

# Importance of Heart Score For Chest Pain Patients Presenting To Emergency Department In The Absence Of Stemi

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## ABSTRACT

**Background:** Chest pain is one of the most common presentations to the emergency department (ED), and the diagnosis of non-ST elevation acute coronary syndrome (ACS) often causes uncertainty. The HEART score is designed to differentiate high-risk and low-risk chest pain patients and serves as an easy, quick, and reliable predictor of outcome rather than mere risk differentiation. In current practice, nearly 60% of chest pain patients have no clear ACS presentation, leading to delayed decision-making and unnecessary hospital admissions. The challenge in the ED is not only to identify high-risk patients but also to safely discharge low-risk patients.

### Aim:

- A. To differentiate high-risk and low-risk patients presenting with chest pain to the ED.
- B. To predict patient outcomes using the HEART score in chest pain patients presenting to the ED.

**Methods:** This prospective study included 200 patients presenting with chest discomfort to the emergency department of PRS Hospital, Kilipalam, Trivandrum, between November 2018 and October 2019. All patients above 18 years presenting with chest pain in the absence of STEMI were included. HEART score was calculated at the time of ED arrival and patients were categorized into high-risk and low-risk groups. Patients were followed up after three days, and various prognostic indicators were recorded. Data were analyzed using SPSS software to evaluate the association between HEART score and patient outcomes.

**Results:** The mean HEART score among patients who required cardiac interventional strategies was 6. HEART score calculated at ED admission showed a strong statistical correlation with patient outcomes. A significant association was observed between HEART score and increasing age, need for PCI, mortality, and duration of hospital stay ( $p < 0.005$ ). HEART score was significantly correlated with overall patient outcome.

**Conclusion:** The HEART score assists physicians in making accurate diagnostic and therapeutic decisions in chest pain patients without ST-elevation myocardial infarction. It is an easy, quick, and reliable predictor of outcome and can be effectively used for triage and risk stratification in the emergency department.

**KEYWORDS:** Chest pain; HEART score; Acute coronary syndrome; Emergency department; Risk stratification; Patient outcome.

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## INTRODUCTION

Chest pain is one of the most common, potentially serious presenting complaints for adult emergency department (ED) visits.[1] A significant proportion of these patients undergo advanced medical evaluation during these visits, resulting in longer and more costly ED stays; during this period, the percentage of these ED presentations with resulting diagnosis of acute coronary syndrome (ACS) decreased. For many years, physicians have sought tools, ranging from specific diagnostic tests to entire strategies of evaluation, to appropriately risk stratify patients suspected of experiencing ACS; these efforts are aimed at preventing major adverse cardiac events (MACE) while reducing unnecessary testing and hospitalizations. A majority of physicians deem a miss rate of <1% for MACE as acceptable in screening tools.[2]

The HEART score was developed as a rapid risk stratification tool for patients with chest pain according to their short-term risk MACE to help identify low-risk patients, suitable for earlier ED discharge.[3] This decision tool is considered rather valuable for several reasons, including its ease of application, ready availability of the variables under consideration, the focus on short-term outcome, appropriate for ED management, and the identification of three discrete sub-populations (low-, moderate-, and high-risk) of ED chest pain patients suspected of ACS.[3]

The HEART score has been extensively studied as a clinical decision tool for risk stratification of patients presenting to the emergency department with chest pain. Backus et al. prospectively validated the HEART score and demonstrated its ability to predict major adverse cardiac events (MACE), including acute myocardial infarction, need for revascularization, and death within six weeks of presentation [4]. Their study highlighted that the HEART score enables accurate clinical decision making without the use of radiation or invasive procedures, supporting its role as a practical triage tool in emergency department settings.

Subsequent investigations focused on real-world implementation and effectiveness of the HEART Pathway. Mahler et al. evaluated real-time use of the HEART Pathway and reported that its implementation was associated with reduced hospitalizations while maintaining a very low rate of missed adverse events among low-risk patients [5]. In a large prospective multicenter study, Mahler et al. further demonstrated that HEART Pathway adoption increased identification of myocardial infarction during the index visit and significantly reduced 30-day hospitalizations, confirming the safety and effectiveness of this approach in routine emergency department practice [6].

Several studies have compared the HEART score with other commonly used risk stratification tools. Sun et al. compared the HEART and TIMI scores and found that the HEART score was more accurate, particularly in identifying low-risk patients suitable for early discharge [7]. Visser et al. compared the diagnostic accuracy of the HEART score with clinical gestalt and showed that both approaches had similar accuracy for diagnosing acute coronary syndrome, while the HEART score provided a more standardized and reproducible assessment [8]. A systematic review and meta-analysis by Phillips et al. further supported these findings, demonstrating that a low-risk HEART score had high sensitivity and negative predictive value for predicting short-term MACE across different patient populations, troponin assays, and follow-up intervals [9].

The integration of high-sensitivity troponin assays with the HEART score has also been evaluated. Willems et al. reported that patients presenting with chest pain who had a low HEART score combined with a negative high-sensitivity troponin could be safely discharged within a short observation period [10]. Parenti et al. assessed the inter-rater reliability of the HEART score among emergency physicians and demonstrated good agreement, particularly in the low- and high-risk categories, reinforcing the practicality and reproducibility of the HEART score in busy emergency department environments [11].

Additional studies have explored specific clinical applications of the HEART score. Frisoli et al. demonstrated that early discharge of low-risk patients based on a modified HEART score, without routine stress testing, significantly reduced length of stay and total healthcare costs [12]. Arslan et al. evaluated the use of the HEART score prior to coronary computed tomography angiography and found that reserving imaging for intermediate-risk patients reduced unnecessary testing without compromising diagnostic accuracy [13]. Sex-based analyses by Six et al. indicated differences in MACE rates between men and women across HEART score categories, suggesting that demographic factors may influence interpretation of HEART score-based risk stratification [14].

Overall, the existing literature supports the HEART score as a reliable, efficient, and clinically useful tool for outcome prediction and risk stratification in emergency department patients presenting with chest pain. Despite extensive validation across diverse populations and healthcare systems, further prospective evaluation remains warranted to assess its performance in different clinical settings.

## HEART SCORE COMPONENTS

### A. History (H)

The “H” component of the HEART score evaluates the clinical history of chest pain and associated symptoms. Patient history is categorized into three levels based on the likelihood of acute coronary syndrome (ACS): **nonspecific**, **mixed**, and **highly suspicious**, corresponding to scores of **0**, **1**, and **2**, respectively. Nonspecific history refers to the absence of classical ischemic features such as exertional chest pain, radiation, associated autonomic symptoms, or response to nitrates. Mixed history includes both typical and atypical features, while highly suspicious history reflects classical characteristics of ACS. It is important to note that certain populations, including elderly and female patients, may present with atypical or non-chest pain symptoms, which may influence scoring and requires careful clinical judgment.

#### Scoring criteria for History:

- Score **0**: Nonspecific history for ACS
- Score **1**: Mixed typical and atypical features
- Score **2**: Highly suspicious history consistent with ACS

### B. Electrocardiogram (E)

The “E” component assesses electrocardiographic findings using predefined objective criteria rather than subjective interpretation. The original HEART score ECG classification is based on the **Manchester scoring criteria**. An entirely normal ECG is assigned **0 points**. Repolarization abnormalities without significant ST-segment deviation are assigned **1 point**; these include findings such as bundle branch block, left ventricular hypertrophy with strain, digoxin effect, ventricular pacing, or unchanged repolarization abnormalities compared to prior ECGs, including those related to previous myocardial infarction. Significant ST-segment deviation, defined as ST-segment depression or elevation not attributable to bundle branch block, ventricular pacing, or digoxin effect, is assigned **2 points**. When prior ECGs are unavailable, abnormalities should be considered new. Patients with ST-segment elevation myocardial infarction (STEMI) are managed independently of the HEART score and were excluded from its original development.

**Scoring criteria for ECG:**

- Score **0**: Normal ECG
- Score **1**: Repolarization abnormalities without significant ST-segment deviation
- Score **2**: Significant ST-segment deviation (new or not known to be old)

**C. Age (A)**

The “A” component reflects the patient’s age in years and represents an objective variable with minimal interobserver variability. Increasing age is associated with higher cardiovascular risk and is weighted accordingly in the HEART score.

**Scoring criteria for Age:**

- Score **0**:  $\leq 45$  years
- Score **1**: 45–64 years
- Score **2**:  $\geq 65$  years

**D. Risk Factors (R)**

The “R” component evaluates traditional risk factors for coronary artery disease (CAD), including **diabetes mellitus, hypertension, hypercholesterolemia, smoking, obesity, and family history of CAD**. Risk factor burden is expressed in a graded manner. Patients with **three or more risk factors**, or with an established history of CAD-related conditions such as peripheral arterial disease, prior myocardial infarction, previous coronary revascularization, or stroke, are assigned **2 points**, irrespective of the number of other risk factors. Only physician-diagnosed conditions prior to ED presentation are considered. Clinical judgment should be exercised when formal medical documentation is unavailable.

**Scoring criteria for Risk Factors:**

- Score **0**: No risk factors
- Score **1**: One or two risk factors
- Score **2**: Three or more risk factors *or* established vascular disease

**E. Troponin (T)**

The “T” component is based on a single serum troponin value obtained during initial emergency department evaluation. Troponin levels are interpreted relative to the local laboratory’s upper reference limit.

In the original HEART score, conventional troponin assays were used; subsequent studies have validated the score using high-sensitivity troponin assays with adjusted thresholds.

**Scoring criteria for Troponin:**

- Score **0**: Troponin  $\leq$  upper reference limit
- Score **1**: Troponin 1–3 times the upper reference limit
- Score **2**: Troponin  $> 3$  times the upper reference limit

**F. Overall HEART Score**

The HEART score is calculated by summing points from all five components (**H, E, A, R, T**), yielding a total score ranging from **0 to 10**, which stratifies patients into **low-, intermediate-, and high-risk categories** for short-term major adverse cardiac events. The components and scoring criteria of the HEART score used for risk stratification are summarized in **Table 1**.

**Table 1. HEART score components and point allocation for risk stratification of chest pain patients presenting to the emergency department**

Component	Criteria	Score
<b>H – History</b>	Highly suspicious	2
	Moderately suspicious	1
	Slightly suspicious	0
<b>E – ECG changes</b>	Significant ST-segment deviation	2
	Non-specific repolarization abnormalities	1
	Normal ECG	0
<b>A – Age</b>	$\geq 65$ years	2
	45–65 years	1
	$\leq 45$ years	0
<b>R – Risk factors</b>	$\geq 3$ risk factors or history of CAD	2
	1–2 risk factors	1
	No risk factors	0
<b>T – Troponin</b>	$\geq 3\times$ upper reference limit	2

Component	Criteria	Score
	1–3× upper reference limit	1
	≤ upper reference limit	0

## I. OBJECTIVE

To evaluate the utility of the HEART score in stratifying chest pain patients presenting to the emergency department into low-risk and high-risk categories and in predicting short-term clinical outcomes.

## METHODS

This was a prospective observational study including 200 patients who presented to the emergency department with chest discomfort. The study was conducted at PRS Hospital, Kilipalam, Trivandrum, a tertiary care center with a well-equipped emergency department, between November 2018 and October 2019. The study was conducted under the guidance of Dr. Danish Salim, Head of the Department of Emergency Medicine.

Data were collected by emergency department physicians from patients presenting with chest pain. All patients aged above 18 years presenting with chest pain in the absence of ST-elevation myocardial infarction were screened, and the HEART score was applied at the time of arrival. Based on the HEART score, patients were categorized into low-risk and high-risk groups. All patients were followed up after three days, and relevant clinical outcomes were recorded.

The collected data were analyzed using SPSS software to assess the ability of the HEART score to stratify risk and predict outcomes in chest pain patients presenting to the emergency department.

### Inclusion Criteria

- Patients aged ≥18 years presenting to the emergency department with chest pain

### Exclusion Criteria

- Patients diagnosed with ST-elevation myocardial infarction
- Patients aged <18 years
- Chest pain due to trauma
- Chest pain following recent surgery
- Costochondral chest pain

## RESULT

### A Baseline Characteristics of the Study Population

The mean age of the study population was  $59.18 \pm 15.46$  years. The majority of patients belonged to the 51–60 years age group (21.5%), followed by the 61–70 years age group. Patients aged above 80 years constituted 5.5%, while 2% were aged below 30 years. The age of the study population ranged from 19 to 91 years (Figure 1). Of the 200 patients included in the study, 101 (50.5%) were males and 99 (49.5%) were females (Table 2).

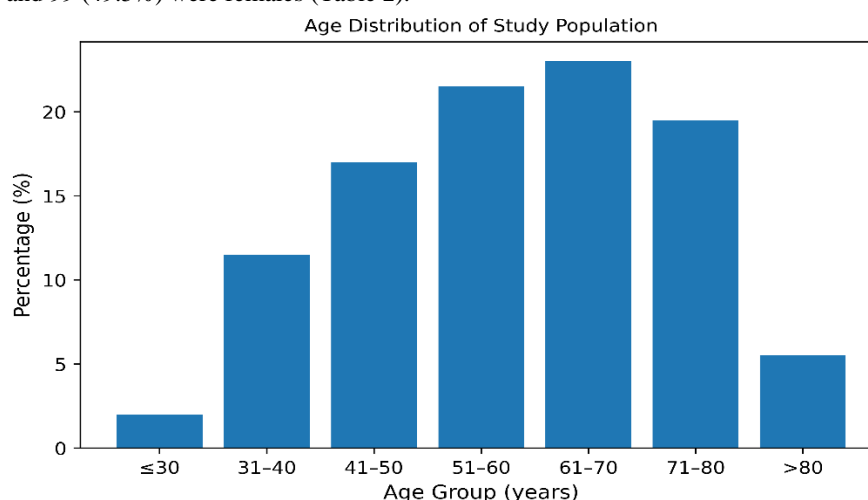


Figure 1. Age distribution of patients presenting with chest pain

**Table 2. Sex distribution of the study population**

Sex	Frequency	Percentage (%)
Male	101	50.5
Female	99	49.5
<b>Total</b>	<b>200</b>	<b>100</b>

**B Clinical Outcomes**

Clinical outcomes of the study population, including hospital admission status, need for percutaneous coronary intervention, duration of hospital stay, and mortality, were recorded during the study period and are summarized in the following tables.

**Table 3. Distribution of patients according to percutaneous coronary intervention (PCI)**

PCI Status	Frequency	Percentage (%)
Yes	109	54.5
No	91	45.5
<b>Total</b>	<b>200</b>	<b>100</b>

Percutaneous coronary intervention was performed in 109 patients (54.5%), while 91 patients (45.5%) did not undergo PCI (Table 3).

**Table 4. Distribution of patients according to length of hospital stay**

Length of Hospital Stay	Frequency	Percentage (%)
No hospital stay	76	38.0
1–4 days	34	17.0
5–7 days	61	30.5
>7 days	29	14.5
<b>Total</b>	<b>200</b>	<b>100</b>

The duration of hospital stay varied among patients, with 38.0% not requiring hospitalization. Among admitted patients, 30.5% stayed for 5–7 days, while 14.5% had a hospital stay exceeding 7 days (Table 4).

**Table 5. Mortality among chest pain patients**

Mortality	Frequency	Percentage (%)
Yes	13	6.5
No	187	93.5
<b>Total</b>	<b>200</b>	<b>100</b>

A total of 13 patients (6.5%) died during the study period, while 187 patients (93.5%) survived (Table 5).

**C Distribution of HEART Score**

Based on HEART score categorization, 112 patients (56.0%) had a HEART score  $\geq 6$ , while 88 patients (44.0%) had a HEART score  $\leq 5$  (Table 6).

**Table 6. Distribution of patients according to HEART score category**

HEART Score Category	Frequency	Percentage (%)
$\geq 6$	112	56.0
$\leq 5$	88	44.0
<b>Total</b>	<b>200</b>	<b>100</b>

The mean HEART score of the study population was  $5.8 \pm 1.7$ , with scores ranging from 2 to 9. The median HEART score was 6, with an interquartile range of 5–7 (Table 7).

**Table 7. Descriptive statistics of HEART score in the study population**

Parameter	Value
Number of patients (N)	200
Mean HEART score	5.8
Standard deviation	1.7
Minimum	2

Parameter	Value
Maximum	9
First quartile (Q1)	5
Median	6
Third quartile (Q3)	7

#### D Association between HEART score and demographic variables

The age of the patients was significantly associated with the HEART score ( $p < 0.001$ ). Higher HEART scores ( $\geq 6$ ) were observed in 11.8% of patients aged 41–50 years, 53.5% of those aged 51–60 years, 82.6% of those aged 61–70 years, 89.7% of those aged 71–80 years, and in all patients aged above 80 years (Table 8). The proportion of patients with higher HEART scores increased progressively across advancing age groups.

**Table 8. Association between HEART score and age group of the patients**

Age group (years)	HEART score $\geq 6$ n (%)	HEART score $\leq 5$ n (%)	Total n (%)
$\leq 30$	1 (25.0)	3 (75.0)	4 (100)
31–40	0 (0.0)	23 (100.0)	23 (100)
41–50	4 (11.8)	30 (88.2)	34 (100)
51–60	23 (53.5)	20 (46.5)	43 (100)
61–70	38 (82.6)	8 (17.4)	46 (100)
71–80	35 (89.7)	4 (10.3)	39 (100)
>80	11 (100.0)	0 (0.0)	11 (100)
<b>Total</b>	<b>112 (56.0)</b>	<b>88 (44.0)</b>	<b>200 (100)</b>

There was no statistically significant association between gender and HEART score ( $\chi^2 = 0.961$ ,  $df = 1$ ,  $p = 0.327$ ). Higher HEART scores ( $\geq 6$ ) were observed in 59.4% of males and 52.5% of females (Table 9).

**Table 9. Association between gender and HEART score**

Sex	HEART score $\geq 6$ n (%)	HEART score $\leq 5$ n (%)	Total n (%)
Male	60 (59.4)	41 (40.6)	101 (100)
Female	52 (52.5)	47 (47.5)	99 (100)
<b>Total</b>	<b>112 (56.0)</b>	<b>88 (44.0)</b>	<b>200 (100)</b>

#### D Association between HEART score and clinical outcomes

A statistically significant association was observed between HEART score and the need for percutaneous coronary intervention (PCI) ( $\chi^2 = 46.98$ ,  $df = 1$ ,  $p < 0.001$ ). Among patients who underwent PCI, 78% had a HEART score  $\geq 6$ , whereas only 29.7% of patients who did not undergo PCI had a HEART score  $\geq 6$ . Patients requiring PCI had significantly higher HEART scores compared to those managed without PCI (Table 10).

**Table 10. Association between HEART score and Percutaneous Coronary Intervention (PCI)**

PCI status	HEART score $\geq 6$ n (%)	HEART score $\leq 5$ n (%)	Total n (%)
Yes	85 (78.0)	24 (22.0)	109 (100)
No	27 (29.7)	64 (70.3)	91 (100)
<b>Total</b>	<b>112 (56.0)</b>	<b>88 (44.0)</b>	<b>200 (100)</b>

Mortality was found to be significantly associated with the HEART score ( $\chi^2 = 10.92$ ,  $df = 1$ ,  $p = 0.001$ ). All patients who died (100%) had a HEART score  $\geq 6$ , whereas no deaths were observed among patients with a HEART score  $\leq 5$ . This finding indicates that higher HEART scores were strongly associated with increased mortality in patients presenting with chest pain to the emergency department (Table 11).



**Table 11. Association between HEART score and mortality**

<b>Mortality</b>	<b>HEART score <math>\geq 6</math> n (%)</b>	<b>HEART score <math>\leq 5</math> n (%)</b>	<b>Total n (%)</b>
Yes	13 (100)	0 (0)	13 (100)
No	99 (52.9)	88 (47.1)	187 (100)
<b>Total</b>	<b>112 (56.0)</b>	<b>88 (44.0)</b>	<b>200 (100)</b>

Hospital admission was significantly associated with the HEART score ( $\chi^2 = 36.71$ ,  $df = 1$ ,  $p < 0.001$ ). Among patients who required admission, 73.7% had a HEART score  $\geq 6$ , whereas only 30.5% of patients who were not admitted had a HEART score  $\geq 6$ . This indicates that patients with higher HEART scores were significantly more likely to require hospital admission for further evaluation (Table 12).

**Table 12. Association between HEART score and hospital admission**

<b>Admission</b>	<b>HEART score <math>\geq 6</math> n (%)</b>	<b>HEART score <math>\leq 5</math> n (%)</b>	<b>Total n (%)</b>
Yes	87 (73.7)	31 (26.3)	118 (100)
No	25 (30.5)	57 (69.5)	82 (100)
<b>Total</b>	<b>112 (56.0)</b>	<b>88 (44.0)</b>	<b>200 (100)</b>

The duration of hospital stay was significantly associated with the HEART score ( $\chi^2 = 63.13$ ,  $df = 3$ ,  $p < 0.001$ ). Among patients who did not require hospital admission, the majority (73.7%) had a HEART score  $\leq 5$ . In contrast, higher HEART scores ( $\geq 6$ ) were increasingly observed with longer hospital stay: 44.1% among patients hospitalized for 1–4 days, 80.3% for 5–7 days, and 96.6% among those hospitalized for more than 7 days. This demonstrates a strong positive association between increasing HEART score and prolonged hospital stay (Table 13).

**Table 13. Association between HEART score and duration of hospital stay**

<b>Hospital stays</b>	<b>HEART score <math>\geq 6</math> n (%)</b>	<b>HEART score <math>\leq 5</math> n (%)</b>	<b>Total n (%)</b>
No hospital stays	20 (26.3)	56 (73.7)	76 (100)
1–4 days	15 (44.1)	19 (55.9)	34 (100)
5–7 days	49 (80.3)	12 (19.7)	61 (100)
>7 days	28 (96.6)	1 (3.4)	29 (100)
<b>Total</b>	<b>112 (56.0)</b>	<b>88 (44.0)</b>	<b>200 (100)</b>

## DISCUSSION

This prospective study evaluated the utility of the HEART score in risk stratification and outcome prediction among 200 patients presenting to the emergency department with chest discomfort in the absence of ST-elevation myocardial infarction. Patients were categorized into low- and high-risk groups based on the HEART score, with the objective of assessing its effectiveness in predicting clinical outcomes, including admission, intervention, length of hospital stay, and mortality.

In the present study, the mean HEART score among admitted patients was 6. This finding is consistent with a multicenter observational study by Parenti N, Lippi G, et al., which demonstrated that patients at risk of developing myocardial infarction who required admission had an average HEART score of 6 [15]. Our findings further reinforce this threshold, as a significant proportion of patients requiring hospital admission belonged to the high-risk HEART score category. Although 73.7% of patients required admission for chest pain evaluation, only 30.5% of admitted patients had a HEART score  $>6$ , highlighting the role of clinical judgment in conjunction with risk scoring.

A prospective cohort study by Visser A, et al., evaluated the diagnostic accuracy of the HEART score in predicting the need for cardiac intervention among emergency department patients [16]. In their study of 255 patients, the mean HEART score among patients undergoing coronary intervention was reported as 6. Similarly, in our study, the overall mean HEART score was 6, and 54.5% of patients underwent percutaneous coronary intervention, supporting the HEART score's role in identifying patients likely to require invasive management.

Gender-based analysis in our study demonstrated no statistically significant association between sex and HEART score. Higher HEART scores ( $\geq 6$ ) were observed in 59.4% of males and 52.5% of females, with no significant difference between groups. This contrasts with findings from a study conducted in Birmingham by Graham CA, et al., which reported higher HEART scores among males undergoing cardiac interventions [17]. Our findings suggest that, in our population, the HEART score performs independently of gender.

Age showed a strong and statistically significant association with HEART score in our study. As patient age increased, HEART scores also increased significantly. High HEART scores were observed in 11.8% of patients aged 41–50 years, 53.5% of those aged 51–60 years, and a substantially higher proportion in patients aged above 60 years. These findings are consistent with observations by Carlton EW, et al., who demonstrated a positive correlation between advancing age and increasing HEART scores, emphasizing age as a key determinant in cardiovascular risk stratification [18].

Backus BE, et al., demonstrated that the HEART score is a reliable predictor of major adverse cardiac events and short-term mortality [19]. In our study, the HEART score proved to be an excellent predictor of mortality, with all patients who died during hospitalization having a HEART score  $\geq 6$ . The optimal cutoff value of HEART score  $>6$  showed a sensitivity of 100%, specificity of 65.24%, positive predictive value of 16.7%, and negative predictive value of 100%, underscoring its utility as a rule-out tool for mortality in emergency department settings.

The duration of hospital stay was also significantly associated with HEART score in our study. Patients with higher HEART scores required prolonged hospitalization, particularly those with scores  $\geq 7$ . Approximately 30.5% of patients required a hospital stay of 5–7 days, and 14.5% required hospitalization beyond 7 days, with most belonging to the high-risk HEART score group. Similar findings were reported by Burke GL, et al., who observed that more than 70% of patients with HEART scores  $>7$  required prolonged hospital stay [20]. Additionally, Brian T. Taylor, et al., reported that patients with higher HEART scores had longer hospital stays, further supporting our findings [21].

Cost-effectiveness and resource utilization associated with HEART score-guided management were highlighted in a study by Riley RF, et al., which demonstrated significant cost savings when low-risk patients were safely discharged without unnecessary testing [22]. Our findings align with this observation, as patients with HEART scores  $\leq 5$  had shorter hospital stays and fewer interventions, reinforcing the role of the HEART score in efficient emergency department triage.

Engel J, et al., emphasized the importance of structured risk stratification tools in managing patients with unstable angina and non-ST-elevation myocardial infarction [23]. Similarly, our study demonstrates that a HEART score  $>6$  effectively identifies high-risk patients, with 73.7% undergoing coronary intervention, allowing clinicians to stratify patients into appropriate risk categories and guide management decisions.

Overall, our findings support the HEART score as a simple, reliable, and effective clinical decision tool for risk stratification, outcome prediction, and triage of patients presenting with chest pain to the emergency department.

#### Financial Support and Sponsorship

Nil.

#### Conflicts of Interest

The authors declare no conflicts of interest.

#### Ethical Approval

This study was conducted in accordance with the ethical standards of the Institutional Ethics Committee. Written informed consent was obtained from all participants prior to inclusion in the study.

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