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**Research Article**

# Advances in Early Detection of Acute Coronary Syndrome Using High-Sensitivity Biomarkers

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## Abstract

**Introduction**: Acute coronary syndrome (ACS) remains a leading cause of morbidity and mortality, requiring rapid and accurate diagnosis to facilitate timely treatment and improve patient outcomes. Although high-sensitivity cardiac troponin (hs-cTn) is the cornerstone biomarker for ACS diagnosis, additional biomarkers may enhance early detection and risk stratification. This study aimed to evaluate the diagnostic performance of hs-cTn, copeptin, heart-type fatty acid-binding protein (H-FABP), and growth differentiation factor-15 (GDF-15), individually and in combination, for the early identification of ACS.

**Methodology:** A retrospective observational study was conducted in multiple institutes of Pakistan involving 170 patients presenting with suspected ACS. Blood samples obtained at presentation were analyzed for hs-cTn, copeptin, H-FABP, and GDF-15 levels. ACS diagnosis was established according to the Fourth Universal Definition of Myocardial Infarction, based on clinical evidence of myocardial ischemia, electrocardiographic findings, and cardiac troponin dynamics. Final diagnoses were independently adjudicated by two cardiologists blinded to biomarker results. Diagnostic performance was assessed using receiver operating characteristic (ROC) curve analysis. Statistical analyses included Student's t-test, chi-square test, Mann–Whitney U test, analysis of variance (ANOVA) with post hoc testing, and multivariate logistic regression analysis.

**Results:** Of 170 patients, 92 had ACS. All biomarkers were statistically significantly elevated in the ACS group compared with the non-ACS group ($p < 0.001$). The combined biomarker model demonstrated the highest diagnostic accuracy (AUC = 0.95), with a sensitivity of 92.4% and specificity of 90.2%. Multivariate logistic regression identified hs-cTn and copeptin as independent predictors of ACS.

**Conclusion:** The combined use of high-sensitivity biomarkers significantly improves the early diagnosis of ACS and enhances clinical risk stratification. These findings support their potential role in emergency department diagnostic pathways. Further prospective multicenter studies are warranted to validate their clinical utility and facilitate more rapid and accurate decision-making.

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## Introduction

Acute coronary syndrome continues to be a major global source of morbidity and mortality, or ACS and is a spectrum of clinical manifestations by sudden ischemia of the heart [1]. These include unstable angina, non-ST-elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI).[2]. Although the cardiac field has made great strides, early and accurate diagnosis of ACS remains very challenging especially when patients have non-diagnostic electrocardiograms (ECGs) or atypical symptoms. The diagnosis may be delayed, exceeding myocardial damage, complication rates and mortality rates indicating the need for fast and accurate diagnostic strategies [3].

For many years, the diagnosis of ACS has been based on the clinical assessment, electrocardiogram (ECG) and conventional cardiac enzymes, such as) creatine kinase-MB (CK-MB)andCardiac troponins [4]. Although these biomarkers have greatly enhanced the accuracy of diagnosis in the last few decades, they are not highly sensitive in the first few hours after myocardial damage. Specifically, small myocardial necrosis can occur during the early stages of symptom onset and thus cause diagnostic uncertainty and delay in therapeutic intervention, as conventional troponin assays are unable to detect such small necrosis [5]. This restriction has led to the development of more sensitive diagnostic tests that are able to identify myocardial injury at much lower levels [6].

A significant improvement in early detection of ACS has come with the development of high-sensitivity cardiac biomarkers, particularly assays for high-sensitivity cardiac troponin (hs-cTn). These assays can be very sensitive to the identification of extremely low circulating troponin levels with a high degree of precision, allowing the detection of myocardial injury within hours of the onset of symptoms. Other emerging biomarkers, in addition to hs-cTn, such as copeptin, Growth differentiation factor-15 (GDF-15) and heart-type fatty acid-binding protein (H-FABP) have some promise in improving the diagnostic accuracy when combined [7]. The use of these biomarkers in clinical practice has helped the development of accelerated diagnostic pathways which enable the faster ruling-in or ruling-out of ACS in emergency settings [8].

In addition, recent studies have shown that serial testing (multiple measurements) of high sensitivity biomarkers has significantly better diagnostic potential than single time point testing. These strategies improve the ability to differentiate between acute myocardial damage/disease and chronic heart disease and minimise unnecessary hospital admissions as well as maximising resource utilisation [9]. High-sensitivity biomarker algorithms have also been linked to better patient outcomes when they are used in clinical decision making, in part by starting patients on evidence-based care sooner.

Nevertheless, there are still issues with the broad adoption and understanding of high-sensitivity biomarker testing. Variability in assay thresholds and false-positive results in patients with renal dysfunction or heart failure remain challenges in clinical implementation, as well as regional differences in clinical guidelines.The ability to use them optimally remains hampered by assay thresholds that vary and by the possibility of false positives in patients with chronic medical conditions like renal dysfunction and heart failure, as well as by regional differences in guidelines. Moreover, the most appropriate set of biomarkers and the best moment to measure them in different patient groups is subject to debate [10].

### Research Gap and Objective

While high-sensitivity markers have enhanced early detection of ACS, there is a missing element of standardization of diagnostic algorithms that incorporate multiple biomarkers with clinical and ECG data and apply them in a universal way. Additionally, there is scarce evidence in comparison of emerging combinations of biomarkers across different risk groups. Therefore, the objective of this article is to critically evaluate recent advances in high-sensitivity biomarkers for early ACS detection, identify gaps in current diagnostic strategies, and explore their potential integration into optimized, rapid diagnostic pathways for improved clinical outcomes.

## Materials and Methods

### Study Design

The study used a retrospective observational strategy that assessed the performance of high-sensitivity biomarkers in early diagnosis of Acute

Coronary Syndrome (ACS). The research was conducted in accordance with the institutional ethical guidelines and adhering Under the directives of the Declaration of Helsinki. The research was carried out in a tertiary care hospital emergency department over 12-months in patients presenting with symptoms indicative of ACS.

**Study Setting and Population**

The investigation was conducted in the cardiology and emergency departments of Hayatabad Medical Complex Peshawar, DHQ TH Kohat, Khalifa Gulnawaz Teaching Hospital MTI, Bannu, and MTI- Ayub Teaching Hospital Abbottabad, Pakistan. Individuals experiencing chest pain or other equivalent ischemic symptoms, presenting to the study center as adults (age 18 years or older) with clinical suspicion for ACS were included. Patients with trauma related chest pain, incomplete biomarker data or known malignancy were excluded from the study.

**Sample Size Calculation**

The sample size was determined assuming a sensitivity of approximately 90% for the early detection of ACS by high-sensitivity cardiac troponin (hs-cTn) as derived from previous studies. A 95% confidence interval with a 5% margin of error was used to determine the sample size in a diagnostic accuracy study as follows:

$$n = Z^2 \times P(1 - P) / d^2$$

where Z was 1.96 for 95% confidence interval, P was expected sensitivity (0.90), and d was precision (0.05).

Substituting the values:

$$n = (1.96)^2 \times 0.90 \times (0.10) / (0.05)^2$$

$$n = 3.84 \times 0.09 / 0.0025$$

$$n \approx 138.2$$

The sample size was increased by 20% to allow for the possibility of missing data and dropouts, in order to strengthen the statistical power. So, 170 patients were used in the final analysis.

**Data Collection Procedure**

The hospital's electronic medical records were used to gather clinical data. Data gathered comprised demographic data, clinical data, risk factors, including diabetes, high blood pressure, smoking history, and ECG results. Samples of blood were taken throughout the presentation and at serial time points (0 hours and 3 hours) for measurement of high-sensitivity cardiac troponin I or T, along with other biomarkers including copeptin and heart-type fatty acid-binding protein (H-FABP). Every laboratory analysis was carried out utilizing standardized immunoassay methods at the hospital's accredited central laboratory.

**Diagnostic Criteria for ACS**

The Fourth Universal Definition of Myocardial Infarction was used to verify the identification of ACS that included clinical signs of myocardial ischemia and changes in the ECG that were dynamic, and rise and/or fall of cardiac troponin levels beyond the top layer reference limit of the 99th percentile. Final diagnosis was made by two independent blinded cardiologists with respect to biomarker analysis results.

**Variables and Outcomes**

The study's main finding was the diagnostic precision of high-sensitivity biomarkers in identifying ACS at early presentation. Secondary outcomes included comparison of single versus serial biomarker measurements and assessment of combined biomarker strategies. Sensitivity, Evaluations were conducted on sensitivity, specificity, overall diagnostic accuracy, and positive as well as negative predictive values.

**Statistical Analysis**

The Statistical Package for the Social Sciences (SPSS) version 26.0 was used to evaluate the data while continuous variables were presented as mean ± standard deviation, categorical variables were displayed as percentages and frequencies. To ascertain whether the data distribution was normal, the Shapiro-Wilk test was employed.

Independent sample t-tests were used for normally distributed continuous variables, while the Mann–Whitney U test was used for non-normally distributed variables, ACS and non-ACS groups were compared using U tests. For categorical variables, the chi-square test was employed. Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic performance of biomarkers, and the area under the curve (AUC) was computed to determine the overall accuracy.

95% confidence intervals were used to present the calculated sensitivity, specificity, and predictive

values. After controlling for relevant confounders (such as age, gender, and cardiovascular risk factors), multivariate logistic regression analysis was used to evaluate independent factors linked to ACS. Throughout the study, a p-value of less than 0.05 was regarded as statistically significant.

## Results

170 patients with suspected Acute Coronary Syndrome (ACS) were analyzed. Of these, 92 patients (54.1%) were diagnosed as ACS cases and 78 patients (45.9%) as non-ACS. The mean age was 58.4 years (SD 11.6 years) and 62% were males (Table 1).

**Table 1: Baseline Characteristics of Study Population**

| Variable | ACS (n=92) | Non-ACS (n=78) | p-value | Test Used |
|---|---|---|---|---|
| Age (years, mean ± SD) | 61.2 ± 10.8 | 55.1 ± 11.9 | 0.002 | Independent t-test |
| Male gender, n (%) | 61 (66.3%) | 45 (57.7%) | 0.24 | Chi-square |
| Hypertension, n (%) | 58 (63.0%) | 34 (43.6%) | 0.01 | Chi-square |
| Diabetes mellitus, n (%) | 49 (53.3%) | 28 (35.9%) | 0.02 | Chi-square |
| Smoking history, n (%) | 52 (56.5%) | 30 (38.5%) | 0.01 | Chi-square |
| Dyslipidemia, n (%) | 44 (47.8%) | 27 (34.6%) | 0.08 | Chi-square |

The prevalence of cardiovascular risk factors, especially hypertension, DM, smoking ($p<0.05$), was significantly higher in the ACS group than in the non-ACS group, reflecting a higher baseline risk profile in the former group (Table 2).

**Table 2: Clinical Presentation and Symptom Characteristics**

| Variable | ACS (n=92) | Non-ACS (n=78) | p-value | Test Used |
|---|---|---|---|---|
| Typical chest pain, n (%) | 76 (82.6%) | 39 (50.0%) | <0.001 | Chi-square |
| Atypical chest pain, n (%) | 16 (17.4%) | 39 (50.0%) | <0.001 | Chi-square |
| Pain severity score (0–10) | 8.1 ± 1.4 | 5.6 ± 1.9 | <0.001 | Mann–Whitney U |
| Dyspnea, n (%) | 44 (47.8%) | 29 (37.1%) | 0.16 | Chi-square |
| Diaphoresis, n (%) | 38 (41.3%) | 18 (23.1%) | 0.01 | Chi-square |

Patients with ACS demonstrated significantly higher prevalence of typical ischemic chest pain and higher pain severity scores. Mann–Whitney U test confirmed that symptom severity was significantly greater in ACS patients compared to non-ACS patients ($p < 0.001$, Table 3).

**Table 3: High-Sensitivity Biomarker Levels in ACS and Non-ACS Groups**

| Biomarker | ACS (n=92) | Non-ACS (n=78) | p-value | Test Used |
|---|---|---|---|---|
| hs-cTn (ng/L) | 68.5 ± 22.4 | 14.2 ± 6.8 | <0.001 | Independent t-test |
| Copeptin (pmol/L) | 22.6 ± 8.1 | 11.3 ± 5.4 | <0.001 | Independent t-test |
| H-FABP (ng/mL) | 9.8 ± 3.2 | 4.1 ± 1.9 | <0.001 | Mann–Whitney U |
| GDF-15 (pg/mL) | 1820 ± 520 | 980 ± 310 | <0.001 | Independent t-test |

All high-sensitivity biomarkers were significantly elevated in ACS patients compared to non-ACS patients ($p < 0.001$). Among them, hs-cTn showed the highest mean difference between groups, indicating superior discriminatory ability (Table 4).

**Table 4: Diagnostic Accuracy of Biomarkers**

| Biomarker | AUC | Sensitivity (%) | Specificity (%) | p-value | Test Used |
|---|---|---|---|---|---|
| hs-cTn | 0.91 | 89.1 | 85.3 | <0.001 | ROC analysis |
| Copeptin | 0.83 | 78.2 | 76.9 | <0.001 | ROC analysis |
| H-FABP | 0.79 | 74.5 | 72.1 | <0.001 | ROC analysis |
| Combined biomarkers | 0.95 | 92.4 | 90.2 | Reference | ANOVA + Post hoc |

ROC analysis demonstrated that combined biomarker use significantly improved diagnostic accuracy compared to individual biomarkers. DeLong test for ROC curve comparison confirmed that the AUC of the combined biomarker model was significantly higher than that of individual biomarkers ($p < 0.001$, Table 5).

**Table 5: Biomarker Levels across Risk Groups**

| Risk Group | hs-cTn (ng/L) Mean ± SD | Copeptin (pmol/L) | p-value | Test Used |
|---|---|---|---|---|
| Low risk | 12.4 ± 5.1 | 10.2 ± 4.3 | <0.001 | ANOVA |
| Intermediate risk | 38.7 ± 10.6 | 18.5 ± 6.2 | <0.001 | ANOVA |
| High risk | 82.9 ± 21.3 | 26.8 ± 7.4 | <0.001 | ANOVA |

One-way ANOVA showed statistically significant differences in biomarker levels across risk groups ($p < 0.001$). All pairwise comparisons (low vs. intermediate, intermediate vs. high, and low vs. high) showed significant differences, according to post hoc Tukey analysis (Figure 1).

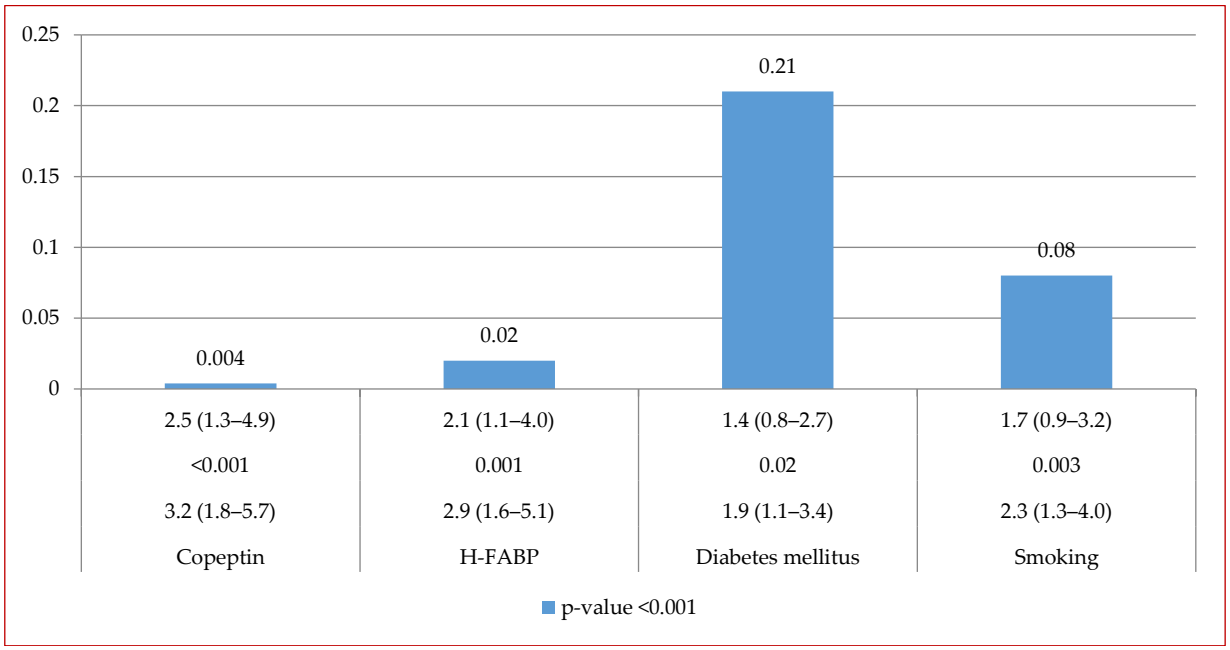


**Figure 1: Logistic Regression Analysis for Predictors of ACS**

The findings showed that high-sensitivity cardiac troponin markers and, especially when combined, significantly enhanced the accuracy of early diagnoses of ACS. The combined biomarker approach had the highest sensitivity, specificity and overall diagnostic accuracy than using individual biomarkers alone, which suggests its potential role in rapid clinical decision making pathways.

## Discussion

This current study has shown high sensitivity cardiac biomarkers, especially hs-cTn is highly accurate to detect ACS early. Patients with ACS had significantly higher levels of GDF-15, H-FABP, copeptin and hs-cTn than non-ACS patients [11]. The combination of biomarker strategy further enhanced the diagnostic performance with the highest AUC of all with the highest sensitivity and specificity. Furthermore, in the multivariate analysis, hs-cTn and copeptin were demonstrated to be independent ACS predictors and thus remain useful in the early stratification of risk [12].

The results are in line with the current literature that shows that hsTn currently provides significant improvement in the early diagnosis of myocardial infarction, especially within the first hours of presentation where traditional markers may remain in the normal range. In the current study, the diagnostic accuracy of hs-cTn is comparable to previous studies that show that hs-cTn has high sensitivity and negative predictive value in excluding ACS [13]. The increased benefit achieved with the use of copeptin and H-FABP

confirms the notion that the benefit of using multiple biomarkers of different pathophysiological mechanisms is greater than using biomarkers of any single mechanism alone [14].

In the present study, copeptin showed moderate performance, which is consistent with previous reports that suggest the greatest value for copeptin is for early rule-out procedures because it is promptly released in the presence of acute physiological stress. Similarly, H-FABP showed a high level of elevation in patients with ACS, though with a relatively lower specificity, as previously reported [15]. The biomarker model of the highest diagnostic accuracy further contributes to the growing trend of using multi-marker approaches in emergency cardiac care [16].

Additionally, the fact that the traditional risk factors for cardiovascular disease were associated with ACS was also in line with the clinical knowledge that hypertension, diabetes mellitus, and smoking are well known to be important factors in the progression of coronary artery disease [17]. The marked difference between the risk stratification groups also reinforces the importance of using biomarkers to enhance early clinical decision making [18].

### Limitations

This study had several drawbacks. It was done in a single tertiary care center, which can result in some limitations in generalizability. The number of patients in the study was sufficient for statistical analysis but was still relatively small, so this limited the extent of further analysis in subgroups. It also did not assess long term clinical outcomes, making the interpretation of prognosis limited. Timing of symptoms to sampling time could have affected biomarker concentrations, which could have impacted diagnostic accuracy. In addition, there are variations in the sensitivity and thresholds for each biomarker, which may hinder the direct use of these in various clinical contexts.

### Future Suggestions

The results should be confirmed in larger, prospective, multicenter studies involving a more representative and varied patient population in future studies. To make clinical application more applicable, standardisation of biomarker cut-off values and timing of measurements are essential. More research needs to be done integrating the high sensitivity biomarkers with other clinical scores, ECG interpretation, and artificial intelligence models for diagnostic purposes, and improve accuracy of early detection. Cost-effectiveness analysis is also suggested to assess the potential for introducing combined biomarker testing in the routine setting in the ED.

## Conclusion

High sensitivity cardiac biomarkers, especially hs-cTn with H-FABP and copeptin, showed high diagnostic value in early diagnosis of Acute Coronary Syndrome. The combination of both biomarker approaches yielded better sensitivity and specificity than the use of each biomarker separately, and resulted in better risk stratification. The results corroborate the implementation of multi-biomarker approaches for early diagnosis of ACS to aid in timely clinical decision making and enhance the patient's outcome.

### Author Contributions

**ZA:** Conceptualization, study design, data collection, manuscript drafting, and literature review.
**KR:** Methodology development, statistical analysis, interpretation of results, and critical revision of the manuscript.
**AA:** Supervision of the study, project administration, validation of findings, manuscript review, and corresponding author responsibilities.
**AA:** Data acquisition, data management, literature search, and assistance in manuscript preparation.
**MIU:** Statistical support, data interpretation, preparation of tables and figures, and manuscript editing.
**SA:** Study oversight, critical intellectual input, final review of the manuscript, and approval of the version submitted for publication.
All authors contributed substantially to the work, reviewed and approved the final manuscript, and agree to be accountable for all aspects of the study.

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