

# Clinical diagnostic value of Stromelysin-2 and High Sensitive-Cardiac Troponin I in the Severity of Coronary Artery Obstruction

Amal A. Hammoodi <sup>1\*</sup> , Basil O. Saleh<sup>1</sup> , Ameen A. Al-Alwany<sup>2</sup> 

<sup>1</sup> Department of Clinical Biochemistry, College of Medicine, University of Baghdad, Baghdad, Iraq.

<sup>2</sup> Department of Internal Medicine, College of Medicine, University of Baghdad, Baghdad, Iraq.



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## Abstract

**Background:** Coronary artery disease is a leading cause of death worldwide. Troponin is released into blood from patients with reversible myocardial ischemia. Stromelysin-2 is also involved in the development and progression of atherosclerosis by tissue remodeling and degradation of extracellular matrix proteins.

**Objectives:** To investigate the role of serum high-sensitive cardiac troponin I (hs-cTn I) and Stromelysin-2 (Str-2) in the diagnosis and determination of the severity of coronary artery obstruction.

**Methods:** This cross-sectional study was conducted on 125 subjects undergoing coronary angiography for suspicion of coronary artery disease in Baghdad Teaching Hospital, Medical City complex/ Baghdad/ Iraq by the Department of Biochemistry, College of Medicine, University of Baghdad between March and December 2024. The participants were divided into three groups: Group I included 48 subjects with no coronary artery lesion or with < 50% stenosis, Group II included 47 subjects with > 50% stenosis in a single or double coronary arteries, and Group III included 30 subjects with > 50% stenosis in ≥ three coronary arteries or ≥ 50% stenosis in the left main stem. Serum levels of hs-cTn I and Str-2 were measured by the ELISA technique.

**Results:** The median and 1<sup>st</sup> – 3<sup>rd</sup> quartile values of hs-cTn I and Str-2 levels of group III and group II were significantly higher than those of groups I ( $p = 0.001$ ). The median value of hs-cTn I was significantly higher in group III than in group II ( $p < 0.001$ ). Receiver operating characteristic curve and area under curve tests revealed that hs-cTn I and Str-2 have a high diagnostic ability of coronary artery obstruction along with a high discriminative ability of hs-cTn I in assessment of severity of coronary artery obstruction.

**Conclusion:** Measurements of serum Str-2 level can be used in the diagnosis of coronary artery obstruction, while serum hs-cTn I level can be used in the diagnosis and differentiation of severity of coronary artery obstruction. Combining both markers add no significant benefit.

**Keywords:** Atherosclerosis; Coronary Angiography; Coronary Artery Disease; Coronary Occlusion, Stromelysin-2.

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## Introduction

Coronary artery disease (CAD) is a leading cause of death worldwide (1). Coronary angiography is the gold standard diagnostic test with high diagnostic accuracy but has limitations, including being invasive, the use of ionizing radiation, high cost, and the need for special facilities (2). The definition of CAD is based on studies showing that a 50% stenosis begins to blunt coronary flow reserve. Angioplasty is recommended for lesions with > 70% stenosis, while lesions with < 50% usually respond to medical therapy (3). In chronic coronary syndromes (CCS), the pathophysiology is fixed atherosclerotic lesions. Prognosis is related to the number of diseased vessels and the degree of LV dysfunction.

Treatment ranges from medical therapy in mildly symptomatic patients to angioplasty for symptomatic patients with one or two - vessel disease despite medical therapy to coronary bypass surgery (CABG) for patients with left main, three-vessel disease with LV dysfunction and diabetics with CAD in two or more vessels including proximal left anterior descending artery. In acute coronary syndromes (ACS), the pathophysiology is an ulcerated or fissured atheromatous plaque with adherent thrombus and is considered the most severe form of CAD regardless of the number of diseased vessels because of the potential for fatal arrhythmia or severe LV dysfunction, and treatment is usually angioplasty or urgent CABG (4,5). The majority of the CAD is related to atherosclerosis, which is a chronic

\* Corresponding author:  
[aamal.abbas2209p@comed.uobaghdad.edu.iq](mailto:aamal.abbas2209p@comed.uobaghdad.edu.iq).

author:

inflammatory process characterized by endothelial dysfunction and subsequent plaque formation of the vascular wall (6). Matrix metalloproteinases (MMPs) are proteolytic enzymes secreted by endothelial cells, vascular smooth muscle, fibroblasts, osteoblasts, macrophages, neutrophils, and lymphocytes. They are involved in tissue remodeling and degradation of extracellular matrix proteins, and changes in their level are associated with an increased risk of cardiovascular disease (7).

Stromelysin-2 (Str-2), also known as MMP-10, breaks down collagen and other tissue substrates. Some studies correlate the absence of Str-2 with delayed plaque progression (8), while others correlate higher levels with atherosclerotic plaques in asymptomatic subjects (9) and with the risk of plaque rupture (10). Despite its potential role in the development and progression of atherosclerosis, no study has specifically explored its association with CAD severity, highlighting the need for further research in this area.

The troponin (Tn) complex, involved in the regulation of muscle contraction, consists of three subunits: I, T, and C. The former is the most heart disease-specific biomarker (11). Several theories clarify troponin release into blood from patients with reversible myocardial ischemia. These include the release of free subunits through bleb formation, transient imbalance of oxygen supply and demand, apoptosis, and myocardial stretching (12).

The aim of the current study was to determine the clinical application of serum high sensitive-cardiac troponin I (hs-cTn I) and stromelysin-2 separately or combined together in the assessment of coronary artery obstruction severity.

### Patients and Methods

This cross-sectional study was carried out in Baghdad Teaching Hospital/ Medical City complex/ Baghdad/ Iraq by the Department of Biochemistry/ College of Medicine/ University of Baghdad between March and December 2024. It included subjects aged between 18 and 80 years, with clinical suspicion of coronary artery disease undergoing elective coronary angiography. A cardiologist performed coronary angiography. After injecting a local anesthetic, a doctor inserts a thin flexible tube called introducer sheath into radial (near the wrist) or femoral artery (near the groin). A left and then right Judkins catheters from Boston Scientific corporation/ USA is threaded over a guide wire toward the heart, to engage the ostium of the left main stem or right coronary artery. During insertion, the doctor uses fluoroscopy (a continuous x-ray procedure) from Philips/ Netherland to observe the progress of the catheter as it is threaded into place. After the catheter tip is in place, a radiopaque contrast agent is injected through the catheter into the coronary arteries, and the outline of the arteries appears on a video screen and is recorded. Images are analyzed to detect the degree of stenosis or occlusions (13).

The participants were divided according to the severity of coronary obstruction into Group I (48

subjects with no coronary artery obstruction or minimal lesion of <50% stenosis), Group II (47 subjects with significant obstruction of  $\geq 50\%$  stenosis in one or two coronary arteries), and Group III (30 subjects with a significant obstruction of > 50% stenosis in three coronary arteries or  $\geq 50\%$  stenosis in the left main stem coronary artery. The sample size of the groups was determined according to the proportionate stratified sampling equation (14), and the power of the sample size was  $\geq 80\%$  to identify medium-to-large effects (15).

This study was approved by the scientific and ethical committees of the Department of Biochemistry/ College of Medicine/ University of Baghdad. Ethical approval was also obtained from Baghdad Teaching Hospital, Medical City Complex, and Ministry of Health/ Iraq (code no. 447). Verbal consent was obtained from the participants.

Exclusion criteria included patients with acute coronary syndrome (ACS), previous percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG), heart failure, myocarditis, cardiomyopathy, pulmonary embolism, sepsis, stroke, chronic kidney disease, chronic liver disease, endocrine disorders, and cancer, based on history, physical examination, and laboratory results.

Five milliliters sample of blood was aspirated from the peripheral vein of each participant just prior to coronary angiography and allowed to clot for 15 minutes, then centrifuged for 10 minutes at 2500 rpm. The separated serum was stored at -45°C until the day of lab testing, which included measurements of hs-cTn I and Str-2, using the enzyme-linked immunosorbent assay (ELISA) sandwich technique Reader [Huma Reader, by the German company Human Diagnostics, Washer (COMBIWASH)]. Both kits are manufactured by Cloud-Clone corp./ USA.

The principle of the ELISA technique with the biotin double antibody sandwich method was used for the evaluation of human hs-cTn I or Str-2. The wells were coated with the hs-cTn I, or Str-2 monoclonal antibody, and allowed to incubate. The next step was to combine streptavidin-HRP with biotin-labeled anti-hs-cTn I, or Str-2 antibodies, to create an immunological complex. After incubation and washing, the enzymes that remained unbound were removed. Substrates A and B were combined. In the presence of acid, the solution would undergo a color shift, first from blue to yellow. The human hs-cTn I, or Str-2 content, was positively correlated with the solution color (16).

**Statistical Analysis** The analysis was carried out by using the SPSS version 25.0 software, which described the data using means, medians, standard deviation (SD), and first- to third-quartile (Q1-Q3) range. Continuous data were subjected to normality test (Shapiro Wilk test). Data with normal distribution were presented as mean and standard deviation. The analysis of variance (ANOVA) test was performed to assess the differences between means of numerical data when more than two means were tested. Data with non-normal distribution were presented as

median and (Q1-Q3) range and analyzed with Mann Whitney U test for two groups comparison or Kruskal Wallis for three groups comparison. Categorical variables were expressed as numbers and percentages and analyzed with Chi-square test. The area under curve (AUC), sensitivity, specificity, and cut-off values for each biomarker were calculated by the receiver operating characteristic curve (ROC). Relative quality (RQ) test was done to compare AUC of biomarkers, separate and combined. The potential link of hs-cTn I and Str-2 with patients' risk factors was investigated by Spearman's correlation test. Multinomial logistic test was performed to analyze the relationship between both biomarkers with varying CAD severity. A difference was deemed

### Results

There were no statistically significant differences between the mean values of age and body mass index (BMI) among and between the three groups. There were significantly more males than females in group III (70%) and group II (72.3%) compared to group I

(43.8%) ( $p=0.008$ ). Smoking was significantly more common among patients in group I (45.8%) and group II (48.9%) compared to group III (20%), ( $p = 0.028$ ). Hypertension was significantly more common in group III (50%) and I (43.8%) than in group II (23.4%), ( $p = 0.034$ ). Type 2 diabetes mellitus (T2DM) was not significantly associated with any of the three groups.

Table (2) and Figures (1, 2) show the median and (Q1-Q3) range values of serum hs-cTn I and Str-2 levels of the three studied groups. The median values of hs-cTn levels of patients in group III were significantly higher than those in group I ( $p = 0.001$ ) and group II ( $p = 0.001$ ). The median value of hs-cTn I of patients in Group II was significantly higher than that of patients in Group I ( $p = 0.001$ ). The median values of serum level of Str-2 in group III and group II were significantly higher than that in group I (for both,  $p=0.001$ ). There were non-significant differences between groups II and II

**Table 1: Description of the study groups by age, BMI gender, smoking, and comorbidity**

Parameter		Group I (n=48)	Group II (n=47)	Group III (n=30)	P value
Gender	Male	21 (43.8%)	34 (72.3%)	21 (70.0%)	0.008**
	Female	27 (56.5%)	13 (27.7%)	9 (30.0%)	
Age (years)		53.7±10.19	55.2±11.54	58.6±8.94	0.134*
BMI (Kg/m <sup>2</sup> )		32.2 ±5.55	31.9 ±5.63	30.2 ±4.98	0.280*
Smoking	Never	26 (54.2%)	24 (51.1%)	24 (80.0%)	0.028**
	Ex/current	22 (45.8%)	23 (48.9%)	6 (20.0%)	
Comorbidity	Hypertension	21 (43.8%)	11 (23.4%)	15 (50.0%)	0.034**
	T2DM	31 (64.6%)	20(42.6%)	3(43.3%)	

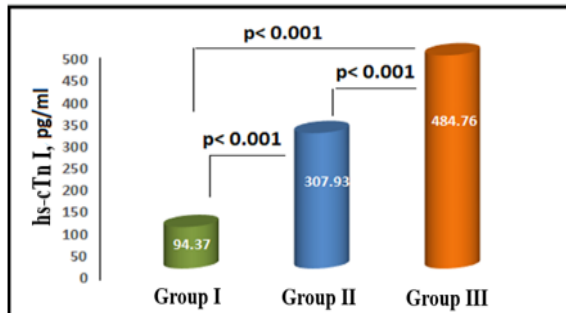
\*Analysis of variance test, \*\*Chi square

**Table 2: Median and (1<sup>st</sup> - 3<sup>rd</sup>) quartile range values of hs-cTn I and Str-2 of the three study groups**

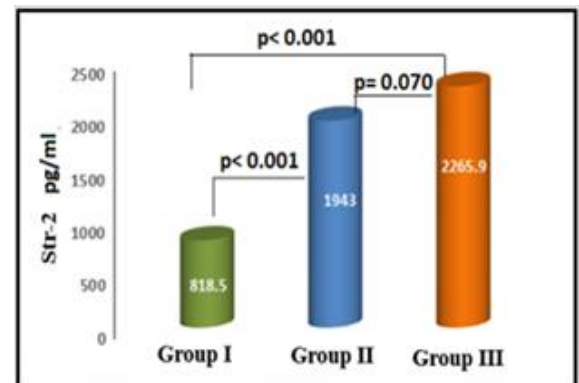
Parameter	Group I (n=48)	Group II (n=47)	Group III (n=30)	P value*
hs-cTnI (pg/mL)	94.37 (22.5 - 337.9)	307.93 (114.1 - 412.6)	484.76 (220 - 622.5)	0.001
Str-2 (pg/mL)	818.5 (347.8- 1649.3)	1943 (864.7- 4204.9)	2265.9 (1597.7- 2976.6)	0.001

\*Non-parametric Kruskal Wallis test

XXX



**Figure 1: Median serum levels of hs-cTn I in the three study groups**



**Figure 2: Median serum levels of Str-2 in the three study groups**

Spearman's correlation test was used to explore the relationship of median serum level of hs-cTn I and Str-2 in relation to sex distribution (female and male), smoking, T2DM, and hypertension in patients of the three study groups was non-significant. The results also revealed a significant positive correlation between hs-cTn I and Str-2 in group I ( $r = 0.335$ ,  $p = 0.02$ ), and group II ( $r = 0.754$ ,  $p < 0.001$ ) as shown in Figure (3, 4).

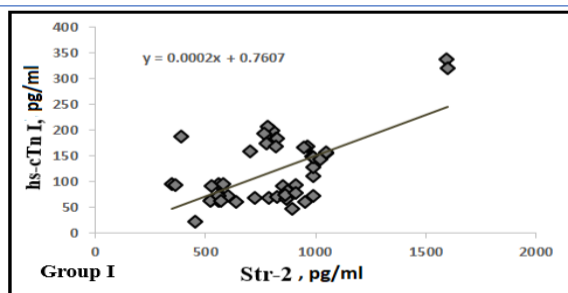


Figure 3: Scatter plot and regression line between Str-2 and hs-cTnI in Group I

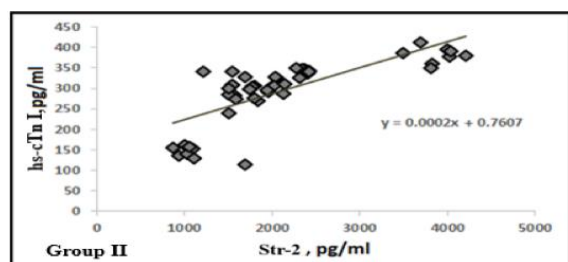


Figure 4: Scatter plot and regression line between Str-2 and hs-cTnI in Group II

Table (3) shows the results of the receiver operating characteristic curve (ROC) and area under curve (AUC) for hs-cTn I and Str-2 in the diagnosis of the coronary artery obstruction and differentiation of its severity. Serum Str-2 has the highest AUC value (0.971), with sensitivity 98% and specificity 73%, at cutoff value (923.5 pg/ml) in the diagnosis of patients with obstructions of > 50% stenosis in single or double coronary arteries (group II). However, serum

hs-cTn I have the highest AUC value (0.997), with sensitivity 97% and specificity 98%, at cutoff value (320.4 pg/ml) in the diagnosis of patients with > 50% stenosis in three or more coronary arteries or ≥ 50% stenosis in left main stem (group III). Serum hs-cTn I have the highest AUC value (0.926), with sensitivity 90% and specificity 92%, at cutoff value (354.4 pg/ml) in differentiation between group II and III. The relative quality ratio (RQ) was calculated using the relative quality equation (AUC combined/Max AUC individual) for group I vs. group II and group I vs. group III, and the result was equal to one, which means there is no added benefit from biomarkers combination. As for group II vs. group III, the relative quality ratio (RQ) was less than one, indicating that the use of the marker with the highest AUC, which is hs-cTn I, is sufficient

Results of the multinomial logistic regression:  $R^2 = 0.682898$  and Adjusted  $R^2_{adj} = 0.68$ , which means that these biomarkers predict 68.3% of the variance of severity. The coefficient of multiple correlation ( $R$ ) = 0.826376, which indicates a very strong relationship between the expected and calculated results of severity. Goodness of fitness ( $F$ ) = 267.040808,  $p$ -value < 0.05. This value means the model is statistically better than separate biomarkers in predicting the variance of severity. Str-2 is not significant alone as a predictor for severity but may be of benefit if used with hs-cTn in contrast to hs-cTn I, which has a strong predictive ability.

Table 3: The Receiver Operating Characteristic curve for hs-cTn I and Str-2

Groups		AUC	Sensitivity %	Specificity %	95% CI	Cut-off value	$p$ value
I	cTnI	0.92	83	96	0.87-0.97	>224	<0.001
vs.	Str-2	0.97	98	73	0.94-0.99	>923	<0.001
II	both	0.97					
I	cTnI	0.99	97	98	0.99-1.0	>320	<0.001
vs.	Str-2	0.99	97	94	0.98-1.0	>1444	<0.001
III	both	0.99					
II	cTnI	0.92	90	92	0.85-0.99	>354	<0.001
vs.	Str-2	0.73	-	-	-	-	NS
III	both	0.77					

## Discussion

The age distribution in the current study is close to that previously reported in the Middle East, which is 12 years earlier than in the western countries, but the occurrence of hypertension, diabetes, and obesity is much higher than that in the Middle East and Gulf region, which may be due to the population lifestyle. The sample size of the referred to study is higher than that of the current study (17).

As for the higher frequency of males than females in groups II and III in the current study, it is well known that men are more likely than women to acquire CAD, and they typically do so at a younger age. Additionally, when compared to age-matched women, they had a higher risk of developing severe CAD (18,19). The significant association between hypertension and coronary artery obstruction in the current study is in agreement with the study of Charach *et al.*, which found that the severity of CAD

was associated with hypertension, diabetes, and dyslipidemia (20). Another study by Aksu and Ahmed stated that risk factors correlate with the occurrence of atherosclerosis but not with its severity (21). In this study, increasing age, although not statistically significant, was associated with multi-vessel CAD similar to a narrative review involving 75 articles (22).

There is a controversy among reported studies about the relationship between smoking and the number of diseased coronary arteries. Five researchers found a high correlation between smoking and the number of damaged arteries, while six studies found no relationship (23). In fact, triple-vessel disease rates were considerably lower in angiographic sub-studies of the Global Utilization of Streptokinase and t-PA for Occluded Coronary Arteries (GUSTO-I), Thrombolysis in Myocardial Infarction (TIMI), and Controlled Abciximab and Device Investigation to



Lower Late Angioplasty Complications (CADILLAC) trial (24-26). According to Acute Catheterization and Urgent Intervention Triage strategy (ACUITY) Trial, smokers are more likely to need PCI and less likely to need CABG because of their good coronary structure (27). Compared to those who do not smoke, CAD smokers are at least ten years younger (28). The results of this study revealed a significant increase in hs-cTn with more diseased coronary arteries, which is in agreement with other studies that concluded a significant association of hs-cTn I with the presence of CAD in CCS (29-31) and with its severity (21,32). Increased myofibrillar troponin Proteolytic degradation may be the cause of elevated cTn I in CCS. This is explained by several mechanisms, including a local increase in the intracellular  $\text{Ca}^{2+}$  concentration after ischemia, due to reduced  $\text{Ca}^{2+}$  extrusion by the Na/Ca exchanger with concomitant reduced  $\text{Ca}^{2+}$  uptake by the sarcoplasmic reticulum, increases the activity of the  $\text{Ca}^{2+}$ -dependent protease calpain, causing chronic proteolytic degradation of myofibrillar cTn (33). Another pathobiological mechanism by which intact troponin may be released during ischemia without necrosis is the formation and release of membranous blebs. Blebs are spherical protrusions of the plasma membrane. Their development is driven by increases in cytoplasmic (intracellular) hydrostatic pressure. Blebbing is considered to be a key characteristic of the execution phase of apoptosis, but strikingly, it is also well known that blebbing is involved in key physiological processes of healthy cells, such as during cytokinesis (34). Another study suggested that small increases in cardiac troponin in some patients with CCS may be caused by increased plaque activity or vulnerability (35).

The results of the current study found that the serum level of Str-2 was significantly associated with coronary artery obstructions at all levels of severity but cannot differentiate between them. A recent study identified a missense variant in the Str-2 gene associated with altered protein activity and an imbalance with its tissue inhibitor may contribute to the pathogenesis of atherosclerosis suggesting a potential link between Str-2 and CAD (36). In contrast another study found no association between Str-2 with the presence of coronary plaques (7).

### Conclusions

Measurement of serum Str-2 can be used in the diagnosis of coronary artery obstruction, while serum hs-cTn I aids in the diagnosis and differentiation of its severity and in improving risk stratification and management strategy. Combining both markers adds no significant benefit.

### Limitations

According to the sample size, this study shows a medium and large effect. Further studies with larger sample sizes might explore smaller or subtler effects.

### Authors' declaration

We confirm that all the figures and tables in the manuscript belong to the current study. Authors sign on ethical consideration's approval-ethical clearance: The project was approved by the local ethical committee in the place where the research was conducted or samples collected according to the code number (447) on 29/1/2025.

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### Authors' contributions

Study conception and design: (Basil O Saleh, Ameen A. Al-Alwany. Literature search: (Amal A. Hammoodi). Data acquisition: (Amal A. Hammoodi). Data analysis and interpretation: (Amal A. Hammoodi and Basil O. Saleh). Manuscript preparation: (Basil O Saleh, Ameen A. Al-Alwany, and Amal A. Hammoodi). Manuscript editing and review: Basil O Saleh, Ameen A. Al-Alwany.

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## القيمة التشخيصية السريرية للستروميلايسين-2 والتروبونين القلبي عالي الحساسية في تحديد شدة إنسداد الشرايين التاجية

امال عباس حمودي<sup>1</sup>، باسل عويد صالح<sup>1</sup>، أمين عبدالحسن العلواني<sup>2</sup>  
<sup>1</sup> فرع الكيمياء الحياتية السريرية، كلية الطب، جامعة بغداد، بغداد، العراق.  
<sup>2</sup> فرع الطب الباطني، كلية الطب، جامعة بغداد، بغداد، العراق.

### الخلاصة:

**خلفية البحث:** يُعتبر مرض الشريان التاجي أحد الأسباب الرئيسية للوفاة على مستوى العالم. يتم إطلاق التروبونين في الدم من المرضى الذين يعانون من نقص تروية عضلة القلب القابل للعكس. يشارك إنزيم الستروميلايسين-2 في تطور وتقدم تصلب الشرايين من خلال إعادة تشكيل الأنسجة وتحلل بروتينات المادة خارج الخلية.

**الهدف:** التحقق من دور التروبونين القلبي عالي الحساسية I وإنزيم الستروميلايسين-2 في تشخيص مرض الشريان التاجي وتحديد مدى شدة إنسداد الشرايين التاجية.

**المنهجية:** تم إجراء دراسة مقطعية على 125 شخصًا خضعوا لقسطرة الشرايين التاجية للإشعاع في إصابتهم بمرض الشريان التاجي في مستشفى بغداد التعليمي/ مجمع مدينة الطب/ بغداد/ العراق، من قبل فرع الكيمياء الحياتية/ كلية الطب/ جامعة بغداد، خلال الفترة من آذار إلى كانون الأول 2024. تم تقسيمهم إلى ثلاث مجموعات: المجموعة الأولى: تضم 48 شخصًا لا يعانون من أية آفة في الشريان التاجي أو لديهم تضيق أقل من 50%. المجموعة الثانية: تضم 47 شخصًا لديهم تضيق أكبر من 50% في شريان واحد أو شرياني تاجيين. المجموعة الثالثة: تضم 30 شخصًا لديهم تضيق أكبر من 50% في ثلاثة شرايين تاجية أو أكثر، أو تضيق  $\leq 50\%$  في الجذع الرئيسي الأيسر. تم قياس مستويات التروبونين القلبي عالي الحساسية I والستروميلايسين-2 في مصل الدم باستخدام تقنية ELISA.

**النتائج:** كانت القيم الوسيطة (المجال بين الربيع الأول والثالث) لمستويات التروبونين القلبي عالي الحساسية I والستروميلايسين-2 في المجموعة الثالثة والمجموعة الثانية أعلى بشكل ملحوظ مقارنة بالمجموعة الأولى ( $p=0.001$ ). كانت القيمة الوسيطة لـ التروبونين القلبي عالي الحساسية I في المجموعة الثالثة أعلى بشكل ملحوظ مقارنة بالمجموعة الثانية ( $p<0.001$ ). أظهرت اختبارات منحني خاصية التشغيل للمستقبل (ROC) ومنطقة تحت المنحنى (AUC) أن التروبونين القلبي عالي الحساسية I والستروميلايسين-2 يتمتعان بقدرة تشخيصية عالية لتحديد إنسداد الشرايين التاجية، كما أن التروبونين القلبي عالي الحساسية I يتميز بقدرة تمييزية عالية في تقييم شدة الإنسداد.

**الاستنتاجات:** يمكن استخدام قياس مستوى الستروميلايسين-2 في تشخيص إنسداد الشرايين التاجية. يمكن استخدام قياس مستوى التروبونين القلبي عالي الحساسية I في التشخيص والتفريق بين درجات شدة إنسداد الشرايين التاجية. لا يضيف الجمع بين كلا المؤشرين فائدة كبيرة من الناحية التشخيصية.

**الكلمات المفتاحية:** شدة إنسداد الشريان التاجي؛ التروبونين القلبي عالي الحساسية I؛ ستروميلايسين-2.