Received: 19 March 2024 | Revised: 30 July 2024 | Accepted: 3 September 2024

DOI: 10.1002/hsr2.70083

ORIGINAL RESEARCH

Health Science Reports Open Access WILEY

Evaluation of Pro-BNP biomarker in heart failure patients and its relationship with complete blood count parameters: A case–control study

Ekhlas Torfi[1] | Seyed S. Bahreiny[2] | Najmaldin Saki[3] | Reyhane Khademi[3] | Ehsan Sarbazjoda[2,3] | Inas A. Nezhad[2] | Mojtaba Aghaei[2,3]

[1]Department of Cardiovascular Disease, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[2]Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[3]Thalassemia & Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Correspondence

Mojtaba Aghaei, Thalassemia & Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
Email: mojtabaaghaei745@gmail.com

Abstract

Background and aims: Heart failure (HF) is a growing global health concern. N-terminal prohormone of brain natriuretic peptide (NT-pro-BNP) is an established biomarker for ventricular dysfunction in heart failure (HF). This case–control study examined the relationship between brain natriuretic peptide (Pro-BNP) levels and complete blood count (CBC) parameters in HF patients and healthy controls, exploring the utility of CBC as a supplementary diagnostic tool for HF.

Methods: The study included 89 participants, divided into 42 HF patients with diagnosed HF (patient group) and 47 healthy individuals (control group). Pro-BNP levels were measured alongside a comprehensive CBC panel, including parameters such as white blood cell count, hemoglobin levels, and platelet count. Demographic, clinical characteristics, and CBC parameters were compared between the two groups, with statistical analyses performed to identify any significant associations.

Results: The analysis demonstrated that HF patients had significantly higher Pro-BNP levels than the control subjects, indicating a strong association between Pro-BNP levels and HF (1052.65 [196.56] vs. 2500.34 [1105.90], $p < 0.001$). Moreover, significant differences in CBC parameters, such as platelet count: 246.96 (82.72) versus 206.45 (57.20), $p = 0.009$; mean corpuscular volume (MCV): 83.74 (5.86) versus 87.12 (4.60), $p < 0.00$; and red cell distribution width: 13.47 (1.29) versus 14.28 (1.35), $p < 0.001$) were observed, with the patient group showing altered levels indicative of cardiac stress and inflammation. Correlation analysis further established the relationship between Pro-BNP levels and CBC parameters, with notable correlations observed with MCV (0.250, $p < 0.020$) and mean corpuscular hemoglobin levels (0.246, $p < 0.045$). These findings suggest a complex interplay between Pro-BNP levels and CBC parameters, underscoring the potential of CBC parameters as auxiliary diagnostic markers in HF.

Conclusion: Pro-BNP exhibits clinical relevance in diagnosing cardiovascular dysfunction, with elevated levels and distinct hematological profiles in HF patients. Pro-BNP's

diagnostic and predictive capabilities for hematocrit and platelet count support its use in risk assessment and treatment decisions for HF.

KEYWORDS

biomarker, complete blood count parameters, ematological profiles, heart failure, heart failure patients, Pro-BNP

1 | INTRODUCTION

Cardiovascular diseases are still one of the most common causes of death worldwide. Accurate diagnosis and risk stratification of heart failure (HF) patients is very important for effective treatment and timely intervention.[1,2] In recent years, there has been increasing interest in the identification of biomarkers that can help in the early detection, prognosis, and monitoring of cardiovascular disease. One such promising biomarker is brain natriuretic peptide (Pro-BNP).[3–5]

Pro-BNP is a precursor peptide synthesized and released by the ventricular myocardium in response to cardiac stress and strain.[5] High Pro-BNP levels are associated with various cardiac diseases such as HF, myocardial infarction, and atrial fibrillation. The measurement of Pro-BNP has become an important tool in clinical practice for the diagnosis and prognosis of HF patients.[4,6,7]

Although Pro-BNP has been shown to be diagnostically and prognostically useful, its relationship to other routine laboratory parameters, such as complete blood count (CBC) parameters, is still relatively unknown.[5,8,9] A CBC is a commonly performed blood test that provides information about the cellular components of the blood, including red blood cells, white blood cells (WBCs), and platelets. Changes in CBC parameters have been observed in various cardiovascular diseases and reflect the underlying pathophysiological processes.[10–13]

The aim of this project is to investigate the relationship between Pro-BNP levels and CBC parameters in HF patients in a case–control study. In particular, we want to find out how changes in CBC parameters such as hemoglobin level, WBC count, and platelet count are related to Pro-BNP levels in different cardiac diseases. By investigating these associations, we could potentially identify other markers or patterns that could improve the diagnostic accuracy and prognostic value of Pro-BNP in HF patients.

This research will involve the use of a well-defined group of HF patients, including both cases and controls. Cases will include patients with confirmed cardiovascular disease, such as HF, myocardial infarction, or atrial fibrillation, while controls will be individuals without known heart disease. Both cases and controls will undergo Pro-BNP measurement and CBC testing.

The findings of this case–control study have the potential to provide valuable insights into the relationship between Pro-BNP and CBC parameters in HF patients. Understanding these connections can increase our understanding of the underlying pathophysiological mechanisms of cardiovascular diseases and may help develop more accurate diagnostic and prognostic algorithms. Finally, this research may have implications for personalized treatment approaches and improving patient outcomes in the cardiovascular field.

2 | MATERIALS AND METHODS

2.1 | Study subjects

In this case–control study, 42 patients diagnosed with HF were included, according to the 2022 European Society of Cardiology guidelines for diagnosing and treating acute and chronic HF. The HF patients had an ejection fraction of <40% and exhibited symptoms such as dyspnea, fatigue, and edema. The control group consisted of 47 healthy individuals with no history of chronic disease or clinical signs of heart disease. All participants underwent Pro-BNP measurement and CBC testing. A priori power analysis was conducted using G*Power software. Based on an expected effect size of 0.5, α of 0.05, and desired power of 0.80, the minimum required sample size was determined to be 88 participants (44 per group). Given the available resources and time frame, we recruited 42 HF patients and 47 healthy controls, which, while lower than the recommended sample size, still provides valuable preliminary data on the relationships between Pro-BNP and CBC parameters in this population. All members of the control group had no history of chronic disease or clinical signs of heart disease. Both cases and controls will be subjected to Pro-BNP measurement and CBC test. To evaluate the value of CBC indexes, we obtained 3 mL of whole blood sample in a K2-EDTA anticoagulated tube from all participants. Then, a point-of-care kit assessed the NT-Pro-BNP level based on a quantitative immunochromatographic assay (Response Biomedical), which used a rapid analyte measurement platform instrument (a fixed wavelength fluorescence spectrophotometer that analyzes a disposable cartridge). CBC information was obtained and recorded using an automated cell counter (Sysmex XS-500i). The study comprised 42 patients diagnosed with HF, including 20 women (47.6%) and 22 men (52.3%), with an average age at diagnosis of 42.87 ± 18.41 years. In comparison, the control group consisted of 47 healthy individuals, 21 males (44.7%) and 26 females (55.3%), with an average age of 47.83 ± 13.77 years, and underwent routine check-up tests at the same hospital. The control group included people close to the patient group in terms of age and gender (but without HF). All members of the control group had no history of chronic disease or clinical signs of heart disease. Body mass index (BMI) in the control and patient groups (25.1 [2.01] vs. 24.7 [4.08]) were measured in almost equal

proportions. This study was approved by the medical research ethics committee of Ahvaz Jundishapur University of Medical Sciences (February 2024, IR.AJUMS.REC.1402.628) and also the Helsinki Declaration of 1964. Also, informed consent was obtained from all participants who joined this study.

2.2 | Data collection

The blood samples were collected from all study participants and subjected to the following laboratory analyses.

Pro-BNP levels were quantified using an enzyme-linked immunosorbent assay kit (a point-of-care kit and Response Biomedical). The assay had a detection range of 10 to 35,000 pg/mL and an intra-assay and inter-assay coefficient of <10% variation.

CBC parameters, including hemoglobin, platelet count, hematocrit (Hct), WBC count, mean corpuscular volume (MCV), red cell distribution width (RDW), neutrophil-to-lymphocyte ratio (NLR), red blood cell (RBC) count, mean corpuscular hemoglobin (MCH), and MCH concentration (MCHC), were measured using an automated hematology analyzer (Sysmex XS-500i). The analyzer was calibrated and maintained according to the manufacturer's recommendations, and quality control measures were implemented to ensure the reliability of the results.

All laboratory analyses were performed by experienced technicians blinded to the study participants' clinical status.

2.3 | Inclusion and exclusion criteria

The inclusion criteria for this study are as follows: patients with a confirmed diagnosis of HF, patients who underwent CBC, patients who underwent measurement of Pro-BNP biomarker levels, and completeness of laboratory/clinical information and characteristics. The healthy control group should be of the same age and gender as the group of heart patients, have no history of cardiovascular disease, have a normal electrocardiogram, and all laboratory tests (CBC, kidney and liver function, lipid profile) should be within normal reference ranges. Subjects with other serious chronic illnesses, acute infections, or medications that could affect the biomarkers or laboratory parameters should be excluded. The healthy control subjects should also give informed consent to participate in the study to ensure a representative sample of the general population without confounding factors that could influence the assessment of the biomarker Pro-BNP and its relationship to the parameters of the CBC. Exclusion criteria include the following: patients with a history of malignancy or other serious systemic diseases, patients with a recent history of blood transfusion or known blood disorders, patients with acute infections or inflammatory conditions, patients with renal failure or undergoing dialysis, patients who are pregnant or breastfeeding, and patients who take immunosuppressive drugs.

2.4 | Data analysis

This case–control study employed advanced statistical methodologies to elucidate the relationship between Pro-BNP levels and CBC parameters in HF patients, compared to a control group. The analysis was meticulously executed using GraphPad Prism version 9.3.1 (GraphPad Software) and R (version 3.6.1), ensuring robustness and comprehensive exploration of the study objectives. Descriptive statistics provided a detailed overview of participant demographics, Pro-BNP levels, and CBC parameters. Continuous variables were presented as means ± SD, while categorical variables were expressed as frequencies and percentages. The Shapiro–Wilk test, a robust method for assessing normality in small to moderate sample sizes,[14,15] confirmed the normal distribution of continuous variables ($p > 0.05$ for all variables). For comparative analyses, independent *t* tests were employed to compare means between HF patients and the control group, given the normal distribution of the data. The selection of independent t-tests is supported by their optimal power under normality assumptions, as outlined by Ruxton.[16,17] Pearson correlation coefficients were calculated to investigate the linear relationships between Pro-BNP levels and CBC parameters. The appropriateness of Pearson correlation was ensured through the normality of data, reaffirmed by the Shapiro-Wilk test. This statistical measure is widely recognized for its sensitivity in detecting linear associations.[18,19] Simple linear regression analyses were conducted to quantify the predictive relationships between Pro-BNP levels and CBC parameters. This method allows for the evaluation of the strength and direction of associations, providing insights into potential causal relationships.

Receiver operating characteristic (ROC) analysis was performed to assess the diagnostic accuracy of Pro-BNP levels. The area under the ROC curve (AUC) was calculated to quantify the test's discriminative ability, with an AUC of 0.7–0.8 considered acceptable, 0.8–0.9 excellent, and >0.9 outstanding.[20,21] A heatmap visualization was created to depict the interrelationships among variables, facilitating the identification of patterns and correlations within the data. This visual representation aids in the intuitive understanding of complex data interactions.[22,23] Statistical significance was set at $p < 0.05$ for all analyses, adhering to the conventional threshold for hypothesis test.

3 | RESULTS

The objective of our case–control study, titled "Pro-BNP biomarker in HF patients and its relationship with CBC parameters," was to explore the association between Pro-BNP levels, a recognized biomarker for HF, and various CBC parameters among HF patients compared to a healthy control group. This section provides a comprehensive summary of our findings, highlighting the distinct hematological profiles observed in HF patients and elucidating the correlation between Pro-BNP levels and CBC parameters.

3.1 | Demographics and participant characteristics

The study encompassed a total of 89 participants, with 42 individuals constituting the cardiac patient group and 47 forming the control group. Both groups exhibited comparable distributions in terms of age and gender, ensuring adequate comparability for subsequent analyses. The mean age of participants was 47.83 years in the cardiac patient group and 42.87 years in the control group, with a balanced representation of genders in each cohort. All demographics and participant characteristics display in Table 1.

3.2 | Descriptive analysis

Descriptive statistics provided a comprehensive overview of participant demographics and baseline characteristics, along with the distribution of Pro-BNP levels and CBC parameters. In the control group, the mean Pro-BNP level was 1052.65 pg/mL, with a SD of 196.56 pg/mL. Conversely, in the cardiac patient group, the mean Pro-BNP level was significantly elevated at 1582.59 pg/mL, accompanied by a notably higher SD of 1723.77 pg/mL, indicating substantial variability within this group. The Violin Plots provide additional insight into the distribution shapes, indicating that Pro-BNP levels have a broader distribution in the case group, suggesting variability in the severity or stage of HF.

The descriptive analysis further delineated the CBC parameters, including hemoglobin, platelet count, Hct, WBC count, MCV, RDW, NLR, RBC count, MCH, and MCHC. These parameters were characterized in both the control and patient groups to provide a comprehensive understanding of their distributions and variations across the cohorts. In addition, Supporting Information S1: Figure 1 displays the distribution histograms of significant parameters, including Pro-BNP, platelet count, MCV, RDW, and MCHC for two groups—the control group and patients with HF. These histograms provide crucial insights into the distribution of data and the variability of each parameter between the two groups (Supporting Information S1: Figure 2).

3.3 | Elevated Pro-BNP levels in HF patients

Significant disparities between the cardiac patient group and the control group were unveiled through comparative analysis. Independent *t* tests and Mann–Whitney *U* tests were employed to discern statistically significant differences in Pro-BNP levels and CBC parameters between the two groups. A pivotal finding of our study was the significant elevation of Pro-BNP levels in HF patients (mean ± SD: (1052.65 [196.56] vs. 2500.34 [1105.90] pg/mL, $p < 0.001$) compared to the control group, underscoring the clinical utility of Pro-BNP as a biomarker for cardiac dysfunction.

Furthermore, several CBC parameters exhibited noteworthy distinctions between the groups. HF patients demonstrated lower hemoglobin levels ($p = 0.22$) and a decreased platelet count ($p = 0.009$) compared to controls. Additionally, significant variations were observed in MCV ($p < 0.001$), RDW ($p < 0.001$), and MCHC ($p = 0.04$), elucidating altered hematological profiles associated with cardiac pathology.

TABLE 1 Demographic, clinical, and outcome characteristics of the 89 studied participants.

Characteristics	Control group ($n = 42$)	HF group ($n = 47$)	*p*
Age (year)	42.87 (18.41)	47.83 (13.77)	0.15
Sex (F/M)	20 (47.6%)/22 (52.3%)	25 (55.3%)/22 (44.7%)	0.20
BMI (kg/m^2)	25.1 (2.01)	24.7 (4.08)	0.57
NT-proBNP, (pg/mL)	1052.65 (196.56)	2500.34 (1105.90)	<0.001
Hb (g/L)	13.23 (1.56)	12.82 (1.62)	0.22
Platelet count ($\times 10^9$/L)	246.96 (82.72)	206.45 (57.20)	0.009
Hct (%)	39.28 (3.83)	39.96 (4.59)	0.45
WBC count (WBC, $\times 10^9$/L)	8.06 (1.89)	8.36 (2.30)	>0.99
MCV(fL)	83.74 (5.86)	87.12 (4.60)	<0.001
RDW (%)	13.47 (1.29)	14.28 (1.35)	<0.001
NLR	1.74 (1.57)	1.95 (0.79)	0.44
RBC count (RBC, $\times 10^{12}$/L)	4.70 (0.47)	4.60 (0.57)	0.34
MCH (pg)	29.34 (7.98)	29.28 (9.02)	0.97
MCHC (g/dL)	34.95 (8.68)	32.07 (1.55)	0.04

Abbreviations: BMI, body mass index; F, female; Hb, hemoglobin; Hct, hematocrit; HF, heart failure; M, male; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NLR, neutrophil-to-lymphocyte ratio; NT-proBNP, pro B type natriuretic peptide; RBC, red blood cell; RDW, red cell distribution width; WBC, white blood cell.

TABLE 2 Correlations between maternal Pro-BNP levels and other clinical characteristics among the HF patients.

Parameter	Group	Correlation coefficient (*r*)	*p*
Hemoglobin	Control	0.033	0.83
	Patients (HF)	−0.162	0.31
Platelet count	Control	0.092	0.54
	Patients (HF)	0.190	0.03
Hct	Control	0.024	0.88
	Patients (HF)	−0.206	0.01
WBC	Control	0.060	0.69
	Patients (HF)	0.001	0.99
MCV	Control	0.250	0.02
	Patients (HF)	0.023	0.88
RDW	Control	0.024	0.87
	Patients (HF)	0.138	0.38
NLR	Control	0.069	0.65
	Patients (HF)	0.061	0.70
RBC	Control	−0.171	0.25
	Patients (HF)	−0.223	0.15
MCH	Control	0.246	0.04
	Patients (HF)	−0.065	0.68
MCHC	Control	−0.003	0.99
	Patients (HF)	−0.079	0.61

Abbreviations: Hct, hematocrit; HF, heart failure; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NLR, neutrophil-to-lymphocyte ratio; Pro-BNP, pro B type natriuretic peptide; RBC, red blood cell; RDW, red cell distribution width; WBC, white blood cell.

3.4 | Correlation analysis

Correlation analysis unveiled the associations between Pro-BNP levels and CBC parameters within the cardiac patient group. Although generally weak, certain correlations exhibited potential clinical relevance. A moderate positive correlation was observed between platelet count and Pro-BNP levels ($r = 0.299$, $p = 0.02$), suggesting a plausible link between elevated Pro-BNP levels and increased platelet counts in cardiac disease. Conversely, a moderate negative correlation was noted between HCT levels and Pro-BNP levels ($r = 0.206$, $p = 0.01$), albeit not reaching statistical significance, implying a potential association between higher Pro-BNP levels and lower hemoglobin levels. In addition, a moderate positive correlation was observed between MCV, MCH, and Pro-BNP levels (Table 2).

3.5 | Regression analysis outcomes

Simple linear regression analyses further elucidated the predictive value of Pro-BNP levels for CBC parameters within the cardiac patient group. Although some relationships were statistically insignificant, notable findings emerged. Hct exhibited a statistically significant predictive relationship with Pro-BNP levels, indicating that variations in Pro-BNP levels could elucidate a proportion of the variability in Hct among HF patients. Similarly, the positive slope in the regression model for platelet count and Pro-BNP levels corroborated the association between elevated Pro-BNP levels and increased platelet counts ($p < 0.05$), signifying a potential link with inflammatory or thrombotic states in cardiac pathology (Table 3).

TABLE 3 The table below presents the detailed analysis of simple linear regression for the group of patients through HF and the relationship between Pro-BNP levels and various CBC parameters.

Independent variable	Slope	Intercept	*R*	*p*	SE
Hemoglobin	−0.000235	13.188	−0.250	0.11	0.000143
Platelet count	0.009937	190.727	0.299	0.03	0.005006
Hct	−0.000693	41.056	−0.260	0.05	0.000407
WBC	0.000060	7.965	0.045	0.78	0.000211
MCV	0.000139	86.903	0.052	0.74	0.000422
RDW	0.000117	14.096	0.150	0.34	0.000122
NLR	0.000083	1.817	0.182	0.25	0.000071
RBC	−0.000090	4.739	−0.270	0.08	0.000051
MCH	0.001041	27.629	0.199	0.20	0.000811
MCHC	−0.000016	32.091	−0.017	0.91	0.000142

Abbreviations: CBC, complete blood count; Hct, hematocrit; HF, heart failure; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NLR, neutrophil-to-lymphocyte ratio; Pro-BNP, pro B type natriuretic peptide; RBC, red blood cell; RDW, red cell distribution width; WBC, white blood cell.

3.6 | ROC analysis and heatmap

ROC analysis and heatmap visualization were employed to further delineate the diagnostic accuracy of Pro-BNP levels and the interrelationships between variables, respectively. These analyses provided additional insights into the discriminatory capacity of Pro-BNP as a biomarker for cardiac dysfunction, Pro-BNP (pg/mL) were significantly good predictors of HF with high the AUC, which was 87.2% for Pro-BNP (pg/mL) (Figure 1).

In this case–control study, multifaceted statistical analyses reveal the complex relationships between Pro-BNP levels, a key biomarker for HF, and various CBC parameters in HF patients compared to a healthy control group. The study elucidates how these factors interact by utilizing a heatmap correlation matrix, enhancing our understanding of cardiac disease pathophysiology (Figure 2). Moreover, these findings highlight the clinical significance of Pro-BNP in diagnostic and prognostic evaluations, underscoring its importance in managing HF.

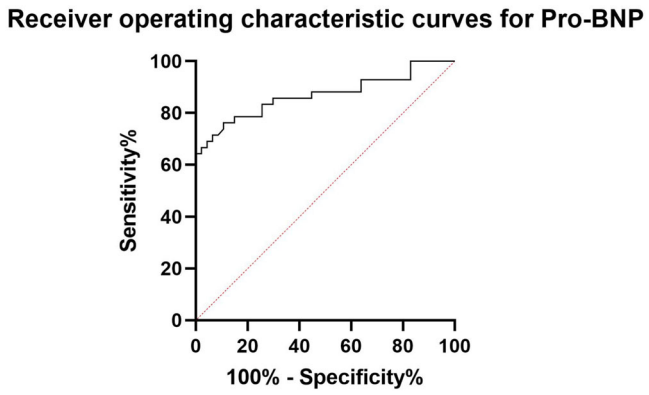

FIGURE 1 Receiver operating characteristic (ROC) curves showing the predictive value of serum pro-brain natriuretic peptide (Pro-BNP) in HF. Area under the curve = 0.872 (0.789–0.943), $p < 0.0001$.

4 | DISCUSSION

The pivotal role of BNP and its N-terminal prohormone (NT-proBNP) as biomarkers for cardiac dysfunction is well-established, offering significant prognostic and diagnostic utility in HF management.[24–26] The objective of our case–control study was to further elucidate the relationship between Pro-BNP levels and CBC parameters in HF patients versus a healthy control group. This exploration aimed to uncover potential interactions that could enhance the understanding of Pro-BNP's role in cardiac pathology and identify new avenues for clinical assessment and management of HF.

The study aimed to elucidate the relationship between Pro-BNP levels, a recognized biomarker for cardiac dysfunction, and various CBC parameters in a case–control setting involving HF patients and healthy controls. Our findings highlight significant differences in Pro-BNP levels, platelet count, MCV, RDW, and MCHC between the two

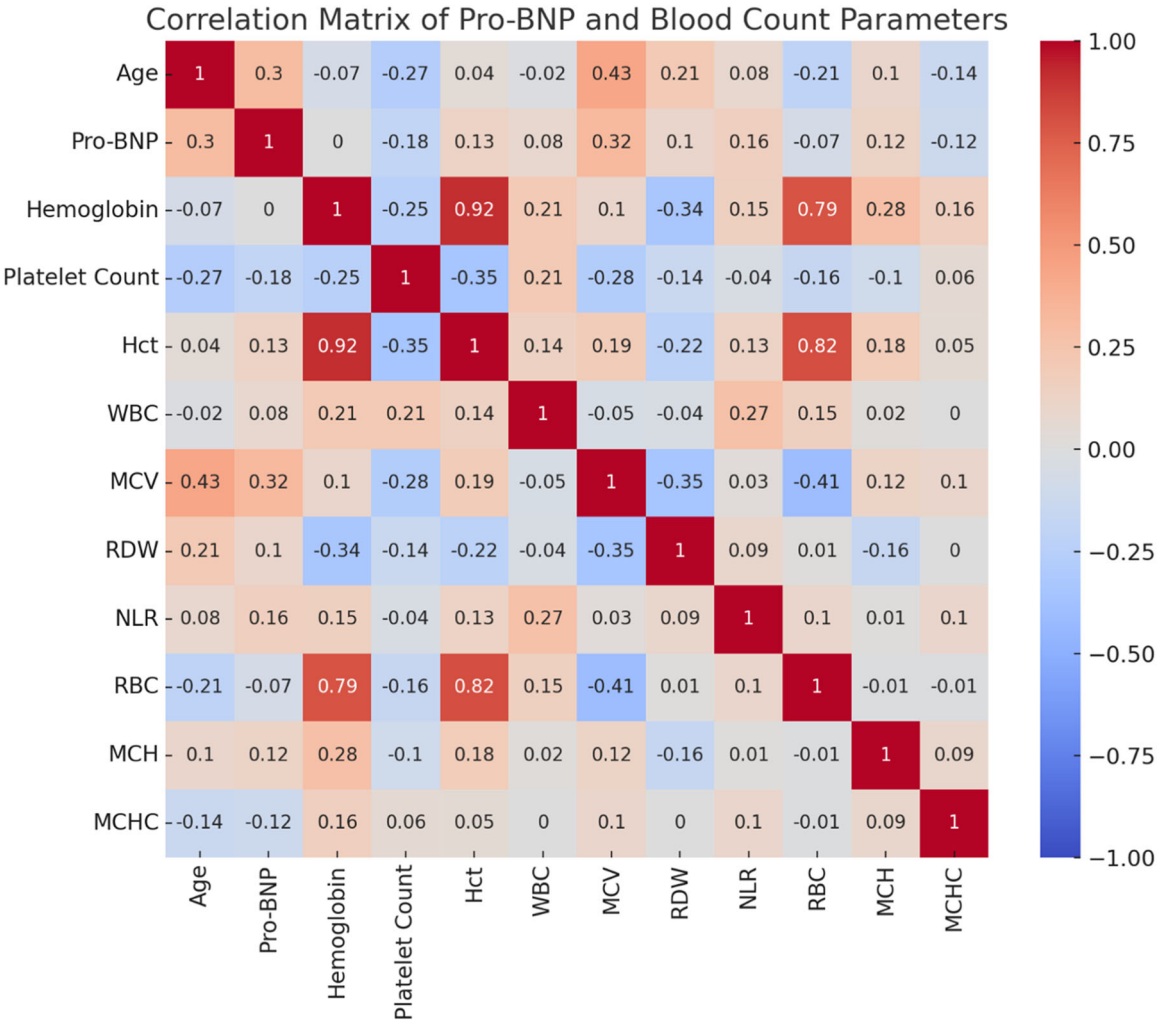

FIGURE 2 The heatmap above displays the correlation matrix of pro-brain natriuretic peptide (Pro-BNP) levels and blood count parameters, including hemoglobin, platelet count, WBC, mean corpuscular volume (MCV), red cell distribution width (RDW), neutrophil-to-lymphocyte ratio (NLR), red blood cell (RBC), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Hct, hematocrit; WBC, white blood cell.

groups, providing insights into the pathophysiological mechanisms underlying cardiac conditions and their impact on hematological profiles.

4.1 | Pro-BNP and CBC parameters: Key findings

Our analysis revealed that Pro-BNP levels were significantly higher in HF patients compared to controls, aligning with existing literature that underscores Pro-BNP as a critical marker for HF and cardiac stress.[27–29] The elevation of Pro-BNP in HF patients reflects the heart's compensatory response to increased ventricular pressure and volume, serving as a diagnostic and prognostic marker.[30]

The study also identified significant differences in platelet count, MCV, RDW, and MCHC between patients and controls. Specifically, platelet count was higher in controls, suggesting a potential thrombopoietic response or altered platelet lifespan in cardiac conditions. This finding could indicate an adaptive or pathologic response to cardiovascular stress, warranting further investigation into platelet dynamics in HF patients.[31–33]

Furthermore, the elevation of MCV and RDW in HF patients points towards anisocytosis and a possible association with adverse cardiac events. RDW, in particular, has been recognized as a prognostic marker in HF, reflecting underlying inflammatory processes, nutritional deficiencies, or bone marrow dysregulation.[34–37] The increased MCV and RDW may signify erythropoietic stress or marrow response to chronic disease states, highlighting the intricate relationship between cardiac function and erythropoiesis.[34,37,38]

Conversely, MCHC values were higher in controls, suggesting alterations in hemoglobin concentration within red cells in HF patients. This variation might reflect changes in red cell hydration or the presence of underlying conditions affecting hemoglobin synthesis or degradation, which could have implications for oxygen transport efficiency and tissue oxygenation in cardiac disease.[34,37–39]

The moderate positive correlation between Pro-BNP levels and platelet count ($r = 0.190$, $p = 0.03$) may indicate an inflammatory or thrombotic response in cardiac disease, supporting findings by Budak et al.[40] that link elevated platelet counts to increased cardiac stress. Conversely, the negative correlation between Pro-BNP levels and HCT or hemoglobin ($r = -0.250$, $p = 0.11$) could reflect the dilutional effect or erythropoiesis suppression commonly seen in HF patients, as discussed by Tominaga et al.[41]

The diagnostic accuracy of Pro-BNP, as demonstrated by our ROC analysis, with high area AUC values (87.2%, $p = 0.001$), reinforces its clinical relevance, consistent with findings by Rørth et al. and demonstrates its superiority over traditional clinical assessments in predicting HF.[30]

4.2 | Clinical and pathophysiological implications

These findings underscore the multifaceted impact of cardiac conditions on hematological parameters, suggesting that CBC alterations may not only result from the disease process but also contribute to its pathophysiology and progression. The interplay between Pro-BNP levels and CBC parameters could offer valuable insights into disease mechanisms, potentially guiding therapeutic interventions aimed at mitigating hematologic abnormalities and improving cardiac function.[4,24,42]

Moreover, the identification of specific CBC parameter alterations in HF patients may enhance the prognostic utility of Pro-BNP, allowing for a more comprehensive risk stratification and monitoring approach. For instance, the combination of elevated Pro-BNP with specific CBC anomalies could identify patients at higher risk for adverse outcomes, informing clinical decision-making and personalized treatment strategies.[24,42]

4.3 | Future directions and limitations

Although the study showed a relatively weak correlation between Pro-BNP levels and the CBC parameters that demonstrated correlation, it has limitations. The primary limitation of our study is the small sample size, which reduces the statistical power and may not be representative of the broader population. A limited number of participants increases the susceptibility to type II errors and reduces the generalizability of our findings. The high SD observed in the case group reflects substantial variability within this group, underscoring the need for larger sample sizes in future studies to achieve more precise estimates and enhance the reliability of the results.[43,44]

The variability in clinical phenotypes of HF within our sample is another limitation. HF is a heterogeneous condition with diverse etiologies and presentations, which can influence biomarker levels and clinical outcomes. This heterogeneity is reflected in the high SD of Pro-BNP levels in the case group. Future research should consider stratifying patients based on HF subtypes or employing larger, more diverse cohorts to better capture this variability and enhance the specificity of the findings.[45,46]

The variability in clinical phenotypes of HF within our sample is another limitation. HF is a heterogeneous condition with diverse etiologies and presentations, which can influence biomarker levels and clinical outcomes. This heterogeneity is reflected in the high SD of Pro-BNP levels in the case group. Future research should consider stratifying patients based on HF subtypes or employing larger, more diverse cohorts to better capture this variability and enhance the specificity of the findings.[46]

Being an observational study, our research is inherently limited in controlling for all potential confounding variables. While we adjusted for major known confounders such as age, sex, and BMI, there may be other unmeasured factors influencing the results. Randomized controlled trials or well-designed cohort studies with comprehensive data collection are needed to mitigate these limitations and provide more definitive evidence.[47–49]

The cross-sectional design precludes causal inferences, and the sample size may limit the generalizability of the findings. Future longitudinal studies with larger cohorts are necessary to elucidate the

temporal relationships between these biomarkers and their prognostic significance in diverse cardiac conditions.[24,50]

Additionally, exploring the underlying mechanisms driving the observed CBC alterations in HF patients, including the role of inflammation, nutritional status, and bone marrow function, could further illuminate the complex interplay between cardiac health and hematologic profiles. Integrating genomic, proteomic, and metabolomic approaches may unravel novel pathways and targets for therapeutic intervention, enhancing patient outcomes in cardiac disease. Another limitation is the lack of disease phenotype or severity stratification, which can cause high variability in Pro-BNP levels. Future studies should consider examining Pro-BNP in more homogeneous HF populations to better understand its relationship with other clinical parameters.[51–53]

5 | CONCLUSION

In conclusion, our study highlights significant associations between Pro-BNP levels and key CBC parameters in HF patients, offering insights into the hematologic manifestations of cardiac dysfunction. These findings emphasize the importance of a multidisciplinary approach to cardiac patient care, incorporating comprehensive hematologic evaluation to enhance diagnostic accuracy, risk assessment, and therapeutic management. Further research is imperative to fully understand the clinical implications of these associations and to harness their potential in improving cardiac patient care.

AUTHOR CONTRIBUTIONS

Ekhlas Torfi: Investigation; methodology; writing—review and editing; writing—original draft. **Seyed S. Bahreiny**: Software; formal analysis; conceptualization; investigation; funding acquisition; writing—original draft. **Najmaldin Saki**: Conceptualization; investigation; validation; writing—original draft; writing—review and editing. **Reyhane Khademi**: Investigation; writing—original draft. **Ehsan Sarbazjoda**: Investigation; methodology; writing—original draft; writing—review and editing. **Inas A. Nezhad**: Investigation; methodology; data curation. **Mojtaba Aghaei**: Conceptualization; investigation; writing—original draft; writing—review and editing; software; formal analysis; methodology.

ACKNOWLEDGMENTS

We wish to thank all our colleagues at Ahvaz Jundishapur University of Medical Sciences.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

There are no data sets available for this specific manuscript.

ETHICS STATEMENT

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

TRANSPARENCY STATEMENT

The lead author Mojtaba Aghaei affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

ORCID

Seyed S. Bahreiny http://orcid.org/0000-0003-2148-1205
Najmaldin Saki http://orcid.org/0000-0001-8494-5594
Mojtaba Aghaei http://orcid.org/0000-0002-6382-6657

REFERENCES

1. Sethi Y, Patel N, Kaka N, et al. precision medicine and the future of cardiovascular diseases: a clinically oriented comprehensive review. *J Clin Med*. 2023;12:1799.
2. Esau D, Abramson BL. Approach to risk stratification of atherosclerotic cardiovascular disease: use of biomarkers and imaging in a Canadian context. *Can Fam Phys* 2022;68:654-660.
3. Ghantous CM, Kamareddine L, Farhat R, et al. Advances in cardiovascular biomarker discovery. *Biomedicines*. 2020;8:552.
4. Cao Z, Jia Y, Zhu B. BNP and NT-proBNP as diagnostic biomarkers for cardiac dysfunction in both clinical and forensic medicine. *Int J Mol Sci*. 2019;20:1820.
5. Panagopoulou V, Deftereos S, Kossyvakis C, et al. NTproBNP: an important biomarker in cardiac diseases. *Curr Top Med Chem*. 2013;13:82-94.
6. Mair J. Comparison of B-type natriuretic peptide and N-terminal proBNP in daily clinical practice. *Eur Cardiol Rev*. 2008;4(2):35-38.
7. Ceriello A, Lalic N, Montanya E, et al. NT-proBNP point-of-care measurement as a screening tool for heart failure and CVD risk in type 2 diabetes with hypertension. *J Diabetes Complications*. 2023;37:108410.
8. Sarzani R, Spannella F, Giulietti F, et al. NT-proBNP and its correlation with in-hospital mortality in the very elderly without an admission diagnosis of heart failure. *PLoS One*. 2016;11:e0153759.
9. Curiati MNC, Silvestre OM, Pires LJT, et al. Comparação entre BNP e NT-proBNP quanto à concordância e quanto à influência das variáveis clínicas e laboratoriais. *Einstein (São Paulo)*. 2013;11:273-277.
10. Melanson SE, Lewandrowski EL. Laboratory testing for B-type natriuretic peptides (BNP and NT-proBNP): clinical usefulness, utilization, and impact on hospital operations. *Am J Clin Pathol*. 2005;124(suppl):S122-S128.
11. Madjid M, Fatemi O. Components of the complete blood count as risk predictors for coronary heart disease: in-depth review and update. *Tex Heart Inst J*. 2013;40:17-29.
12. Lassale C, Curtis A, Abete I, et al. Elements of the complete blood count associated with cardiovascular disease incidence: findings from the EPIC-NL cohort study. *Sci Rep*. 2018;8:3290.
13. Haybar H, Pezeshki SMS, Saki N. Evaluation of complete blood count parameters in cardiovascular diseases: an early indicator of prognosis? *Exp Mol Pathol*. 2019;110:104267.
14. Souza RR, Toebe M, Mello AC, Bittencourt KC. Sample size and Shapiro-Wilk test: an analysis for soybean grain yield. *Eur J Agron*. 2023;142:126666.
15. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). *Biometrika*. 1965;52(3-4):591-611. doi:10.1093/biomet/52.3-4.591

16. Delacre M, Lakens D, Leys C. Why psychologists should by default use Welch's t-test instead of Student's t-test. *Int Rev Social Psychol*. 2017;30:92-101.
17. Ruxton GD. The unequal variance t-test is an underused alternative to student's t-test and the Mann–Whitney U test. *Behav Ecol*. 2006;17(4):688-690. doi:10.1093/beheco/ark016
18. Ruxton GD, Wilkinson DM, Neuhäuser M. Advice on testing the null hypothesis that a sample is drawn from a normal distribution. *Anim Behav*. 2015;107:249-252.
19. Mukaka MM. A guide to appropriate use of correlation coefficient in medical research. *Malawi Med J*. 2012;24(3):69-71.
20. Swets JA. *Signal Detection Theory and ROC Analysis in Psychology and Diagnostics: Collected Papers*. Psychology Press; 2014.
21. Swets JA. Measuring the accuracy of diagnostic systems. *Science*. 1988;240(4857):1285-1293.
22. Feltrin L, Bertelli M. Using clustered heat maps in mineral exploration to visualize volcanic-hosted massive sulfide alteration and mineralization. *Nat Resour Res*. 2020;29:311-344.
23. Wilkinson L, Friendly M. The history of the cluster heat map. *Am Stat*. 2009;63(2):179-184.
24. McKie PM, Burnett Jr. JC. B-type natriuretic peptide as a biomarker beyond heart failure: speculations and opportunities. *Mayo Clin Proc*. 2005;80:1029-1036.
25. Januzzi, Jr. J.L. Natriuretic peptides as biomarkers in heart failure. *J Investig Med*. 2013;61:950-955.
26. Gaggin HK, Januzzi Jr. JL. Biomarkers and diagnostics in heart failure. *Biochim Biophys Acta Mol Basis Dis*. 2013;1832:2442-2450.
27. Ragonese M, Di Bella G, Spagnolo F, et al. Serum NT-pro-BNP levels predict cardiovascular events in acromegaly patients. *Exp Clin Endocrinol Diabetes*. 2022;130:229-236.
28. Isah IA, Sadoh WE, Iduoriyekemwen NJ. Usefulness of amino terminal pro-B-type natriuretic peptide in evaluating children with cardiac failure. *Cardiovasc Diagn Ther*. 2017;7:380-388.
29. Noori N, Teimouri A, Shahramian I. Comparison between brain natriuretic peptide and calcitonin gene-related peptide in children with dilated cardiomyopathy and controls. *Niger Med J*. 2017;58:37.
30. Rørth R, Jhund PS, Yilmaz MB, et al. Comparison of BNP and NT-proBNP in patients with heart failure and reduced ejection fraction. *Circ Heart Fail*. 2020;13:e006541.
31. Getawa S, Bayleyegn B. Platelet, neutrophil and lymphocyte quantitative abnormalities in patients with heart failure: a retrospective study. *Vasc Health Risk Manag*. 2023;19:69-78.
32. Chung I, Lip GYH. Platelets and heart failure. *Eur Heart J*. 2006;27:2623-2631.
33. Mojadidi MK, Galeas JN, Goodman-Meza D, et al. Thrombocytopaenia as a prognostic indicator in heart failure with reduced ejection fraction. *Heart, Lung Circ*. 2016;25:568-575.
34. Ji X, Ke W. Red blood cell distribution width and all-cause mortality in congestive heart failure patients: a retrospective cohort study based on the Mimic-III database. *Front Cardiovasc Med*. 2023;10:1126718.
35. Wang H, Yang G, Zhao J, Wang M. Association between mean corpuscular volume and severity of coronary artery disease in the Northern Chinese population: a cross-sectional study. *J Int Med Res*. 2020;48:030006051989671.
36. Lippi G, Turcato G, Cervellin G, Sanchis-Gomar F. Red blood cell distribution width in heart failure: a narrative review. *World J Cardiol*. 2018;10:6-14.
37. Danese E, Lippi G, Montagnana M. Red blood cell distribution width and cardiovascular diseases. *J Thorac Dis*. 2015;7:402-411.
38. Arkew M, Gemechu K, Haile K, Asmerom H. Red blood cell distribution width as novel biomarker in cardiovascular diseases: a literature review. *J Blood Med*. 2022;13:413-424.
39. Choy M, Zhen Z, Dong B, et al. Mean corpuscular haemoglobin concentration and outcomes in heart failure with preserved ejection fraction. *ESC Heart Failure*. 2023;10:1214-1221.
40. Budak YU, Huysal K, Demirci H. Correlation between mean platelet volume and B-type natriuretic peptide concentration in emergency patients with heart failure. *Biochem Med*. 2015;25:97-102.
41. Tominaga M, Kawai M, Minai K, et al. Association between plasma B-type natriuretic peptide and anaemia in heart failure with or without ischaemic heart disease: a retrospective study. *BMJ Open*. 2019;9:e024194.
42. Vuolteenaho O, Ala-Kopsala M, Ruskoaho H. BNP as a biomarker in heart disease. *Adv Clin Chem*. 2005;40:1-36.
43. Nayak B. Understanding the relevance of sample size calculation. *Indian J Ophthalmol*. 2010;58:469-470.
44. Faber J, Fonseca LM. How sample size influences research outcomes. *Dental Press J Orthod*. 2014;19:27-29.
45. Bozkurt B, Ahmad T, Alexander KM, et al. Heart failure epidemiology and outcomes statistics: a report of the Heart Failure Society of America. *J Card Failure*. 2023;29:1412-1451.
46. Papadimitriou L, Moore CK, Butler J, Long RC. The limitations of symptom-based heart failure management. *Card Fail Rev*. 2019;5:74-77.
47. Ostrowska B, Lind L, Blomström-Lundqvist C. An association between heart rate variability and incident heart failure in an elderly cohort. *Clin Cardiol*. 2024;47:e24241.
48. Baig M, Moafi-Madani M, Qureshi R, et al. Heart rate variability and the risk of heart failure and its subtypes in post-menopausal women: the Women's Health Initiative study. *PLoS One*. 2022;17:e0276585.
49. Saki N, Haybar H, Aghaei M. Subject: motivation can be suppressed, but scientific ability cannot and should not be ignored. *J Transl Med*. 2023;21:520.
50. Weber M. Role of B-type natriuretic peptide (BNP) and NT-proBNP in clinical routine. *Heart*. 2005;92:843-849.
51. Yaluri N, Yaluri AS, Žeňuch P, Žeňuchová Z, Tóth Š, Kalanin P. Cardiac biomarkers and their role in identifying increased risk of cardiovascular complications in COVID-19 patients. *Diagnostics*. 2023;13:2508.
52. de Moura Monteiro Júnior JG, de Oliveira Cipriano Torres D, Filho DCS. Hematological parameters as prognostic biomarkers in patients with cardiovascular diseases. *Curr Cardiol Rev*. 2019;15:274-282.
53. van den Bosch E, Bossers SSM, Kamphuis VP, et al. Associations between blood biomarkers, cardiac function, and adverse outcome in a young Fontan cohort. *J Am Heart Assoc*. 2021;10:e015022.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Torfi E, Bahreiny SS, Saki N, et al. Evaluation of Pro-BNP biomarker in heart failure patients and its relationship with complete blood count parameters: A case–control study. *Health Sci Rep*. 2024;7:e70083. doi:10.1002/hsr2.70083