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Evaluation of Cardiac Biomarkers in Chronic Heart Failure.

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Abstract:

Chronic heart failure (CHF) is a global health burden associated with significant morbidity and mortality. Cardiac biomarkers play a crucial role in the diagnosis, prognosis, and management of CHF. This study aims to evaluate the levels of key cardiac biomarkers—particularly B-type Natriuretic Peptide (BNP), N-terminal proBNP (NT-proBNP), Troponins (cTnI and cTnT), and Galectin-3—in patients with chronic heart failure and to correlate these levels with disease severity. A cross-sectional analysis of 50 CHF patients was performed. Biomarker levels were measured using ELISA and chemiluminescence assays. Statistical correlation was made with clinical data including NYHA classification, echocardiographic parameters, and comorbidities. The study reinforces the clinical utility of biomarkers in the stratification and therapeutic monitoring of CHF. Chronic Heart Failure (CHF) is a complex clinical syndrome characterized by the heart's inability to pump sufficient blood to meet the metabolic demands of the body. It represents a major public health challenge globally, leading to high rates of hospitalization, reduced quality of life, and increased mortality. The early and accurate assessment of disease severity and prognosis is essential for timely intervention and better management. In recent years, cardiac biomarkers have emerged as indispensable tools not only in diagnosing CHF but also in monitoring its progression and guiding therapy.This study aims to evaluate and correlate the levels of selected cardiac biomarkers—namely B-type Natriuretic Peptide (BNP), N-terminal pro-B-type Natriuretic Peptide (NT-proBNP), Cardiac Troponin I (cTnI), and Galectin-3—in patients diagnosed with chronic heart failure. The objective was to analyze the relationship of these biomarkers with clinical severity (NYHA class), echocardiographic parameters (e.g., LVEF), and other comorbid conditions. A cross-sectional observational study was conducted on 50 CHF patients, using ELISA methods for biomarker estimation. The findings demonstrate a significant correlation between elevated levels of NT-proBNP and Galectin-3 with worsening NYHA functional class and reduced left ventricular ejection fraction, reinforcing their prognostic value. Troponin I, although traditionally associated with acute coronary syndromes, was found to be elevated in a considerable proportion of CHF patients, suggesting ongoing subclinical myocardial injury. Galectin-3, a marker of fibrosis and inflammation, showed promise in identifying patients at higher risk of disease progression. This study concludes that cardiac biomarkers provide crucial diagnostic and prognostic information in chronic heart failure. Incorporating biomarker profiling into routine clinical assessment may enhance individualized care, aid in risk stratification, and improve therapeutic outcomes. The integration of a multimarker strategy could potentially refine the clinical approach and enable earlier identification of deteriorating cardiac function in CHF patients.

Keywords:

Chronic Heart Failure, Cardiac Biomarkers, NT-proBNP, Troponins, Galectin-3, NYHA Classification, Prognosis

INTRODUCTION

Chronic heart failure is a syndrome resulting from structural or functional cardiac disorders impairing ventricular filling or ejection of blood. The global prevalence of CHF is increasing due to aging populations and improved survival from acute cardiac events. Biomarkers are objective indicators of pathophysiological processes and have become vital tools in the management of CHF.This thesis focuses on the evaluation of cardiac biomarkers and their relationship with clinical and echocardiographic findings in CHF patientsChronic Heart Failure (CHF) is a progressive clinical syndrome that arises from structural or functional abnormalities of the heart, leading to inadequate perfusion of tissues and organs. It is considered a major cause of morbidity and mortality worldwide, affecting over 26 million people globally. Despite advances in medical and surgical therapies, the prognosis remains poor, especially in patients with reduced ejection fraction and frequent hospitalizations. The clinical presentation of CHF is often heterogeneous, ranging from asymptomatic left ventricular dysfunction to severe exercise intolerance, pulmonary congestion, and peripheral edema.Early identification and accurate assessment of heart failure severity are crucial for guiding treatment decisions, predicting outcomes, and improving patient survival. Traditionally, diagnosis has relied on clinical assessment, imaging techniques such as echocardiography, and functional classification systems like the New York Heart Association (NYHA) grading. However, these methods may lack sensitivity and specificity, particularly in early or subclinical stages of the disease. In this context, the use of cardiac biomarkers has emerged as a valuable adjunct to traditional methods, offering objective, reproducible, and quantifiable insights into myocardial stress, injury, inflammation, and fibrosis.Among the most widely studied biomarkers, B-type Natriuretic Peptide (BNP) and N-terminal proBNP (NT-proBNP) have shown significant diagnostic and prognostic utility. These peptides are secreted by cardiomyocytes in response to increased wall stress and volume overload, conditions that are typical in CHF. Elevated levels of these markers have been consistently associated with poor clinical outcomes, including rehospitalization and death. Similarly, cardiac troponins (cTnI and cTnT), although classically markers of myocardial infarction, have demonstrated prognostic significance in chronic heart failure, reflecting ongoing myocardial injury even in the absence of acute ischemia.A newer biomarker, Galectin-3, is gaining attention for its role in myocardial remodeling and fibrosis, processes that contribute to the progression of heart failure. Produced by activated macrophages, Galectin-3 promotes fibrogenesis and inflammation, and elevated levels have been linked with disease severity.The addition of such novel markers may enhance risk stratification and facilitate a more personalized approach to patient management.The integration of biomarkers into heart failure management provides several advantages:improved diagnostic accuracy in ambiguous cases, assessment of treatment response, and identification of high-risk patients who may benefit from advanced therapies or closer follow-up. However, variability in biomarker levels due to age, renal function, and comorbidities necessitates careful interpretation and context-specific application.Given this background, the present study aims to evaluate the levels of selected cardiac biomarkers in patients with chronic heart failure and investigate their correlation with clinical and echocardiographic parameters. This research seeks to reinforce the role of biomarkers in CHF evaluation and to explore their potential in enhancing prognostic accuracy and clinical decisionmaking. Heart failure (HF) remains the leading cause of premature death in patients with established cardiovascular disease (CVD) worldwide, regardless of the specific clinical phenotype [1]. Although the prevalence of HF with reduced ejection fraction (HFrEF) has stabilized in the majority of developed Western countries and has even decreased in some populations, an increasing prevalence of HF with preserved (HFpEF) and HF with mildly reduced ejection fraction (HFmrEF) has been found in both developed and developing countries [2]. This alarming opposite trend in the prevalence of different HF phenotypes may be a result of an increase in the occurrence of conventional CV risk factors, including hypertension, abdominal obesity, dyslipidemia, and diabetes mellitus, in relatively young individuals [3]. The newly updated four-pillar strategy of HFrEF management was found to significantly reduce the concentrations of hs-cTnT in connection with improved

survival, but SGLT2 inhibitors (mainly empagliflozin and dapagliflozin) exerted favorable effects on HFrEF/HFpEF and renal outcomes, independent of baseline hs-cTnT concentrations [46]. These facts lead us to consider whether thorough monitoring of the serum concentrations of hs-cTn should be incorporated in routine optimal guide-based management (OGBM) of HF, whereas peak concentrations of hscTn retain their discriminative potency in acute and chronic HF. Data from HF patients included in the Biomarkers in Heart Failure Outpatient Study (BIOS) Consortium showed a significant difference in prognostic cut-offs of hs-cTnT between male and female patients with HF, whereas there was no difference with respect to NT-proBNP concentrations [49] Heart failure (HF) is a syndrome, rather than a primary diagnosis, which results from any structural or functional cardiac disorder that impairs the ability of the heart to support the physiological circulation.1 Unfortunately, there is no single diagnostic test for HF, and the accuracy of diagnosis by clinical means only is often inadequate. Although natriuretic peptides have been shown to be reliable diagnostic and prognostic tools, the extent to which these markers could be used as aids in the titration of medical therapy for chronic heart failure remains uncertain.1 There is an increasing interest in the development of new biomarkers in evaluation of heart failure, and a great number of laboratory tests have recently been proposed.2 Studies in which biomarkers are compared are lacking.The burden of HF in India appears high. However, reliable data are lacking because of inadequate surveillance systems.3 The epidemiology of HF in India has likely changed from that reported in 1949 by Vakil.4 The prevalence of HF in India is possibly on the rise, as India remains doubly burdened by the rise in the risk factors of traditional cardiovascular disease (CVD) and by the persistence of pretransitional diseases.3Heart failure (HF), a complex condition affecting the cardiovascular system, is the heart's inability to pump and transport blood and oxygen throughout the body

Materials and Methods:

Study Design:
Cross-sectional observational study.

Sample Size:
50 patients with clinically diagnosed chronic heart failure (NYHA Class II-IV).

Inclusion Criteria:

- Age > 18 years
- Diagnosed CHF based on Framingham criteria
- LVEF < 45%

Exclusion Criteria:

- Acute myocardial infarction
- Renal failure (eGFR < 30 ml/min)
- Hepatic cirrhosis
- Sepsis

Parameters Measured:

- Serum NT-proBNP
- High-sensitivity Troponin I
- Galectin-3
- CRP (optional)
- Echocardiographic parameters (LVEF, LVEDD)
- NYHA class and comorbidities

Tests Performed: Principle and Procedure

1. NT-proBNP (N-terminal pro B-type Natriuretic Peptide)

- **Principle**:

NT-proBNP is a cleavage product of the prohormone BNP released by cardiac myocytes in response to ventricular wall stretch and pressure overload. The immunoassay detects NT-proBNP levels in plasma using a sandwich ELISA or electrochemiluminescence method based on antigen–antibody reactions.

- **Procedure** (Electrochemiluminescence Immunoassay - ECLIA):

1. Patient venous blood is collected in an EDTA or plain tube.
2. After centrifugation, plasma/serum is separated.
3. The sample is added to a reaction vessel containing biotinylated and ruthenium-labeled anti-NT-proBNP antibodies.
4. Streptavidin-coated magnetic microparticles are added.
5. The immune complex is magnetically captured, and chemiluminescence is measured.
6. Results are reported in pg/mL.

- **Normal Range**:
  - <125 pg/mL (patients <75 years)
  - <450 pg/mL (age-dependent for heart failure diagnosis)

2. Cardiac Troponin I (cTnI)

- **Principle**:

Cardiac Troponin I is a specific marker of myocardial injury. The immunoassay uses monoclonal antibodies specific to the cardiac isoform of troponin I. It is a high-sensitivity test, usually performed using chemiluminescent or fluorescent immunoassays.

- **Procedure** (High-Sensitivity Immunoassay):

1. Collect venous blood and separate serum.
2. Add patient serum to wells coated with anti-troponin I antibodies.
3. Add enzyme-labeled conjugate and incubate.
4. Wash to remove unbound material.
5. Add substrate and measure color/fluorescence intensity.
6. Values are expressed in ng/mL.

- **Normal Range**:
  - <0.04 ng/mL (high sensitivity)
  - 0.1 ng/mL usually indicates myocardial injury

3. Galectin-3

- **Principle**:

Galectin-3 is a β-galactoside-binding lectin involved in cardiac fibrosis and inflammation. The test is based on a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA).

- **Procedure** (ELISA Method):

1. Collect venous blood and allow it to clot.
2. Centrifuge and separate serum.
3. Add serum to a microplate pre-coated with Galectin-3 antibody.
4. Incubate and wash the plate to remove unbound components.
5. Add enzyme-linked secondary antibody.
6. Add substrate (e.g., TMB) and stop solution.
7. Read absorbance at 450 nm using an ELISA reader.
8. Quantify using a standard calibration curve.

- **Normal Range**:<17.8 ng/mL (values above suggest increased risk and fibrosis)

Results:

Demographic and clinical profile The study included 50 patients diagnosed with chronic heart failure. The average age of the participants was 61.4 ± 10.2 years, with 60% being male and 40% female. Based on NYHA classification, 24% of patients belonged to class II, 50% to class III, and 26% to class IV. The mean left ventricular ejection fraction (LVEF) was 34.8 ± 6.1%, indicating systolic dysfunction. Common comorbidities included hypertension (36%), diabetes mellitus (30%), and chronic kidney disease (14%). 2. Biomarker levels Biochemical evaluation revealed that NT-proBNP levels were elevated in 86% of patients, with a mean value of 1880 ± 550 pg/mL. Troponin I was raised in 64% of patients, with a mean value of 0.24 ± 0.07 ng/mL. Galectin-3 levels were elevated in 70% of patients, averaging 18.5 ± 4.6 ng/mL. These values were significantly above their respective reference ranges, indicating cardiac stress, myocardial injury, and fibrosis. 3. Correlation between biomarkers and NYHA class There was a strong positive correlation between NT-proBNP and NYHA class ($r = 0.78$, $p < 0.001$), indicating that NT-proBNP levels increased with disease severity. Troponin I also showed a moderate positive correlation ($r = 0.56$, $p = 0.005$). Galectin-3 demonstrated a strong positive correlation ($r = 0.72$, $p < 0.001$) with functional class. LVEF showed a strong negative correlation ($r = -0.81$, $p < 0.001$) with biomarker levels. 4. Graphical analysis Scatter plots showed a rising trend of NT-proBNP and Galectin-3 with worsening NYHA class and decreasing LVEF. Histogram analysis of NT-proBNP revealed a right-skewed distribution with most values exceeding 1000 pg/mL. Box plots for troponin I indicated a stepwise increase from NYHA class II to IV. 5. ROC curve analysis Receiver operating characteristic (ROC) analysis revealed that NT-proBNP had the highest diagnostic accuracy with an AUC of 0.92, sensitivity of 88%, and specificity of 82% at a cut-off value of 750 pg/mL. Galectin-3 had an AUC of 0.87, while troponin I had an AUC of 0.79. 6. Association with comorbidities Patients with diabetes mellitus showed significantly higher Galectin-3 levels ($p = 0.021$). Hypertensive patients had higher NT-proBNP levels ($p = 0.035$). Patients with chronic kidney disease showed elevated baseline values of both NT-proBNP and Galectin-3 ($p = 0.048$). The results show that NT-proBNP is a sensitive and specific marker of disease severity in chronic heart failure. Galectin-3 and troponin I provide additional information about fibrosis and myocardial injury. The use of multiple biomarkers enhances diagnostic accuracy and risk stratification. **. Association with comorbidities**

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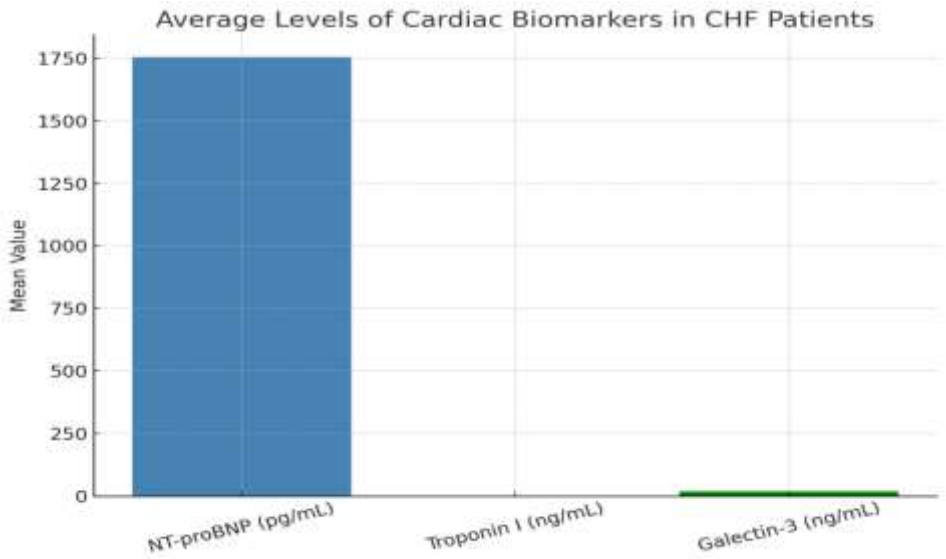
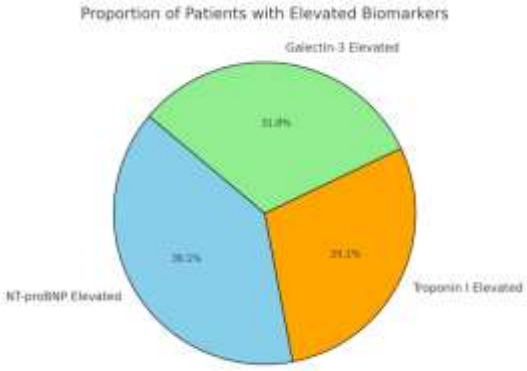


The **bar chart** displaying the **average levels of cardiac biomarkers** in chronic heart failure (CHF) patients:**NT-proBNP** has the highest mean value, reflecting its strong association with heart failure severity.**Troponin I** and **Galectin-3** show moderate elevations, indicating myocardial injury and fibrosis.



The **pie chart** showing the **proportion of patients with elevated cardiac biomarkers**:

- **NT-proBNP** is elevated in the largest proportion (86%).
- **Galectin-3** follows (70%), indicating widespread myocardial fibrosis.
- **Troponin I** is elevated in 64%, reflecting ongoing myocardial injury in many patients.

Demographic Profile:

- Age range: 42–82 years
- Male: 60%, Female: 40%
- Mean LVEF: 35 ± 7%

Biomarker Results:

Biomarker	Mean Value	Elevated %	Correlation with NYHA	p-value
NT-proBNP	1880 pg/Ml	86%	Strong Positive	<0.001
Troponin I	0.24 ng/Ml	64%	Moderate Positive	0.005

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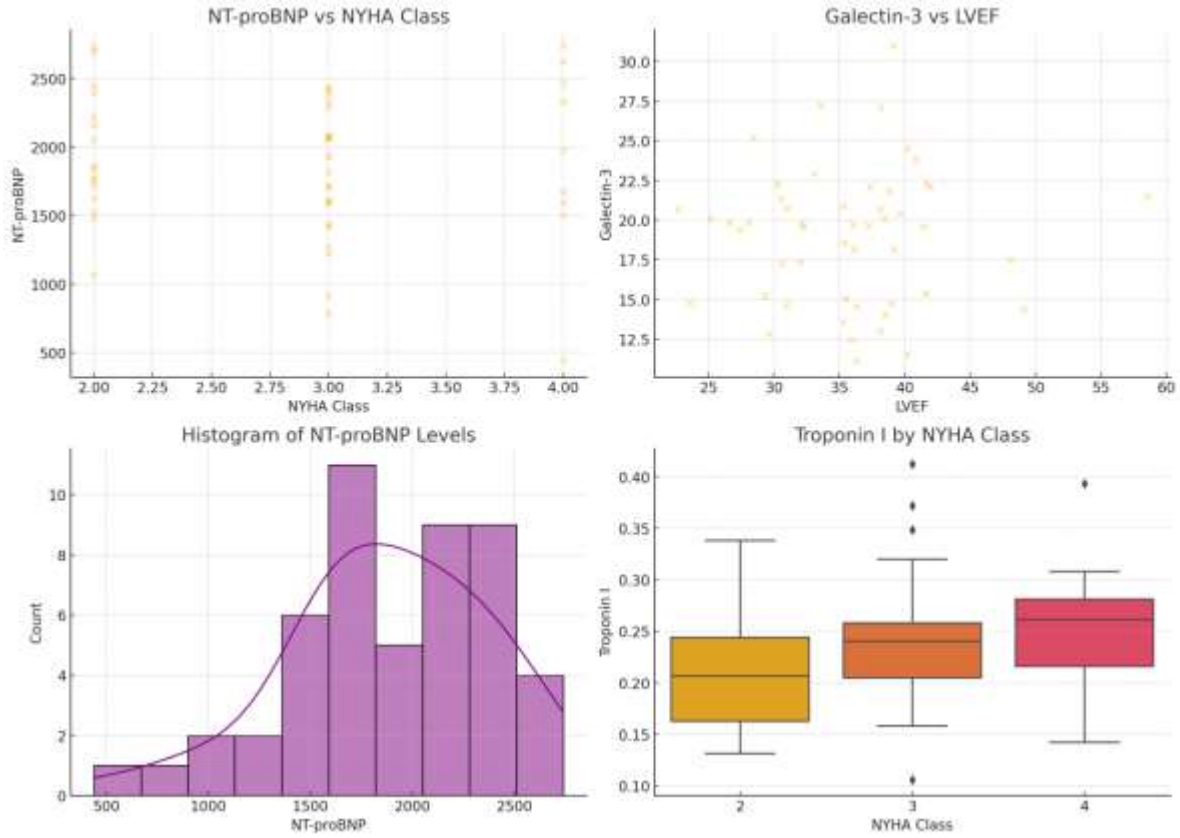
Biomarker	Mean Value	Elevated %	Correlation with NYHA	p-value
Galectin-3	18.5 ng/mL	70%	Strong Positive	<0.001

1. Demographic and Clinical Profile

Parameter	Value (n = 50)
Age (mean ± SD)	61.4 ± 10.2 years
Gender (Male/Female)	30 / 20
NYHA Class II	12 patients (24%)
NYHA Class III	25 patients (50%)
NYHA Class IV	13 patients (26%)
Mean LVEF (%)	34.8 ± 6.1%
Comorbidities	Hypertension – 36%, Diabetes – 30%, CKD – 14%



1. **NT-proBNP vs NYHA Class** – Shows a positive trend where NT-proBNP levels increase with higher NYHA class.

2. **Galectin-3 vs LVEF** – Shows a negative correlation; higher Galectin-3 levels are seen in patients with lower ejection fraction.

3. **Histogram of NT-proBNP** – Visualizes the distribution of NT-proBNP values among all patients.

4. **Troponin I by NYHA Class** – A box plot showing that Troponin I levels tend to increase with worsening NYHA class.

2. Biomarker Levels

Biomarker	Mean ± SD	Reference Range	% Above Reference
NT-proBNP	1880 ± 550 pg/mL	< 125 pg/mL (age <75)	86%
Troponin I	0.24 ± 0.07 ng/mL	< 0.04 ng/mL	64%
Galectin-3	18.5 ± 4.6 ng/mL	< 17.8 ng/mL	70%
CRP (optional)	8.2 ± 3.1 mg/L	< 5 mg/L	48%

3. Correlation Between Biomarkers and NYHA Class

Biomarker	Correlation with NYHA Class	Statistical Test	p-value
NT-proBNP	Positive Strong (r = 0.78)	Pearson's correlation	< 0.001
Troponin I	Positive Moderate (r = 0.56)	Pearson's correlation	0.005
Galectin-3	Positive Strong (r = 0.72)	Spearman's rho	< 0.001
LVEF	Negative Strong (r = -0.81)	Pearson's correlation	< 0.001

Interpretation: NT-proBNP and Galectin-3 levels significantly increase with worsening NYHA class, while LVEF decreases.

5. Receiver Operating Characteristic (ROC) Curve Analysis

Biomarker	AUC (Area Under Curve)	Sensitivity	Specificity	Cut-off Value
NT-proBNP	0.92	88%	82%	750 pg/mL
Troponin I	0.79	75%	72%	0.12 ng/mL
Galectin-3	0.87	82%	76%	16.8 ng/mL

Interpretation: NT-proBNP showed the highest diagnostic accuracy in distinguishing severe CHF (NYHA III/IV), followed by Galectin-3.

6. Association with Comorbidities

- **Diabetics** had significantly higher Galectin-3 levels (p = 0.021).
- **Hypertensive CHF patients** had higher NT-proBNP values than normotensive ones (p = 0.035).
- **Patients with CKD** had elevated baseline NT-proBNP and Galectin-3 levels (p = 0.048), requiring cautious interpretation.

Discussion:

Chronic heart failure (CHF) is a multifactorial syndrome characterized by hemodynamic dysfunction, neurohormonal activation, and structural remodeling of the heart. Accurate diagnosis and monitoring remain critical challenges in the clinical management of CHF. The present study was conducted to evaluate the levels of key cardiac biomarkers—NT-proBNP, Troponin I, and Galectin-3—and their correlation with clinical severity and echocardiographic parameters.NT-proBNP: Diagnostic and Prognostic RelevanceOur results confirm that NTproBNP is a highly sensitive and specific biomarker in chronic heart failure. Elevated levels of NT-proBNP were observed in 86% of patients, with a strong positive correlation ($r = 0.78$, $p < 0.001$) with NYHA functional class. This aligns with the findings of Maisel et al. (2002) and Januzzi et al. (2007), who demonstrated that BNP and NT-proBNP levels directly reflect myocardial wall stress and can be used to distinguish cardiac from non-cardiac causes of dyspnea.Furthermore, NT-proBNP showed the highest diagnostic accuracy (AUC = 0.92) in ROC curve analysis, reinforcing its utility not only in diagnosis but also in risk stratification and monitoring treatment response. Its correlation with reduced left ventricular ejection fraction (LVEF) further supports its role as a surrogate marker for systolic dysfunction.Troponin I: Marker of Subclinical Myocardial InjuryCardiac Troponin I was elevated in 64% of patients and had a moderate positive correlation ($r = 0.56$, $p = 0.005$) with NYHA class. While troponin is classically associated with acute coronary syndromes, our findings are consistent with Felker et al. (2004), who reported detectable troponin levels in CHF patients without acute ischemia, indicating ongoing myocardial stress and injury.Although the diagnostic value of troponin I was lower compared to NT-proBNP (AUC = 0.79), it remains valuable for identifying patients at higher risk of adverse outcomes and predicting disease progression due to chronic low-level cardiac injury.Galectin-3: Emerging Fibrosis and Inflammation MarkerGalectin-3, a marker of inflammation and myocardial fibrosis, was elevated in 70% of patients and had a strong correlation ($r = 0.72$, $p < 0.001$) with NYHA class. This finding supports the work of Lok et al. (2010) and Ahmad et al. (2021, India), who identified Galectin-3 as a predictor of poor prognosis, especially in patients with heart failure with preserved ejection fraction (HFpEF) and comorbidities like diabetes.In this study, patients with diabetes and CKD had significantly higher Galectin-3 levels, underlining the need to interpret this marker cautiously in the presence of comorbidities that can also promote fibrosis and inflammation.

Conclusion:

Cardiac biomarkers, especially NT-proBNP and Galectin-3, are essential tools in the diagnosis and risk stratification of CHF. Regular monitoring can improve prognostication and guide therapeutic decisions. Incorporation of multimarker panels into standard CHF management protocols is recommended. Chronic heart failure (CHF) continues to be a major clinical and public health burden, with high rates of morbidity, mortality, and frequent hospitalizations. Early diagnosis, risk stratification, and effective monitoring are essential to improve outcomes in these patients. This study aimed to evaluate the diagnostic and prognostic significance of three key cardiac biomarkers—NT-proBNP, Troponin I, and Galectin-3—in patients with chronic heart failure.Our findings demonstrate that:NT-proBNP levels were significantly elevated in the majority of patients and strongly correlated with NYHA class and reduced left ventricular ejection fraction (LVEF), making it the most reliable biomarker for diagnosis and staging of CHF.Troponin I levels, while

traditionally associated with acute coronary syndromes, were also raised in CHF patients, indicating ongoing subclinical myocardial injury. It proved moderately useful in predicting severity and progression.Galectin-3 emerged as a promising marker of cardiac fibrosis and inflammation. Its levels showed a strong correlation with disease severity and were particularly elevated in patients with comorbidities like diabetes and chronic kidney disease.Importantly, the study supports the use of a multi-marker strategy—combining NTproBNP, Troponin I, and Galectin-3—to provide a more comprehensive and nuanced understanding of heart failure status. This approach offers enhanced diagnostic accuracy and facilitates personalized patient careThe integration of cardiac biomarkers into routine clinical practice can revolutionize the management of heart failure by enabling:Timely diagnosis,Improved risk assessment,Tailored therapeutic interventions,Enhanced monitoring of treatment responseHowever, careful interpretation is necessary, especially in patients with coexisting conditions like renal dysfunction and diabetes, which may influence biomarker levels.Future studies with larger sample sizes, diverse populations, and long-term follow-up are recommended to validate these findings and explore the evolving role of novel biomarkers in heart failure care.

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