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Evaluation of dual marker approach using heart-type fatty acid binding protein and high sensitivity troponin-I as an alternative to serial sampling for diagnosis of acute myocardial infarction

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ABSTRACT

Objective

An early rule in (high specificity and high PPV) and early rule out (high sensitivity and high NPV) is essential for diagnosing acute myocardial infarction (AMI) to provide better utilization of resources, cost-effectiveness, and to reduce mortality.

Methods

Consecutive chest pain patients (n=80) with symptoms indicative of coronary artery disease reported to the emergency room within 6 hours after onset of symptoms. An alternate Dual Marker Approach (DMA; both Heart-type Fatty Acid Binding Protein (H-FABP) and High sensitive Troponin-I (hsTnI) at 0 h) was compared

to the Double Sampling approach (DSA; hsTnI at 0 h and 3 h (ESC guidelines)).

Results

If both biomarkers were increased (n=17; 77.5%: 11 STEMI and 6 NSTEMI) above their respective cut-off value (HFABP 6.3 ng/mL and hsTnI 20.24 ng/L) at presentation, AMI ensued (100% PPV). Also, if both the markers were below their respective cut-offs at presentation, AMI was safely ruled out (n=41; with only 1 false negative). However, among the patients with either of these markers above their respective cut-off at presentation (n=22), DSA was required to find remaining AMI cases (n=4). Overall, DMA stands best for rule out (sensitivity 95.5%, NPV 97.6%) while DSA is superior for rule in (98.2% specificity, 95.2% PPV).

Conclusion

With the use of the proposed DMA, 58/80 (72.5%) patients with acute chest pain were reliably ruled in/ruled out for AMI at the presentation itself, while the remaining patients still required serial monitoring (DSA) for confirmation.

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INTRODUCTION

Early diagnosis and treatment of acute myocardial infarction (AMI) cases during the first hour (golden hour) after symptoms may reduce mortality from 9% to 3% [1]. Similarly, identifying patients without AMI and safely sending them home may result in considerable advantages for both patients and hospitals. ECG changes (ST elevation) and cardiac troponins (cTn), though highly specific, may not be apparent in the initial hours. Previous studies revealed that ECG misinterpretation might result in inappropriate clinical management in about 3-18% of patients[1-3]. Likewise, an inadequate increase in cTn and increasing duration may occur in cases of microinfarction[4].

The continuous improvement of the analytical sensitivity and assay precision in the low measuring range of cardiac troponin (cTn) assays has ultimately led to the development of 'high-sensitivity troponin (hsTnI and hsTnT) assays[5]. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) task force suggested that cTn should be reliably measurable in >80% (preferably >95%) of healthy subjects, and the total imprecision (CV) at the 99th percentile value should be ≤10% [5-7]. High-sensitivity cardiac troponin I (hsTnI) assays measure the same analyte as its predecessor cardiac Troponin I (cTn-I) with limits of detection (LoD) in ranges that were previously undetectable.

This additional sensitivity leads to the detection of cTn release at an earlier time point than the previous generations of cTn assays, especially in patients with a recent onset of chest pain. Most patients with AMI can be reliably identified within 3 h after admission, which indicates that the observation time in the emergency department may be reduced to rule out AMI[8]. However, in patients with 3 h values unchanged, in whom the pre-test likelihood of AMI is high, additional subsequent sampling (e.g., after 2 or 3 h) may still be advisable[5]. However, the sensitivity of the 99th percentile to rule-out AMI is too low for clinical use[9], and diagnosis of AMI cannot be made solely based on troponin I individual test results[5]. Thus, the role of early rule in (high positive predictive value) and early rule out (high sensitivity) algorithms is critical.

Heart-type fatty acid-binding protein (H-FABP), a novel biomarker, is one of the most abundant proteins in cardiomyocytes, comprising 5-15% of the total cytosolic protein pool. It leaks out of myocardial tissue. The concentration increases in the blood within 2 hours and is reported to peak at approximately 4-6 hours and returns to

the normal baseline value in 20 hours[10]. Recent studies have shown that combined with well-established markers such as hsTnI, H-FABP may allow early and accurate rule out compared to the currently available diagnostic tests[11].

The present study aimed at two different algorithms in terms of diagnostic performance and ROC analysis among the acute chest pain patients presenting within 6 hours of onset of symptoms. First, the Double Sampling Approach (DSA), i.e., collecting two samples for hsTnI drawn at presentation (at 0 hour) and after 3 hours. The second, Dual Marker Approach (DMA) estimates hsTnI and HFABP simultaneously at presentation (at 0 hour).

MATERIAL AND METHODS

Patient's selection

This prospective observational study included 80 consecutive cases of chest pain suggestive of coronary origin according to the 2015 American Heart Association guidelines[12] and presenting within 6 hours of the onset of symptoms at the emergency ward of Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow. Patients were enrolled after obtaining written informed consent from the patient's attendant in the format approved by the institutional ethical committee (IEC-31/16). Patients <18 years, eGFR<60 ml/min, unwillingness to provide consent, history of previous AMI, or acute muscle injury/ trauma were excluded from this study, i.e. their samples were not further assessed for HFABP. Comprehensive history taking included the patient's symptoms and time of onset. Past medical history such as diabetes mellitus (DM), hypertension (HTN), and previous ischemic events and general clinical examination, ECG, and laboratory investigations were documented at admission using medical records. However, patients with ST-elevation were analyzed separately.

Diagnostic criteria for acute myocardial infarction (AMI)

The definitive diagnosis was made after a critical review of all the clinical features and relevant information by a panel of two cardiologists. AMI was defined according to the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC)/American Heart Association (AHA)/World Heart Federation (WHF) Task Force for the Universal Definition of Myocardial Infarction. Presence of at least one element of clinical evidence of acute myocardial ischemia (symptoms of ischemia, ECG findings, coronary angiography, or imaging evidence) and with acute myocardial injury characterized by detection of a rise and/or fall of cTnI values with at least one value above the 99th percentile URL (0.2 ng/ml for Siemens Advia Centaur XP c-TnI assay)[13].

Sample collection

Venous samples (3mL) were taken at presentation ('0' hour) and post-admission (at 3 h) serially for hsTnI estimation (Double Sampling Approach; DSA). After accomplishing routine analysis, including cardiac troponin I (cTnI), the first sample ('0' hour) was stored at -80ºC for H-FABP estimation.

Estimation of hsTnI and H-FABP biomarkers

The first sample (0h) was analyzed for hsTnI and H-FABP both (Dual Marker Approach; DMA), while the second sample at post-admission (3 h) was analyzed for hsTnI only. According to the manufacturer's protocols, the hsTnI was analyzed on Access-2, using a commercial kits electrochemiluminescence assay (Beckman oulter, USA). H-FABP levels were determined by enzyme-linked immunosorbent assay (ELISA) using the commercial kit(Biovendor R&D, CZ) as described by the manufacturer's protocol.

Diagnostic outcomes were categorized as one of the following: ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI), unstable angina (UA), and non-cardiac chest pain (NCCP).

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 21.0. The data presented in number (N) and percentage (%). The two strategies/approaches namely DSA and DMA, were compared for their early rule in (high positive predictive value and specificity) and early rule out (high sensitivity and negative predictive value) capacity to diagnose AMI using sensitivity, specificity, positive predictive value, negative predictive value and area under curve (AUC). The optimum cut-offs for HFABP (>6.3 ng/ml) were taken from ROC analysis, defined as the biomarker level at which sum of sensitivity and specificity was highest to diagnose AMI[13]. P value <0.005 was considered as statistically significant. Sensitivity and specificity were calculated according to Gijsberts et al[14].

RESULTS

Out of the 80 enrolled patients with acute chest pain, 22 cases (27.5%) were confirmed to have AMI. Both acute chest pain patients (n=61(76.2%)) and AMI (n=19 (86.3%)) were more prevalent in males. Male: female ratio in AMI patients was significantly higher (6.3:1) than that in non-AMI patients (2.6:1; p<0.0001). The median age at presentation for AMI was 67.0 (IQR=15.25) years, which was significantly higher than acute chest pain patients (Median 57.5 years; IQR=21.75). NSTEMI was significantly associated with AMI in 11 patients (50 %) (p<0.0001). (Table 1)

Single biomarker assays

To achieve the maximum sum of sensitivity (86.4%) and specificity (94.2%), the optimal threshold (Cut-off) of H-FABP was 6.3 ng/ml as guided by ROC analysis. Upper Reference Limit (URL) for hsTnI at admission was 20.24 ng/L (as calculated from the non-AMI population; n=58) in our study.

Among the single biomarker assays, hsTnI and HFABP provide equal sensitivity (81.8%) to diagnose AMI at presentation (0 hour) as compared to cardiac troponin I (cTnI; sensitivity 63.6%). However, their specificity was much lower (89.6% for hsTnI and 86.2% for HFABP) in comparison to cTnI (100%) Fig 2A. The cut-off of cardiac troponin I was taken as 0.2 ng/ml as per manufacturer's instruction. On ROC analysis, hsTnI showed a slightly better area under the curve (0.87) than HFABP (0.84) to diagnose AMI at presentation (Table 2; Fig.1 A, B & C). The sensitivity of both the tests was 86.4% to diagnose AMI at presentation (Fig.2A & 2B).

DSA and DMA approach

When the same cut-offs as described above were applied in our two diagnostic algorithms, namely DSA and DMA, the sensitivity (95.5%), NPV (97.6%), and PPV (100%) was highest for DMA. At the same time, specificity was maximum (98.2%) for the DSA irrespective of the time of presentation since the onset of symptoms and ST elevation (Fig. 2A, 2B).

On the further assessment of the DMA approach, three findings were noteworthy: Firstly, if both markers were increased above their respective cut-offs i.e. H-FABP>6.3 ng/ml; hsTnI >20.24 ng/L (DMA: both positive; n=17), the PPV (100%) was maximium. Secondly, if neither of the markers were increased (both negative; n=41) above their respective cut-offs, NPV was 97.5% (Table 3A Fig. 2B). Thirdly, there was not

even a single AMI case where only one marker was raised if presentation time was > 3 hours. This indicates that if AMI occurs, both the markers would be elevated in 3 hours of onset of symptoms.

Since all the cases with STEMI were confirmed as AMI in our study group, we further focused on the patients with non ST elevation, where all the parameters of diagnostic performance remained same for DMA (specificity 86.5%, PPV 100%, NPV 97.6%) except sensitivity which drops down to 90.9%. It out performs DSA except for specificity (98.2%) (Fig. 2A, 2B). All the patients with both markers above cut-offs (DMA: both positive; n=6) were finally diagnosed as AMI (TP) while only one case (FN) was detected among patients with both the markers below cut-offs. Out of 10 DSA positive patients, only one was false positive while only two patients were diagnosed as AMI (FN) out of 59 patients with DSA (Table 3B).

Diagrammatic representation of rule-out/rule-in of AMI patients by applying DSA and DMA approach is summarized in Figure 3.

Table 1 **Descriptive characteristics of the study population**

	Suspected AMI patients (n= 80)		p value
	AMI (No); n=58	AMI (Yes); n=22	
Age Median (Years)(IQR)	67.0 (15.25)	57.5 (21.75)	
Gender Male Female (M:F)	 42 16 2.6:1	 19 3 6.3:1	<0.0001*
Diabetics	6	7	0.027*
Smokers	16	8	0.307
Hypertension	27	16	0.031*
Non-ST elevation	58	11	<0.001*
ST elevation MI (STEMI)	0	11	NA
Unstable Angina	14	0	NA
Non-Cardiac Chest Pain (NCCP)	44	0	NA

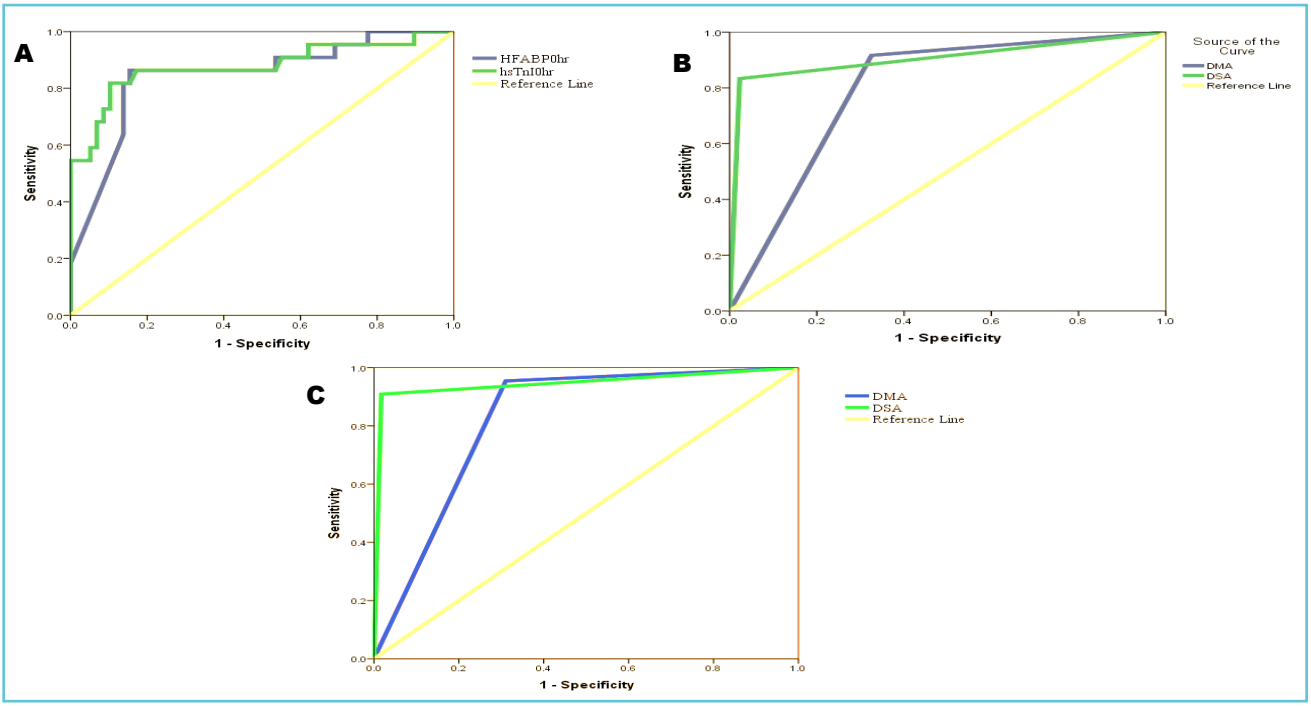
Abbreviation: IQR; Inter quartile range.

Table 2 ROC analysis of individual and combine approaches: within 3 hours of the onset of symptoms, within 3-6 hours of the onset of symptoms and overall

Variable(s)	AUC	SE	p-value	95% CI
Group A - Presentation within 3 hours of the onset of symptoms				
H-FABP>6.3 ng/ml at admission	0.73	0.10	0.03*	0.520-0.942
hsTnI>URL at admission	0.74	0.11	0.02*	0.521-0.967
H-FABP >6.3 ng/ml or hsTnI 20.24 ng/ L i.e. >URL at presentation (0 h)	0.78	0.07	0.008*	0.630-0.934
hsTnI at 0 & 3 hour (ESC 3-h guideline)	0.87	0.08	<0.0001*	0.712-1.000
Group B - Presentation within 3-6 hours of the onset of symptoms				
H-FABP>6.3 ng/ml at admission	0.85	0.09	0.11	0.661-1.000
hsTnI>URL at admission	0.93	0.07	0.05	0.793-1.000
H-FABP >6.3 ng/ml or hsTnI 20.24 ng/ L i.e. >URL at presentation (0 h)	0.86	0.09	0.10	0.681-1.000
hsTnI at 3 & 6 hour (ESC 3-h guideline)	1.00	0.00	0.02*	1.000-1.000
Group C - Overall performance (presentation combining both time groups)				
H-FABP>6.3 ng/ml at admission	0.84	0.05	<0.0001*	0.743-0.948
hsTnI>URL at admission	0.87	0.05	<0.0001*	0.774-980
H-FABP >6.3 ng/ml or hsTnI 20.24 ng/ L i.e. >URL at presentation (0 h)	0.82	0.04	<0.0001*	0.728-916
hsTnI at 0 & 3 hour (ESC 3-h guideline)	0.94	0.03	<0.0001*	0.873-1.000

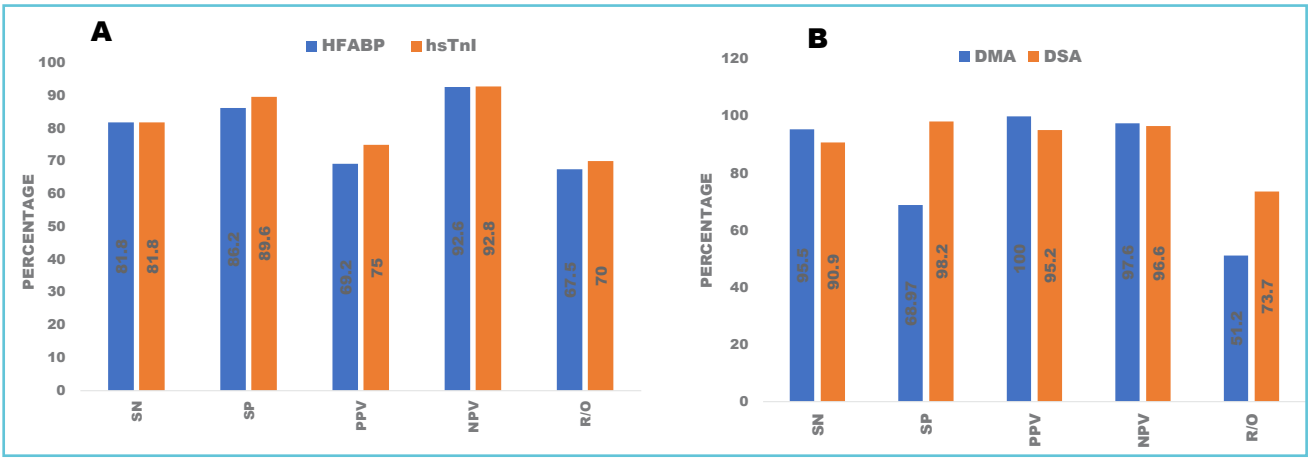
Abbreviations. H-FABP: Heart-type Fatty Acid Binding Protein, hsTnI: High sensitive Troponin-I, URL: Upper Reference Limit, CI: Confidence Interval, AUC: area under the curve<p 0.05 considered as significant.

Figure 1 ROC analysis of DMA & DSA approach: (A) Diagnostic performance of individual marker: hsTnI & HFABP at 0 hr; (B) Within 3 hours of onset of symptoms; (C) After 6 hours of the onset of symptoms



*Abbreviations: HFABP: Heart-type Fatty Acid Binding Protein, hsTnI: High sensitive Troponin-I, DMA: Dual Marker Approach, DSA: Double Sampling approach. *All SN, SP, PPV and NPV were 100% in STEMI cases not included.*

Figure 2 Overall performance (presentation combining both time groups; both NSTEMI and STEMI): (A) Comparison of diagnostic performances of HFABP & hsTnI at 0 hr; (B) Comparison of diagnostic performances of DMA & DSA approaches



*Abbreviations: HFABP: Heart-type Fatty Acid Binding Protein, hsTnI: High sensitive Troponin-I, DMA: Dual Marker Approach, DSA: Double Sampling approach, SN: Sensitivity, SP: Specificity, PPV: Positive Predictive Values, NPV: Negative Predictive Values, R/O: Ruled out patient.*All SN, SP, PPV and NPV were 100% in STEMI cases (excluded in the graph).*

Table 3A **Comparison of diagnostic performance using different approaches of marker individually and in combination (in all studied cases)**

Approach			AMI	No AMI
cTnI alone	c TnI>0.2 ng/ml	Positive	15	01
		Negative	07	57
H-FABP alone	H-FABP>6.3 ng/ml at admission	Positive	18	08
		Negative	04	50
hsTnI alone	hsTnI>URL at admission	Positive	18	06
		Negative	04	52
DMA	H-FABP >6.3 ng/ml or hsTnI 20.24 ng/L i.e. >URL at presentation (0 hour)	Both Positive	17(TP)	00(FP)
		Either Positive	04(TP)	06(FP)
		Both Negative	01(FN)	40(FN)
DSA	hsTnI at 0 & 3 hour (ESC 3-hour guideline)	Positive	20	01
		Negative	02	57

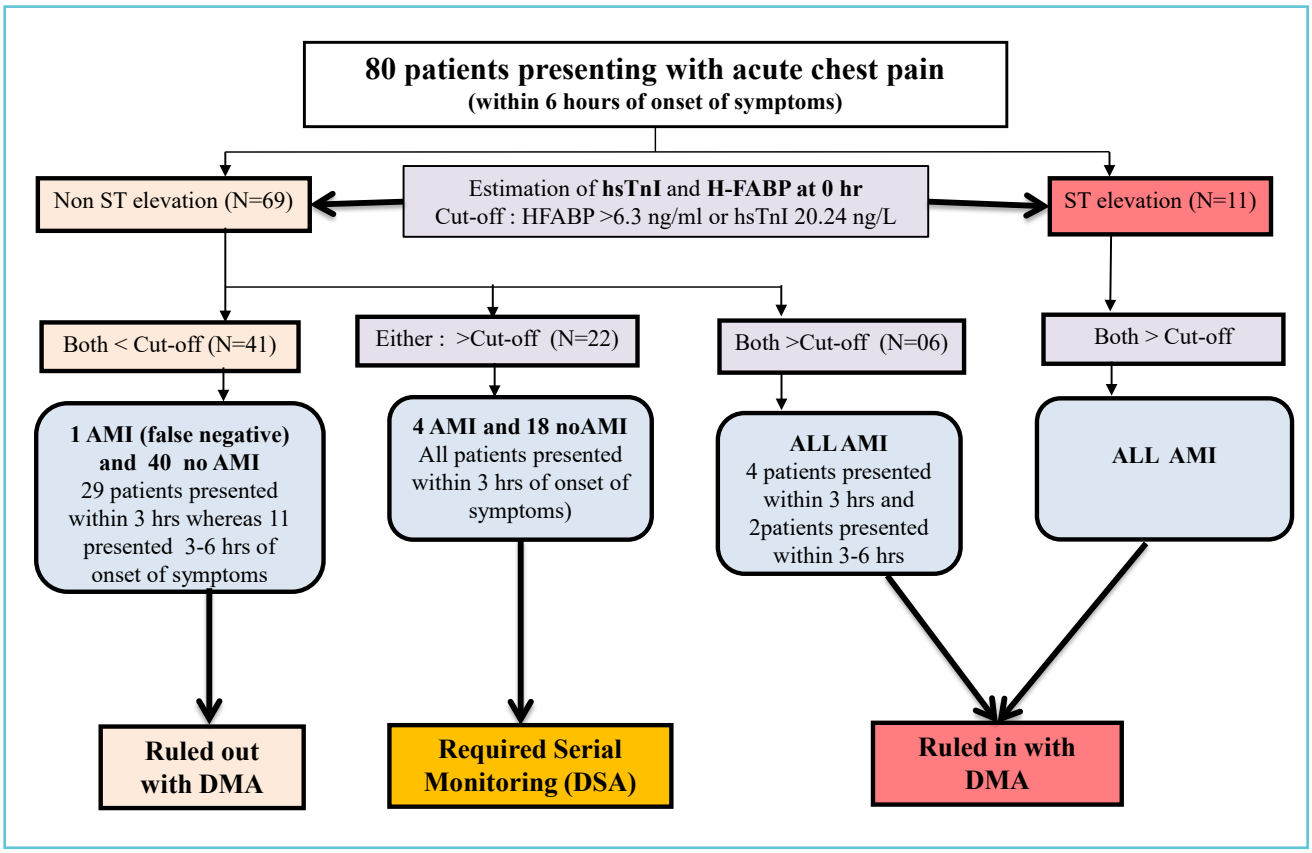
Table 3B **Comparison of diagnostic performance using different approaches of marker individually and in combination (only in patients with non ST elevation)**

Approach			AMI	No AMI
cTnI alone	cTnI >0.2 ng/ml	Positive	04	01
		Negative	07	57
H-FABP alone	H-FABP>6.3 ng/ml at admission	Positive	07	08
		Negative	04	50

hsTnI alone	hsTnI>URL at admission	Positive	07	06
		Negative	04	52
DMA	H-FABP >6.3 ng/ml or hsTnI 20.24 ng/L i.e. >URL at presentation (0 hour)	Both Positive	06(TP)	00(FP)
		Either Positive	04(TP)	06(FP)
		Both Negative	01(FN)	40(TN)
DSA	hsTnI at 0 & 3 hour (ESC 3-hour guideline)	Positive	09 (TP)	01 (FP)
		Negative	02 (FN)	57 (TN)

Abbreviations: H-FABP: Heart-type Fatty Acid Binding Protein, hsTnI: High sensitive Troponin-I, DMA: Dual Marker Approach, DSA: Double Sampling approach, URL: Upper Reference Limit, ESC: European Society of Cardiology, AMI: Acute Myocardial Infarction, TP: True Positive, FP: False Positive, TN:True Negative, FN: False Negative.

Figure 3 **Diagrammatic representation of rule-out/rule-in of AMI patients by applying DSA & DMA approach**



Abbreviations: HFABP: Heart-type Fatty Acid Binding Protein, hsTnI: High sensitive Troponin-I, AMI: Acute Myocardial Infarction, DMA: Dual Marker Approach, DSA: Double Sampling approach.

DISCUSSION

Diabetes mellitus and hypertension are well-known risk factors[14,15] for AMI, consistent with our findings (Table 1). Though smokers prevalence was not higher in our AMI patients, it has been well associated with other causes of cardiac/non-cardiac chest pain (NCCP), evident from our data. The total prevalence of smoking among our study subjects was 30 % (n=24).

Our study took the cut-off for H-FABP as 6.3 ng/ml from ROC analysis. Certain single-center Indian studies[15-17] have also described very similar thresholds (6.3, 6.4, and 6.32 ng/ml), respectively. Similar results ranging from 5.0 to 7.0 ng/ml have also been published by others[18-21], these slight variations may be due to the difference in the sampling timing from the onset of symptoms, methodology used, demographics, and ethnicity. Our findings agree with the opinion of McCann et al., who declared that assessment of H-FABP within the first 4 h of symptom onset is superior to that of cardiac Troponin T (cTnT) for the detection of AMI[21].

In this study, we compared the performance of hsTnI or H-FABP alone as well as their combinations (DMA) at admission (0 hrs) along with the DSA (using hsTnI at 0 hr and 3-hr sampling) to accurately rule in/rule out AMI in eighty acute chest pain patients in an emergency settings.

Our results showed that among patients with non-ST-elevation, the PPV of DMA was 100% if both markers were elevated. It efficiently rules out 51.2% cases (sensitivity 95.5%, NPV 97.6%; only one false negative) for AMI. None of the biomarkers (cTnI, hsTnI, or HFABP) at presentation was sufficient for a reliable rule in/ rule out of AMI when used alone.

On serial monitoring of hsTnI at 0 hr and 3 hr interval (DSA), both sensitivity (90.9%) and specificity (98.2%) and area under the curve (AUC) are significantly enhanced in comparison to when URL of hsTnI or cTnI (at cut-off of 0.2 ng/ml) were used alone at presentation (0 hour). A single negative cTnI test is not sufficient to disregard the presence of AMI because of its low sensitivity in the first 3 h of chest pain onset as per previous reports[22, 23].

In ROC analysis our data revealed that area under curve (AUC) for HFABP was 0.84 (95% Confidence Interval; 0.743-0.948) to diagnose AMI at presentation. These findings were parallel to previous studies 0.830 (95% CI; 0.770–0.890)[20] and 0.800 (95% CI; 0.760-0.840)[22] while slightly deviated from findings of Reddy et al[17] i.e. 0.728(95% CI; 0.622-0.817) and Ruff et al[24] i.e. 0.780 (95% CI; 0.720-0.840). Similarly, area under curve (AUC) for hsTnI was 0.87 (95% CI; 0.774-0.980) in our study which was consistent with Eggers et al[22] 0.840 (95% CI; 0.800-0.880) while Kellens et al[20] and Ruff et al[24] reported it as 0.790 (95% CI of 0.73–0.85) and 0.956 (95% CI; 0.930-0.990) respectively. These minor deviations might be due to heterogenity of presentation since the onset of symptoms, methodology, instrumentation and ethnicity.Eleven (11) subjects presented with ST-elevation (n=11) on ECG; all had an elevation of both hsTnI and HFABP at 0 h sample and were later confirmed as AMI cases in our study group. Thus, both the biomarkers may be used reliably as supportive evidence in such cases to rule out any subjective variation. We further focused on non-ST-elevation subjects where the role of DMA seems pertinent with PPV same as that in overall study population (presenting with or without ST elevation). Though the sensitivity decreased slightly, DMA same proportion of patients owing to same NPV.

Our results for DSA for the overall patient population (irrespective of ST-elevation and time of presentation; Table-3A) complied with that depicted by Pickering et al.[5] who noted sensitivity (95.4%), specificity (96.5%), PPV (91.3%) and NPV (98.2%).

When both markers were elevated, the diagnosis of AMI was made irrespective of the presentation time since the onset of symptoms. Among cases (n=41) with both markers below the cut-off values, only one false negative case was found.

The history of the false-negative patient was further investigated in detail. He was a 68-year-old male who lived nearby and reached the hospital within 20 minutes of developing acute chest pain. His pain was relieved by itself within 30 minutes and recurred after 15 minutes of the first episode. On the first sample taken subsequently, i.e., at admission ('0' hour), both hsTnI (2.3 ng/L) and H-FABP (1.05 ng/ml) were very low while hsTnI was raised to 14.0 ng/L at 3 hours post-admission sample. Thus, this type of case (with very early presentation) may not be ruled out in with single sample since it is also possible that ischemia might have started after the first sampling (as suggested by clinical history). The role of careful history taking and a high index of suspicion must not be overlooked in such cases, which may occur in clinical practice.

Our study focused on the strategic evaluation of different approaches for effective rule out/rules in for the diagnosis of AMI. It was done on a limited population at a single center. A multicentric study with a large number of subjects, preferably with a multimarker approach, is warranted for further evaluation.

Further, ELISA based HFABP estimation is time consuming though robust. Commercial kits on fully automated analyzers are recently available claiming remarkable analytical sensitivity. In future trials, it may be included in DMA algorithm to get rapid result and enhance practical utility in emergency settings.

CONCLUSION

A single marker assay (hsTnI or HFABP) is associated with false positives as well as false negatives. The proposed dual marker approach using H-FABP in tandem with hsTnI enhances sensitivity, positive and negative predictive values. With the use of the proposed DMA, 58/80 (72.5%) patients with acute chest pain were ruled in/ruled out for AMI at the time of their presentation itself, while the remaining 27.5% of patients still required serial monitoring (DSA) for confirmation.

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Authors' contribution

Dr. Manish Raj Kulshrestha: Conceptualization and drafting of manuscript graphs and tables preparation.

Dr. Apoorv Raj: Sample collection, history & data collection of enrolled patients.

Dr. Vandana Tiwari: Conceptualisation, Experimentation, Manuscript writing, Result analysis and manuscript reviewing and editing.

Dr. Bhuwan Chandra Tiwari: Patient enrollment, Clinical evaluation and data collection.

Dr. Ashish Jha: Patient enrollment and Clinical evaluation.

Dr. Subrat Chandra: Data collection and manuscript formatting.

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