LVEF%, however, only 1/4th of the CAD patients had an echocardiographic finding of LV systolic dysfunctions in terms of lower levels of LVEF% (table-1).

Biomarkers and Lipidemic Analyses
There were significant higher concentrations of the all four markers in CAD than in control group (table-1): (7.9±8.7 vis 0.02±0.05 ng/ml, *P*-0.001), (361.1±173.8 vis 268.3±180.9 pmol/l, *P*-0.005), (1.98±2.3 vis 0.67±0.06 mg/L, *P*-0.001), and (8.8±7.2 vis 5.15±4.3 mg/ml, *P*-0.001) for TnI, NT-ProANP, CyC, and HSCRP consecutively. Equally, the mean total cholesterol, low-density, very low-density, and were higher significantly in patients than controls.

Table 1: Baseline Characteristics of the Studied Subjects

Characteristics		Total	CAD (136)	Control (44)	P-value
Age		56.8	61.3	35.8	0.05
Active Smoking - No (%)		96 (53.3)	72 (52.9)	24 (54.5)	0.05
BMI (kg/m²)		27.1±5.0	27.2 ± 5.16	26.7±5.4	>0.05
Female Sex - No (%)		50 (27.8)	44 (32.4)	12 (27)	0.05
Left Ventricular Ejection Fraction%		50.4±11.8	50.4	67.9	0.05
Category of LVEF% In CAD Patients	< 40%	34 (26.9)			
	> 40%	92 (73.8)			
Blood Urea (mmol/l)		7.1±3.0	7.3±3.0	6.9±4.5	> 0.05
Creatinine (mmol/l)		86.1±0.9	81.8±27.4	77.6±16.8	> 0.05
No of Affected Coronary Arteries		2.3±0.9			
Sites of Ischemic Cardiac Segment					

Inferior Segment	39%			
Anterior Segment	39%			
Inferolateral Segment	13%			
Unclassified sites	9%			
Troponin-I (ng/ml)	6.31±8.4	7.9±8.7	0.02±0.05	0.001
NT-ProANP (pmol/l)	329.7±181.0	361.1±173.8	268.3±180.9	0.005
Cystatin-C (mg/L)	1.59±1.6	1.98±2.3	0.67±0.06	0.001
HSCRP (mg/ml)	7.4±6.7	8.8±7.2	5.15±4.3	0.001
Hypertension - No (%)	65 (%)	60 (44.8)	5 (11.4)	0.05
Diabetes Mellitus - No (%)	55 (%)	51 (38.1)	4 (9.1)	0.05
Triglycerides	2.4±0.6	2.8±03	2.4±0.6	> 0.05
Cholesterol	4.9±1.1	5.6±0.7	4.6±1.1	0.05
High Density Lipoprotein	4.8±1.1	5.6±0.7	4.9±1.0	> 0.05
Very Low-Density Lipoprotein	0.48±0.1	0.5±0.1	0.4±0.1	0.05
Low Density Lipoprotein	2.9±1.8	4.3±0.8	2.8±1.9	0.05

Isolated Diagnostic Performance of The Four Biomarkers
Studies of isolated ROC curves of all biomarkers were shown in both figure-1 and table-2. The AUCs of TnI, NT-ProANP, CyC and HSCRP were, respectively, 0.93 (*p*-0.001), 0.69 (*p*-0.001), 0.82 (*p*-0.4) and 0.69 (*p*-0.001); the highest was for TnI. There was no statistically significant difference between the diagnostic performance power (differences in AUC) of NT-ProANP and HSCRP. Nevertheless, TnI has the highest AUC followed by CyC. The sensitivity and specificity of both TnI and NP-ProANP were parallel (77% and 63.6%) for the diagnosis of CAD. Whereas, sensitivity CyC was higher than HSCRP and specificity of (70.5 vis 67.7), which is not the case for the specificity (65.9 vis 71.4). These results could be caused to some extent by the early presentation of CAD patients besides rapid blood sampling on arrival following the initial complaint. Youden Index J, for all four biomarkers, were comparable ($\approx$ 0.40).
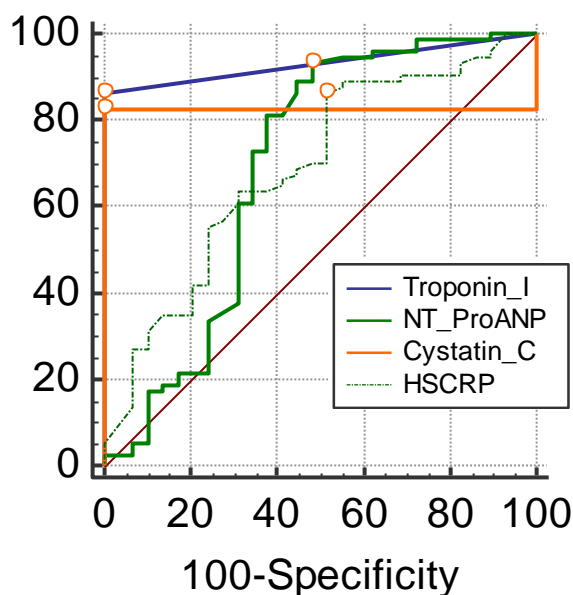

Figure 1: ROC Study of four biomarkers: Troponin-I, NT-ProANP, Cystatin-C, and HSCRP for the diagnostic values of Coronary Heart Diseases.

Table 2: Area under the ROC curve (AUC) for Diagnostic Accuracy of the Four Biomarkers for Coronary Heart Diseases

	Troponin-I	NT-ProANP	Cystatin-C	HSCRP
AUC	0.93	0.69	0.82	0.69
Significance	0.001	0.001	0.4	0.001
95% Confidence Interval	0.87 to 0.97	0.59 to 0.78	0.74 to 0.89	0.59 to 0.78
Sensitivity	77.9%	77.9%	70.5%	67.7%
Specificity	63.64%	63.6%	65.9%	71.4%
Cut-off Value	2.06	211.5	1.6	5.49

Youden Index J	0.42	0.42	0.36	0.39

Combined Diagnostic Performance of The Four Biomarkers

Based on the pairwise comparison of ROC studies for the diagnostic ability of the combined biomarkers for CAD calculation, the combination of TnI and HSCRP appeared to perform best in predicting the event of CAD (P-0.0001). the next best performing couple is the addition of TnI and NT-ProANP (P-0.001). Using HSCRP with TnI and NT-ProANP in a three-markers panel, or in a four-markers panel with CyC yielded no further diagnostic value (results not shown). Combining either CyC or HSCRP and NT-ProANP, provided no extra information in the diagnosis of CAD than it would if either were studied uncoupled (table-3).

Table 3: Pairwise Comparison of ROC for Diagnostic Significance of the Multiple Biomarkers for Coronary Heart Diseases.

	Troponin-I	NT-ProANP	Cystatin-C	HSCRP
Troponin-I (AUC: 0.93%)	—	P-0.001	P-0.03	P-0.0001
NT-ProANP (AUC: 0.69%)	—	—	P-0.1	P-0.9
Cystatin-C (AUC: 0.82%)	—	—	—	P-0.08
HSCRP (AUC: 0.69%)	—	—	—	—

Spearman's-correlation revealed that patients with a calculated multi-marker in the highest measures showed a higher diagnostic power of CAD (results not shown). Whereas there were positive non-significant correlations among all biomarkers with both LVEF and number of diseased coronary arteries by angiography apart from TnI that showed a negative significant correlation with LVEF (table-4).

Table 4: Correlation of the Four Biomarkers to LVEF% and Number of Diseased Coronary Arteries

	Troponin-I		NT-ProANP		Cystatin-C		HSCRP	
	r	p	r	p	r	p	r	p
LVEF%	-0.14	0.1	0.02	0.8	0.02	0.8	0.03	0.7
No of Diseased Coronaries	-0.01	0.9	0.07	0.5	0.02	0.8	0.07	0.5

DISCUSSION

The study revealed that initial plasma values of TnI, NT-ProANP, CyC, and HSCRP on admission were higher in patients than in the control group, and these raised biomarkers have a diagnostic accuracy for CAD. Moreover, concentrations of biomarkers at admission were not correlated with the LVEF% and angiographic findings. Of note, these four biomarkers had been selected in such a way (13) to evaluate cardiomyocyte damage, LV dysfunction, inflammation, and renal dysfunction, (TnI, NT-ProANP, CyC, and HSCRP). Though TnI is currently the hallmark biomarker for CAD, it is typically within normal during the first few hours after the onset of ischemia (14). Hence, for early presented patients, cardiologists must wait a few hours prior to final management can be started (15). These findings highpoint the incremental efficacy of combined biomarkers approach that mirror harmonizing axes of CAD (16). Supporting our findings of the multi-marker approach can increase the diagnostic accuracy and provide more information for the risk stratification of CAD are several scholars have published beneficial effect of combining multimarkers dogma (17-20). In contrast, there were non-significant variances of a paired comparison of the ROC curve study among cardiomarker tests in other analyses (21). The most noteworthy remark in this study is that HSCRP above all other cardiomarkers tested enhances TnI in diagnosing patients with CAD. It is notable that TnI was found to be independently superior to the other three biomarkers in predicting CAD (22). The current finding in our smaller cohort of patients supports this observation. There are several main arguments that can be advanced to support our finding of HSCRP as a possible diagnostic marker in CAD *per se* (7-9, 23) or combined with other biomarkers (8, 9, 24, 25). Clinical trials and laboratory studies have exposed that inflammations play a dynamic role in the coronary-sclerosis. High levels of CRP are perhaps pointing to ongoing inflammation and vascular events besides a higher likelihood of plaque instability (7-9). Then again raised HSCRP is a substantial prognostic pointer for initial risk stratification in patients with CAD (3, 8) and correlated to the risk of recurrent attack (7). In contrast, the nonsignificant statistical variation observed between stable angina and healthy groups (31).

In agreement with preceding studies (11, 22) elevated levels of TnI and atrial peptides were associated with higher rates of adverse cardiac events. When used in combination, TnI and NT-ProANP appeared to perform second-best in diagnosing CAD. It is identified that ANP and BNP are released from cardiocytes during cardiac necrosis. The extent of the response in ANP or BNP has been exposed by some authors (32). Basically, assuming that cardionecrosis and neurohumoral stimulation are the overriding pathways that fortify the etiology of CAD. As claimed by other survey (11), we find that NT-ProANP and TnI collectively, perform better than either marker *per se* in predicting CAD. Pathophysiologically, ventricular stressing during cardiac

necrosis, releases neurohormones which might be measured even when cardiac necrosis is not existing or is yet to ensue. In this respect, previous studies established the additional value of neuropeptides when added to the old markers to increase sensitivity for ischemic pain (26) were completed in the era of classical TnI analysis and very little evidence is available on the utility of these biomarkers in the existing milieu of highly sensitive TnI use (15). These studies comprised various factors disturbing the normal levels of neuropeptides (e.g., age, gender, BMI, kidney function), all of which are hardly adjusted with small sample sizes (15).

By contrast, TnI and neuropeptides were not independent predictors of CAD although they performed better when combined together as a diagnostic or a predictor for CAD. This inconsistency with prior works possibly clarified by the rather small sample size (11) or timing of blood sampling. These additive effects might be clarified by the diverse etiopathological pathways of these two markers in CAD, i.e., high TnI specify cardiocyte necrosis and NT-ProANP is elevated in response to atrial stretch (6).

This study showed that CyC alone is the weakest significant marker in predicting CAD. Moreover, using CyC, within any variation for biomarkers (except with TnI) or as part of a complete four-markers panel provided no further performance in diagnosis of CAD. Eriksson et al., determined that CyC may reflect confidently the presence or absence of CAD as well Abed et al, reported such association (3, 27). Yet, the precise explanations for such associations are indefinite (28).

Current researches demonstrate that multi-biomarker cardiac tests can decrease ED interval time from (24-48 h) to (3-4 h) (29) and can reduce patient medical costs owing to increase the speediness of diagnossis (30).

A paradoxical correlation of the study biomarkers with LVEF and thenumber of diseased coronary arteries can not be explained precisely, but the timing of blood collection from admission or the onset of the ischemic symptoms might be one the acceptable possibilities.

To the best of the authors' knowledge, this is the first study in Iraq; comparing TnI, NT-ProANP, CyC, and HSCRP along with angiography and echo exam in patients with CAD admitted to the ED. The authors observed that the ROC curve did not express a good pattern as anticipated, which pointed out study limitations. A sample size of 136 patients might not practically large adequately. In view of the above, our belief is that the case is clearly not so far closed. Further analogous study with a higher number of patients is desirable to afford robust evidence to select biomarkers with high diagnostic, predictive, and prognostic capacity for CAD. Nevertheless, we are a long way from this ultimate target.

The outcomes of this study designate that multi-markers tests are more useful than a single TnI test. With its reasonably high rates of sensitivity, multi-panel cardiomarkers testing can support physicians in precisely ruling in and out doubtful patients with ischemic chest pain.

CONCLUSIONS
This study suggests an association among high blood levels of TnI, NT-ProANP, CyC, and HSCRP with a diagnosis of CAD at admission. Combined assessments of two biomarkers for diagnostic performance for CAD were better than single biomarkers for TnI, and NT-ProANP, TnI and HSCRP, but not for other combinations owing to multifaceted pathophysiology of CAD. Additional works are mandated to expose the perfect model of combined markers besides conventional risk factors.

LIMITATIONS
There are several limitations in the present work. Single-center based and the small number of enrolled patients may increase the likelihood of selection bias and low statistical influence can interrupt results explanation. An additional weak point is this study not include the effects of medications taken by the patients. Adding BNP and NT-ProBNP can further augment study outcomes in future works.

CONFIDENTIALITY OF DATA
The authors declare that they have followed the protocols of their work center on the publication of patient data and that all the patients included in the study received sufficient information and gave their written informed consent to participate in the study.

RIGHT TO PRIVACY AND INFORMED CONSENT
The authors declare that no patient data appear in this article.

CONFLICTS OF INTEREST
The authors have no conflicts of interest to declare.

FINANCIAL SUPPORT AND SPONSORSHIP
Nil.

REFERENCES
1. Hayder Abdul- Amir Maki Al-hindy ASAM, Basim MH Zwain. Decayed, Missed, Filled Teeth Scores in Iraqi Patients with Acute Myocardial Infarctio. Al-Kufa University Journal for Biology. 2016;8(3):7.
2. Hayder Abdul- Amir Maki Al-hindi SFA-S, Basim MH Zwain, Thekra Abid Al-Kashwan Jaber Relationship of Salivary & Plasma Troponin Levels of Patients with AMI in Merjan medical city of Babylon Province: Cross-Sectional Clinical Stud. Al-Kufa University Journal for Biology. 2016;8 (3):6.
3. Abed DA, Jasim R, Al-Hindy HA-A, Obaid AW. Cystatin-C in patients with acute coronary syndrome: Correlation with ventricular dysfunction, and affected coronary vessels. Journal of Contemporary Medical Sciences. 2020;6(1).
4. Catarina Vieira ea. Multimarker approach with cystatin C, N-terminal pro-brain natriuretic peptide, C-reactive protein and red blood cell distribution width in risk stratification of patients with acute coronarysyndromes. Portuguesa de Cardiologia. 2014;33(3):10.
5. Aydin S UK, Aydin S, Sahin İ, Yardim M. Biomarkers in acute myocardial infarction: current perspectives. Vasc Health Risk Manag. 2019;15:10.

6. Amir Al-Mumin MJM, Nawrass J Alsalihi. Circulating Atrial Neuropeptides (NT-pANP) vis Cardiac-Troponin (cT-I) After Acute Coronary Syndrome: Relationship with Left Ventricular Ejection Fraction and Number of Affected Vessels by Coronary Angiography". Biochemical and Cellular Archives. 2020;20(2).

7. Hayder Abdul-Amir Maki Al-hindi MJM, Thekra Abid Jaber Al-kashwan, Ahmed Sudan, Saja Ahmed Abdul-Razzaq. On Admission Levels of High Sensitive C-Reactive Protein as A Biomarker in Acute Myocardial Infarction: A Case-Control Study. Indian Journal of Public Health Research & Development. 2019;10(4):5.

8. Asseel K. Shaker RA-S, Raad Jasim, Hayder Abdul-Amir Makki Al-Hindy. Biochemical Significance of Cystatin-C and High-Sensitive CRP in Patients with Acute Coronary Syndrome; any Clinical Correlation with Diagnosis and Ejection Fraction. Systematic Review Pharmacy. 2020;11(3):8.

9. Hajir Karim Abdul-Husseein FSD, Ameera Jasim Al-Aaraji, Hayder Abdul-Amir Makki Al-Hindy, Mazin Jaafar Mousa. Biochemical causal-effect of circulatory uric acid, and HSCRP and their diagnostic correlation in admitted patients with ischemic heart diseases. Journal of Cardiovascular Disease Research 2020;11(4).

10. Hayder Abdul-Amir Maki Al-Hindi MJM, Thekra Abid Jaber Al-Kashwan, Ahmed Sudan, Saja Ahmed Abdul-Razzaq. Correlation of on Admission Levels of Serum Uric Acid with Acute Myocardial Infarction: Case : Control Study. Journal of Global Pharma Technology. 2019;11:6.

11. Jong Shin Woo JMC, Soo Joong Kim, Myeong Kon Kim, and Chong Jin Kim. Combined Assessments of Biochemical Markers and ST-Segment Resolution Provide Additional Prognostic Information for Patients With ST-Segment Elevation Myocardial Infarction. Korean Circ J. 2011;41:7.

12. Mentzer G, Hsich EM. Heart Failure with Reduced Ejection Fraction in Women: Epidemiology, Outcomes, and Treatment. Heart Fail Clin. 2019;15(1):19-27.

13. Lind L. Population-based cardiovascular cohort studies in Uppsala. Upsala Journal of Medical Sciences. 2019;124(1):16-20.

14. Morrow DA, Braunwald E. Future of biomarkers in acute coronary syndromes: moving toward a multimarker strategy. Circulation. 2003;108(3):250-2.

15. Fernández-Jiménez R, Fernández-Ortiz A. Multimarker Panel for Patients With Chest Pain: Case Closed? Revista Española de Cardiología. 2013;66(7):523-5.

16. McCord J, Nowak RM, McCullough PA, Foreback C, Borzak S, Tokarski G, et al. Ninety-minute exclusion of acute myocardial infarction by use of quantitative point-of-care testing of myoglobin and troponin I. Circulation. 2001;104(13):1483-8.

17. Silva D. Multimarker approach in risk stratification of patients with acute coronary syndromes: Towards the ideal stratification. Revista Portuguesa de Cardiologia (English edition). 2014;33(3):137-8.

18. Chen Y, Tao Y, Zhang L, Xu W, Zhou X. Diagnostic and prognostic value of biomarkers in acute myocardial infarction. Postgrad Med J. 2019;95(1122):210-6.

19. Schernthaner C, Lichtenauer M, Wernly B, Paar V, Pistulli R, Rohm I, et al. Multibiomarker analysis in patients with acute myocardial infarction. Eur J Clin Invest. 2017;47(9):638-48.

20. Sabatine MS, Morrow DA, de Lemos JA, Gibson CM, Murphy SA, Rifai N, et al. Multimarker approach to risk stratification in non-ST elevation acute coronary syndromes: simultaneous assessment of troponin I, C-reactive protein, and B-type natriuretic peptide. Circulation. 2002;105(15):1760-3.

21. Tsai W, Chien D-K, Huang C-H, Shih S-C, Chang W-H. Multiple Cardiac Biomarkers Used in Clinical Guideline for Elderly Patients with Acute Coronary Syndrome. International Journal of Gerontology. 2017;11(2):104-8.

22. Cameron SJ, Sokoll LJ, Laterza OF, Shah S, Green GB. A multi-marker approach for the prediction of adverse events in patients with acute coronary syndromes. Clin Chim Acta. 2007;376(1-2):168-73.

23. Wang J, Tang B, Liu X, Wu X, Wang H, Xu D, et al. Increased monomeric CRP levels in acute myocardial infarction: a possible new and specific biomarker for diagnosis and severity assessment of disease. Atherosclerosis. 2015;239(2):343-9.

24. Kilic T, Oner G, Ural E, Yumuk Z, Sahin T, Bildirici U, et al. Comparison of the long-term prognostic value of cystatin C to other indicators of renal function, markers of inflammation and systolic dysfunction among patients with acute coronary syndrome. Atherosclerosis. 2009;207(2):552-8.

25. Inanc M. Copeptin, Troponin-I, Pro-BNP and hs-CRP levels in Diagnosing Acute Coronary Syndromes. Angiology: Open Access. 2016;04.

26. Bassan R, Potsch A, Maisel A, Tura B, Villacorta H, Nogueira MV, et al. B-type natriuretic peptide: a novel early blood marker of acute myocardial infarction in patients with chest pain and no ST-segment elevation. Eur Heart J. 2005;26(3):234-40.

27. Eriksson P JK, Brown LC, Greenhalgh RM, Hamsten A, Powell JT. Genetic approach to the role of cysteine proteases in the expansion of abdominal aortic aneurysms. Br J Surg. 2004;91(1):4.

28. Keller T MC, Lubos E, Nicaud V, Wild PS, Rupprecht HJ, et al. Cystatin C and cardiovascular mortality in patients with coronary artery disease and normal or mildly reduced kidney function: Results from the AtheroGene study. Eur Heart J. 2009;30(3):7.

29. Than M, Cullen L, Reid CM, Lim SH, Aldous S, Ardagh MW, et al. A 2-h diagnostic protocol to assess patients with chest pain symptoms in the Asia-Pacific region (ASPECT): a prospective observational validation study. Lancet. 2011;377(9771):1077-84.

30. Birkhahn RH, Haines E, Wen W, Reddy L, Briggs WM, Datillo PA. Estimating the clinical impact of bringing a multimarker cardiac panel to the bedside in the ED. Am J Emerg Med. 2011;29(3):304-8.

31. Wang XH, Liu SQ, Wang YL, Jin Y. Correlation of serum high-sensitivity C-reactive protein and interleukin-6 in patients with acute coronary syndrome. *Genet Mol Res.* 2014;13(2):4260-4266. Published 2014 Jun 9. doi:10.4238/2014.June.9.11.

32. Inanc MT, Cerit N, Elcik D, Kalay N, Kaya MG, et al. Copeptin, Troponin-I, Pro-BNP and hs-CRP levels in Diagnosing AcuteCoronary Syndromes. Angiology: 2016, 4:1 169. DOI: 10.4172/2329-9495.1000169