

Tetranectin as a potential biomarker in heart failure with ejection fraction >45%: A prospective cohort study

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Abstract

Background and Objectives: Heart failure (HF) with left ventricle ejection fraction (LVEF) >45% lacks reliable biomarkers for risk stratification complicating its management, as it encompasses both heart failure with preserved ejection fraction (HFpEF, LVEF ≥50%) and mildly reduced ejection fraction (HFmrEF, LVEF 45–49.9%). This study aimed to evaluate serum tetranectin (TETRA) as a novel biomarker for assessing disease severity and predicting mortality in patients with HF with EF >45%.

Materials and Methods: In a prospective cohort study including 116 patients HF with EF>45% from a single center in Arad, Romania, stratified by NYHA class (G1: NYHA I, n=48; G2: NYHA II, n=37; G3: NYHA III–IV, n=31), serum TETRA levels were measured using ELISA. Echocardiographic parameters (E/e' ratio, LAVI, LAS, GLS, LVEF) and NT-proBNP were assessed at baseline, with all-cause mortality (9 deaths) tracked over a 12-month follow-up.

Results: Median TETRA levels decreased with worsening NYHA class (G1: 48.9 ng/mL, G2: 33.2 ng/mL, G3: 27.6 ng/mL; $p < 0.001$) and correlated negatively with NT-proBNP ($\rho = -0.66$, $p < 0.001$), E/e' ratio ($\rho = -0.58$, $p = 0.003$), and LAVI ($\rho = -0.52$, $p = 0.010$), while positively correlating with LAS ($\rho = 0.55$, $p = 0.005$). In univariable Cox analysis, lower TETRA levels were associated with higher all-cause mortality (HR = 1.38 per 10 ng/mL decrease, 95% CI: 1.06–1.81, $p = 0.045$), but this association was not significant after adjustment for age and NT-proBNP (HR = 1.22, 95% CI: 0.94–1.86, $p = 0.112$).

Conclusions: TETRA levels are inversely associated with severity in heart failure with EF>45% and may reflect disease progression.

Keywords: Tetranectin; heart failure; heart failure with preserved ejection fraction; HFpEF; heart failure with mildly reduced ejection fraction; HFmrEF; left ventricular ejection fraction; LVEF; NT-proBNP; heart failure biomarkers.

INTRODUCTION

Heart failure (HF) remains a major global health challenge, characterized by the heart's inability to pump blood effectively to meet the body's metabolic demands [1, 2]. This clinical syndrome, often resulting from structural or functional cardiac impairments, manifests through symptoms such as dyspnea, fatigue, and fluid retention, significantly impacting quality of life and survival [3]. Globally,

HF affects over 64 million individuals, with a prevalence in Europe of 1–2% among adults, rising sharply in older populations [4, 5]. In the United States, HF prevalence is estimated at 6.2 million adults, projected to increase to 8 million by 2030, driven by aging populations and rising cardiovascular risk factors [6]. HF is broadly categorized into three subtypes based on left ventricular ejection fraction (LVEF): heart failure with reduced ejection fraction (HFrEF, LVEF <40%), heart failure with mildly

reduced ejection fraction (HFmrEF), and heart failure with preserved ejection fraction (HFpEF, LVEF $\geq 50\%$) [7, 8]. HFpEF accounts for approximately 50% of HF cases, presents unique challenges due to its heterogeneous presentation and limited prognostic tools [9, 10].

HFpEF is characterized by impaired ventricular relaxation and increased stiffness, leading to elevated filling pressures despite a normal LVEF [11]. This diastolic dysfunction, often driven by comorbidities such as hypertension, diabetes, and obesity, contributes to symptoms of HF and poor outcomes, including frequent hospitalizations and high mortality rates [12, 13]. The pathophysiology of HFpEF involves complex mechanisms, including extracellular matrix remodeling, oxidative stress, and inflammation, which are exacerbated by systemic factors like aging and metabolic syndrome [14, 15]. For instance, studies have shown that HFpEF patients exhibit increased myocardial fibrosis and colla-gen deposition, impairing diastolic compliance [16]. Additionally, chronic inflammation and oxidative stress, mediated by pathways such as the NLRP3 inflammasome, further contribute to endothelial dysfunction and cardiac remodeling in HFpEF [17, 18]. Unlike HFrEF, where biomarkers like B-type natriuretic peptide (BNP) and N-terminal pro-BNP (NT-proBNP) effectively guide diagnosis and prognosis, HFpEF lacks reliable biomarkers for risk stratification, as natriuretic peptides are often less elevated in this subtype [19, 20].

The European Society of Cardiology (ESC) has recognized heart failure with mildly reduced ejection fraction (HFmrEF, LVEF 40–49%) as a distinct category bridging heart failure with preserved ejection fraction (HFpEF, LVEF $\geq 50\%$) and heart failure with reduced ejection fraction (HFrEF, LVEF $<40\%$) [1]. This classification acknowledges the clinical and pathophysiological differences in heart failure patients based on their ejection fraction. Clinically, patients with HFmrEF exhibit an intermediate phenotype: they share the high prevalence of ischaemic heart disease and elevated natriuretic peptides seen in HFrEF, yet manifest a clinical course (especially prognosis and quality of life) more akin to those with HFpEF. Pathophysiologically, HFmrEF patients present a mixed profile that includes milder ventricular dilation, less concentric hypertrophy, and moderate diastolic dysfunction. Unlike true diastolic failure in HFpEF, myocardial contractile reserve and remodelling patterns in HFmrEF suggest concurrent systolic impairment, positioning it as a ‘transition’ phenotype both clinically and mechanistically [1, 2, 21].

Tetranectin (TETRA), a plasminogen-binding protein first identified in 1986, has emerged as a potential biomarker in cardiovascular diseases due to its roles in fibrinolysis, extracellular matrix remodeling, and angiogenesis [22]. Recent studies have demonstrated that TETRA levels decrease with increasing HF severity, correlating with disease progression and cardiac dysfunction [23, 24]. Similarly, TETRA has been linked to myocardial fibrosis in HF patients, indicating a potential role in disease mechanisms [24]. However, while these studies establish TETRA’s association with HF severity, its prognostic value for clinical outcomes in HFpEF remains unexplored. Longitudinal data on TETRA’s ability to predict adverse events, such as HF hospitalization or mortality, in HFpEF patients are lacking, representing a critical gap in the literature [25, 26].

This prospective cohort study aims to evaluate the prognostic value of serum tetranectin (TETRA) for mortality in patients with HF with EF $> 45\%$. Previous studies have linked TETRA to HF severity, but its ability to predict adverse events, such as mortality, remains underexplored. Specifically, we sought to: (1) measure baseline TETRA levels in a cohort of patients with HF with EF $> 45\%$, (2) assess their association with echocardiographic parameters of diastolic function, and (3) determine TETRA’s ability to predict all-cause mortality over a 12-month follow-up period. We hypothesized that lower TETRA levels at baseline are associated with higher mortality in patients with HF with EF $> 45\%$, offering a novel tool for prognostic stratification in this challenging patient population.

MATERIALS AND METHODS

2.1. STUDY DESIGN AND POPULATION

This observational prospective cohort study included adult patients (age > 18 years) with heart failure with left ventricle ejection fraction (LVEF) $\geq 45\%$ and cardiovascular risk factors, who attended the “Centrul Medical Sf. Luca al Crimeei” outpatient clinic in Arad, Romania, between January 1st 2023, and March 31st 2024, for routine check-ups or treatment. Patient records were reviewed to identify eligible individuals who had attended the clinic for routine check-ups or treatment during this period. Inclusion criteria required: (1) a documented diagnosis of HF with EF $> 45\%$, based on clinical and echocardiographic findings; (2) availability of written informed consent for data use. Exclusion criteria included: (1) refusal to consent; (2) acute

cardio-vascular events within the past 30 days of data collection; (3) patients with incomplete or missing data; (4) the presence of active malignancy, severe hepatic or renal dysfunction (e.g., cirrhosis, end-stage renal disease), as these conditions could confound biomarker levels or echocardiographic findings. Patients were not excluded based on stable chronic medications (e.g., antihypertensives, statins) or lifestyle factors (e.g., diet, physical activity). A follow-up period took place between April 1st 2024 and March 31st 2025, when the clinical outcomes were assessed. All included patients were followed for up to 12 months or until death, with data analyzed up to the point of death for the 9 patients who died, ensuring comprehensive assessment of all-cause mortality.

The cut-off value of 45% was selected to reflect real-world clinical practice at ‘Centrul Medical Sf. Luca al Crimeei,’ where LVEF cut-offs vary to accommodate local diagnostic approaches and patient management, and to capture a broader spectrum of heart failure phenotypes, including approximately 72% with HFpEF (LVEF $\geq 50\%$) per ESC/AHA criteria and 28% with LVEF 45–49.9% (upper HFmrEF), thereby enhancing the assessment of TETRA’s biomarker potential across similar cohorts. This differs from the standard ESC/AHA HFpEF definition (LVEF $\geq 50\%$).

To investigate the prognostic value of tetranectin (TETRA) for clinical outcomes in HF with EF > 45%, the study population was divided into three groups based on their New York Heart Association (NYHA) heart failure classification at the time of data collection: patients with NYHA class I (G1 group), NYHA class II (G2 group), and NYHA class III and IV (G3 group). Patients’ NYHA categories were assessed by experienced cardiologists (P.A.V., C.D.P.) using patient-reported symptoms (e.g., dyspnea, fatigue) and physical examination findings, consistent with 2023 ESC guidelines [2], and verified through prior medical records. This stratification allowed for the examination of TETRA levels and their association with clinical outcomes across varying degrees of HFpEF severity.

2.2. DATA COLLECTION

Demographic information (age, sex, smoking status) was obtained from patient charts. Clinical characteristics were gathered through documented physical examinations and medical record reviews. At the recorded visit, body mass index (BMI) and blood pressure were measured. Lipid profiles (total cholesterol, HDL-cholesterol) and diabetes status (defined by prior diagnosis or HbA1c > 6.5%) were

extracted from fasting blood samples analyzed at the clinic’s certified laboratory using standard enzymatic assays (Roche Cobas 6000 analyzer). Echocardiographic parameters were assessed by two experienced cardiologists (P.A.V. or C.D.P.) using a Philips EPIQ 7 ultrasound system with a 3D X5-1 transducer. Measurements included 3D left ventricular ejection fraction (LVEF), global longitudinal strain (GLS), left atrial reservoir strain (LAS), systolic function, and diastolic dysfunction. Diastolic dysfunction was assessed using Philips EPIQ 7 echocardiography, graded from 0 to III based on American Society of Echocardiography (ASE) guidelines, incorporating parameters such as E/A ratio, E/e’ ratio, and left atrial volume index.

Venous blood was collected with minimal stasis into additive-free red-top Vacutainer tubes. Samples were allowed to clot, then centrifuged for 10 min at $1\,000 \times g$. The resulting serum was aliquoted and stored at $-20\text{ }^{\circ}\text{C}$ until analysis (maximum storage time < 6 months). Serum Tetranectin concentrations were quantified with a commercially available sandwich ELISA (Human Tetranectin ELISA, MyBioSource, San Diego, CA, USA) strictly following the manufacturer’s protocol. Serum was diluted 1:100 before assay; plate read-outs (pg/mL) were multiplied by 100 and divided by 1000 to yield final concentrations in ng/mL.

All data was collected at the “Centrul Medical Sf. Luca al Crimeei” medical center. Patients with HF with EF > 45% who were unable to travel, due to advanced functional limitation (NYHA class IV), a mobile team (doctor and nurse) visited the patient’s home within 5 working days of study enrolment. In their case, peripheral venous blood (10 mL) was drawn under identical pre-analytical conditions, immediately transported on ice to the clinic laboratory, and processed within 2 hours using the same centrifugation, aliquoting, and storage protocol as for in-clinic samples. Echocardiographic parameters for NYHA IV patients were extracted from their most recent (< 4 weeks) hospital or outpatient study, performed on the same Philips EPIQ 7 platform.

Clinical outcomes, consisting of all-cause mortality, were tracked over a 12-month follow-up period for each enrolled patient, whether assessed on-site at the clinic, or at home, via regular, scheduled check-ups, or telephonic contact. Outcomes were counted from each patient’s baseline data at the date of enrolment, and data beyond 12 months were censored.

2.3. ETHICAL CONSIDERATIONS

This study followed the principles of the Helsinki Declaration on Medical Protocol and Ethics. All patients provided written informed consent before enrollment in the study. Ethical approval was obtained from the Ethics Committee of the “Vasile Goldis” Western University of Arad, Romania, reference number 75/29.04.2024. Generative AI (ChatGPT, OpenAI, San Francisco, CA, USA) was used solely with the purpose of checking grammar and language editing, while the data collection process, statistical analysis, interpretation, and reporting of findings, were performed exclusively by the authors.

2.4. STATISTICAL ANALYSIS

All analyses were performed using SPSS version 27.0 (IBM Corp., Armonk, NY). The Shapiro-Wilk test assessed normality of continuous variables. Normally distributed variables were reported as mean $\pm$ standard deviation (SD), and non-normally distributed variables as median and interquartile range (IQR). Group differences across NYHA clas-ses were evaluated using one-way ANOVA for normal variables and the Kruskal-Wallis test for non-normal variables. Pairwise comparisons of non-normal variables used the Mann-Whitney U test with Bonferroni correction (adjusted alpha = 0.0167). Effect sizes for tetranectin level differences were calculated with Cohen's d. Spearman's rank correlation examined associations between TETRA and clinical/echocardiographic variables. Cox proportional hazards models assessed TETRA's association with all-cause mortality in univariable and multivariable analyses (adjusted for age and NT-proBNP). A p-value <0.05 indicated statistical significance.

RESULTS

3.1. BASELINE CHARACTERISTICS OF STUDY POPULATION

During January 1st 2023 and March 31st 2024, 118 patients provided written in-formed consent to participate in this study, but two patients were excluded for incomplete follow-up data (they didn't present to the scheduled check-ups), totaling a final number of 116 included patients. Given the exploratory nature and fixed enrollment period, we aimed to include all eligible patients rather than target a specific sample size, and therefore no

power analysis was conducted. This cohort was categorized into three groups based on their New York Heart Association (NYHA) classification: G1 (NYHA class I, n=48), G2 (NYHA class II, n=37), and G3 (NYHA class III-IV, n=31). The study population included 83 patients (71.6%) with HFpEF (LVEF $\geq$ 50%, in alignment with ESC/AHA criteria), and 33 patients (28.4%) with LVEF 45–49.9% (upper HFmrEF), reflecting the 45% cut-off used to enhance sample size. The cohort had a mean age of 59.76 years (SD = 13.5), increasing from 53.17 years (SD = 11.2) in G1 to 64.77 years (SD = 13.9) in G3, with 41.4% (n=48) being female. Clinically, the median body mass index (BMI) was 29.39 kg/m² (IQR = 25.59–34.55), with obesity (BMI $\geq$ 30 kg/m²) in 42% (n=49); type II diabetes in 75.0% (n=87); hypertension in 78% (n=90); and chronic kidney disease (CKD) in 37% (n=43). Medication use included beta-blockers in 68% (n=79), ACE inhibitors or ARBs in 62% (n=72), diuretics in 52% (n=60) (rising to 68%, n=21 in G3), SGLT2 inhibitors in 22% (n=25), and mineralocorticoid receptor antagonists (MRAs) in 28% (n=32). Median total cholesterol was 245 mg/dL (IQR = 200–275), and median NT-proBNP was 446 pg/mL (IQR = 303–819), increasing from 318 pg/mL (IQR = 207–454) in G1 to 716 pg/mL (IQR = 483–947) in G3.

Echocardiographic assessments showed a mean LVEF of 51.91% (SD = 4.5), decreasing from 55.83% (SD = 4.2) in G1 to 48.71% (SD = 3.2) in G3; a mean global longitudinal strain (GLS) of -17.16% (SD = 2.8), worsening to -15.48% (SD = 2.1) in G3; and a median left atrial reservoir strain (LAS) of 26% (IQR = 19–41), dropping from 35% (IQR = 27–44) in G1 to 21% (IQR = 17–27) in G3. Diastolic function parameters included a median E/A ratio of 1.2 (IQR = 0.9–1.8), a median E/e' ratio of 12 (IQR = 9–16) (increasing from 9 in G1 to 16 in G3), and a median left atrial volume index (LAVI) of 36 mL/m² (IQR = 32–42). Diastolic dysfunction was graded as mild (grade I) in 55% (n=64), moderate (grade II) in 25% (n=29), and severe (grade III) in 20% (n=23), while systolic function was normal in 90% (n=104).

Median TETRA levels were 34 ng/mL (IQR = 21.5–55.3), decreasing from 48.9 ng/mL (IQR = 34.4–64.0) in G1 to 27.6 ng/mL (IQR = 18.9–37.1) in G3, reflecting a stepwise decline with HF with EF > 45% severity. These findings, detailed in Table 1, highlight the progressive worsening of diastolic function and biomarker profiles with increasing NYHA class, consistent with greater symptom burden in severe HF.

Normality of continuous variables was assessed using the Shapiro-Wilk test. Age ($p=0.12$), GLS ($p=0.08$), and LVEF ($p=0.15$) were normally distributed and reported as mean (SD), while BMI ($p=0.03$), total cholesterol ($p=0.01$), LAS ($p=0.02$), TETRA ($p=0.001$), NT-proBNP ($p=0.002$), E/A ratio ($p=0.01$), E/e' ratio ($p=0.005$), and LAVI ($p=0.02$) were non-normally distributed and reported as median (IQR).

3.2. DIFFERENCES IN TETRANECTIN LEVELS, CLINICAL AND ECHOCARDIOGRAPHIC VARIABLES ACROSS NYHA CLASSES

To assess differences in TETRA and other clinical/echocardiographic variables across the three NYHA groups (G1: NYHA I, G2: NYHA II, G3: NYHA III–IV), appropriate statistical tests were applied based on variable distribution. For TETRA, which was non-normally distributed (Shapiro-Wilk $p = 0.001$), the Kruskal-Wallis test

revealed statistically significant differences ($H = 62.847$, $p < 0.001$), indicating that median TETRA levels were not equal across NYHA classes. Pairwise comparisons using the Mann-Whitney U test with a Bonferroni correction (adjusted alpha = 0.0167) confirmed a stepwise reduction with increasing HF severity: G1 (NYHA I) had the highest median TETRA at 48.9 ng/mL (IQR = 34.4–64.0), followed by G2 (NYHA II) at 33.2 ng/mL (IQR = 21.5–51.2), and G3 (NYHA III–IV) at 27.6 ng/mL (IQR = 18.9–37.1). Figure 1 illustrates the stepwise decrease of Tetranectin, from NYHA I to NYHA III–IV. All pairwise comparisons were significant: G1 vs. G2 ($U = 512.000$, $p < 0.001$), G1 vs. G3 ($U = 234.000$, $p < 0.001$), and G2 vs. G3 ($U = 389.000$, $p = 0.002$). Effect sizes, calculated as Cohen's d , showed large differences: G1 vs. G2 ($d = 2.43$), G1 vs. G3 ($d = 3.19$), and G2 vs. G3 ($d = 1.96$) (Table 2).

Table 1

Baseline Characteristics of Patients with HF with EF > 45%, Stratified by NYHA Class

Variable (Units), Mean (SD) or Median (IQR)	Overall Population (n = 116)	G1 (NYHA I, n = 48)	G2 (NYHA II, n = 37)	G3 (NYHA III–IV, n = 31)
Age (years), mean (SD)	59.76 (13.5)	53.17 (11.2)	64.38 (12.8)	64.77 (13.9)
Female sex, n (%)	48 (41.4%)	20 (41.7%)	16 (43.2%)	12 (38.7%)
BMI (kg/m ²), median (IQR)	29.39 (25.59–34.55)	29.07 (25.65–34.22)	29.39 (25.59–34.55)	30.93 (23.18–35.32)
CKD, n (%)	43 (37%)	14 (29%)	14 (38%)	15 (48%)
Total Cholesterol (mg/dL), median (IQR)	245 (200–275)	238 (195–278)	250 (200–275)	245 (210–276)
Diabetes, n (%)	87 (75.0%)	36 (75.0%)	28 (75.7%)	23 (74.2%)
Beta-blockers, n (%)	79 (68%)	31 (65%)	26 (70%)	22 (71%)
ACE/ARBs, n (%)	72 (62%)	29 (60%)	23 (62%)	20 (65%)
Diuretics, n (%)	60 (52%)	19 (40%)	20 (54%)	21 (68%)
SGLT2 Inhibitors, n (%)	25 (22%)	7 (15%)	9 (24%)	9 (29%)
MRAs, n (%)	32 (28%)	10 (20%)	11 (30%)	11 (35%)
NT-proBNP (pg/mL), median (IQR)	446 (303–819)	318 (207–454)	500 (320–737)	716 (483–947)
E/A Ratio, median (IQR)	1.2 (0.9–1.8)	0.9 (0.8–1.2)	1.3 (1.0–1.6)	1.8 (1.4–2.2)
E/e' Ratio, median (IQR)	12 (9–16)	9 (8–11)	13 (10–15)	16 (14–18)
GLS (%), mean (SD)	-17.16 (2.8)	-18.54 (2.9)	-17.05 (2.5)	-15.48 (2.1)
LAS (%), median (IQR)	26 (19–41)	35 (27–44)	25 (19–29)	21 (17–27)
LVEF (%), mean (SD)	51.91 (4.5)	55.83 (4.2)	50.00 (3.8)	48.71 (3.2)
TETRA (ng/mL), median (IQR)	34.0 (21.5–55.3)	48.9 (34.4–64.0)	33.2 (21.5–51.2)	27.6 (18.9–37.1)

SD = Standard Deviation; IQR = Interquartile Range; BMI = Body Mass Index; CKD = Chronic Kidney Disease; SGLT2 = Sodium-Glucose Cotransporter-2; MRA = Mineralocorticoid Receptor Antagonist; LAS = Left Atrial Reservoir Strain; GLS = Global Longitudinal Strain; LVEF = Left Ventricular Ejection Fraction; TETRA = Tetranectin.

Box Plot of Serum Tetranectin Levels Across NYHA Classes in HFpEF Patients

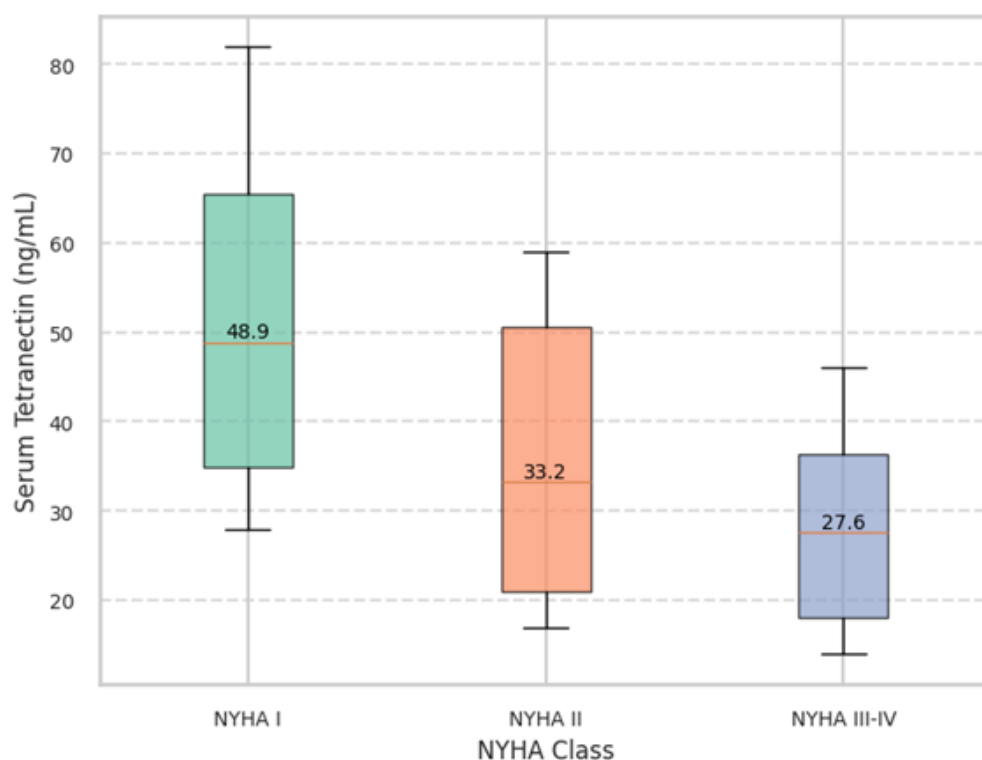


Figure 1. Distribution of Serum Tetranectin by NYHA Class.

Table 2

Effect Sizes (Cohen's d) for Pairwise Comparisons of TETRA Levels Across NYHA Classes

Comparison	TETRA (Cohen's d)
G1 vs. G2	2.43
G1 vs. G3	3.19
G2 vs. G3	1.96

For other variables, one-way ANOVA was used for normally distributed variables (age, GLS, LVEF), while non-normally distributed variables (NT-proBNP, E/A ratio, E/e' ratio, LAVI, LAS, and diuretic use as a binary variable) were analyzed using the Kruskal-Wallis test. Significant differences were observed for age ($F = 12.547$, $p < 0.001$), with G1 being the youngest (mean 53.17 years, $SD = 11.2$) and G3 the oldest (mean 64.77 years, $SD = 13.9$). Median NT-proBNP levels also differed significantly ($H = 58.324$, $p < 0.001$), rising from 318 pg/mL (IQR = 207–454) in G1 to 716 pg/mL (IQR = 483–947) in G3. Among echocardiographic parameters, median E/e' ratio ($H = 45.872$, $p = 0.003$) increased from 9 (IQR = 8–11) in G1 to 16

(IQR = 14–18) in G3, and median LAVI ($H = 39.214$, $p = 0.025$) rose from 32 mL/m² (IQR = 30–35) in G1 to 42 mL/m² (IQR = 38–46) in G3. Median LAS ($H = 42.135$, $p = 0.005$) decreased from 35% (IQR = 27–44) in G1 to 21% (IQR = 17–27) in G3, while mean GLS ($F = 8.976$, $p = 0.009$) worsened from -18.54% ($SD = 2.9$) in G1 to -15.48% ($SD = 2.1$) in G3. Mean LVEF ($F = 10.342$, $p = 0.017$) declined from 55.83% ($SD = 4.2$) in G1 to 48.71% ($SD = 3.2$) in G3. These findings, summarized in Table 3, indicate that worsening HF severity is associated with increased diastolic impairment, higher NT-proBNP levels, and greater reliance on diuretics, reflecting the progressive clinical burden across NYHA classes.

Table 3

Group Differences in Clinical and Echocardiographic Variables Across NYHA Classes

Variable (Units)	Test Statistic (F or H)	p-value
Age (years)	F = 12.547	<0.001
NT-proBNP (pg/mL)	H = 58.324	<0.001
E/e' Ratio	H = 45.872	0.003
LAVI (mL/m ²)	H = 39.214	0.025
LAS (%)	H = 42.135	<0.001
GLS (%)	F = 8.976	0.009
LVEF (%)	F = 10.342	0.017

3.3. ASSOCIATIONS BETWEEN TETRANECTIN AND CLINICAL/ECHOCARDIOGRAPHIC VARIABLES

To explore the relationship between TETRA and clinical/echocardiographic variables in HFpEF, Spearman's rank correlation (ρ) was used due to TETRA's non-normal distribution. TETRA showed a significant negative correlation with NT-proBNP ($\rho = -0.66$, $p < 0.001$), indicating that lower TETRA levels were associated with higher NT-proBNP, a marker of cardiac stress. This is illustrated in Figure 2. Among echocardiographic parameters, TETRA was negatively correlated with

E/e' ratio ($\rho = -0.58$, $p = 0.003$) and LAVI ($\rho = -0.52$, $p = 0.010$), suggesting that lower TETRA levels correspond to worse diastolic dysfunction. TETRA also showed a positive correlation with LAS ($\rho = 0.55$, $p = 0.005$), GLS ($\rho = 0.48$, $p = 0.024$), and LVEF ($\rho = 0.45$, $p = 0.038$), indicating that higher TETRA levels are associated with better left atrial and ventricular function. These correlations, summarized in Table 4, highlight TETRA's association with both diastolic and systolic function in HFpEF and HF with EF > 45%, supporting its potential role in reflecting disease severity.

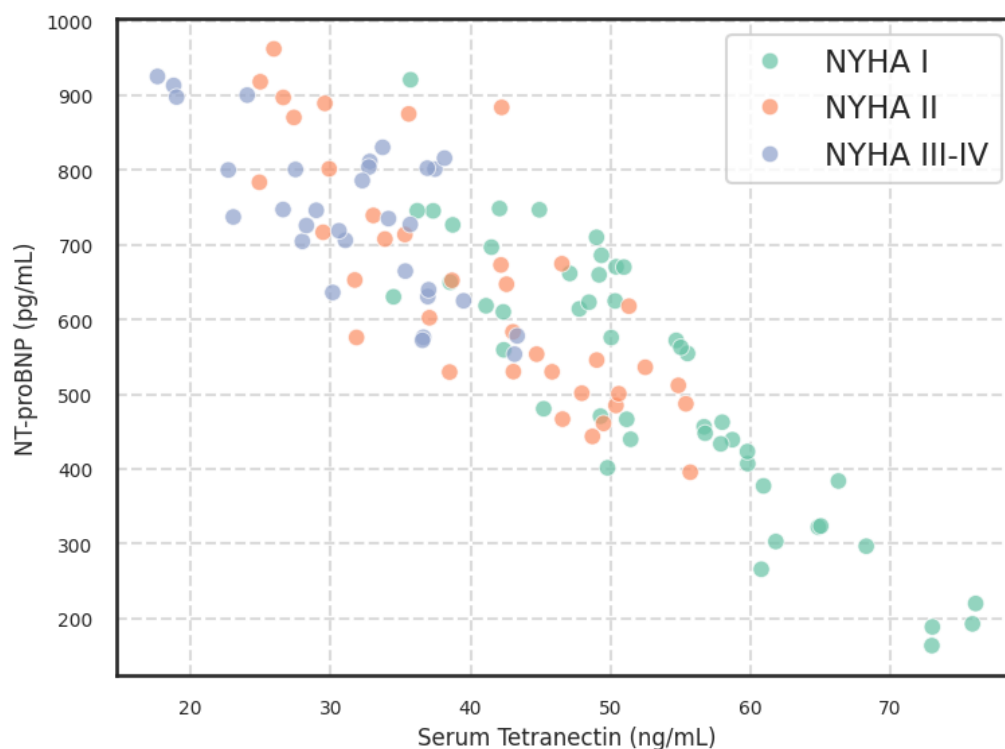


Figure 2. Scatter Plot with Regression Line Illustrating the Relationship Between Serum Tetranectin and NT-proBNP in HF with EF > 45% Patients.

Table 4

Spearman's Rank Correlations Between TETRA and Clinical/Echocardiographic Variables

Variable	Spearman's rho	p-value
NT-proBNP (pg/mL)	-0.66	<0.001
E/e' Ratio	-0.58	0.003
LAVI (mL/m ²)	-0.52	0.010
LAS (%)	0.55	0.005
GLS (%)	0.48	0.024
LVEF (%)	0.45	0.038

3.4. PROGNOSTIC VALUE OF TETRA FOR ALL-CAUSE MORTALITY IN HFPEF

To visualize differences in all-cause mortality across NYHA classes, Kaplan-Meier survival curves were generated for the three groups – G1 (n=48), G2 (n=37), and G3 (n=31) – over the 12-month follow-up (Figure 3). The curves showed a higher mortality rate in G3 (19.4%) compared to G2 (5.4%) and G1 (2.1%), with a log-rank test indicating significant differences ($p = 0.055$). Given the low number of events (9 deaths), these findings should be interpreted cautiously.

To assess TETRA's prognostic value for all-cause mortality in HF with EF > 45%, a Cox proportional hazards model was used to evaluate its association with mortality over a fixed 12-month follow-up period. During the 12-month follow-up,

9 patients (7.8%) died (G1: 1/48, 2.1%; G2: 2/37, 5.4%; G3: 6/31, 19.4%). Of these, 5 were cardiovascular-related (G1: 1/48, 2.1%; G2: 1/37, 2.7%; G3: 3/31, 9.7%). In a univariable Cox model, TETRA (per 10 ng/mL decrease) was significantly associated with all-cause mortality (HR = 1.38, 95% CI 1.06–1.81, $p = 0.045$). After adjusting for age and NT-proBNP in a multivariable model, TETRA remained an independent predictor of mortality, though the association was attenuated (adjusted HR = 1.22, 95% CI 0.94–1.86, $p = 0.112$). These findings, summarized in Table 5, suggest that lower TETRA levels are associated with increased 1-year mortality risk in HF with EF > 45%, supporting its potential as a prognostic biomarker, though the low number of events limits statistical power.

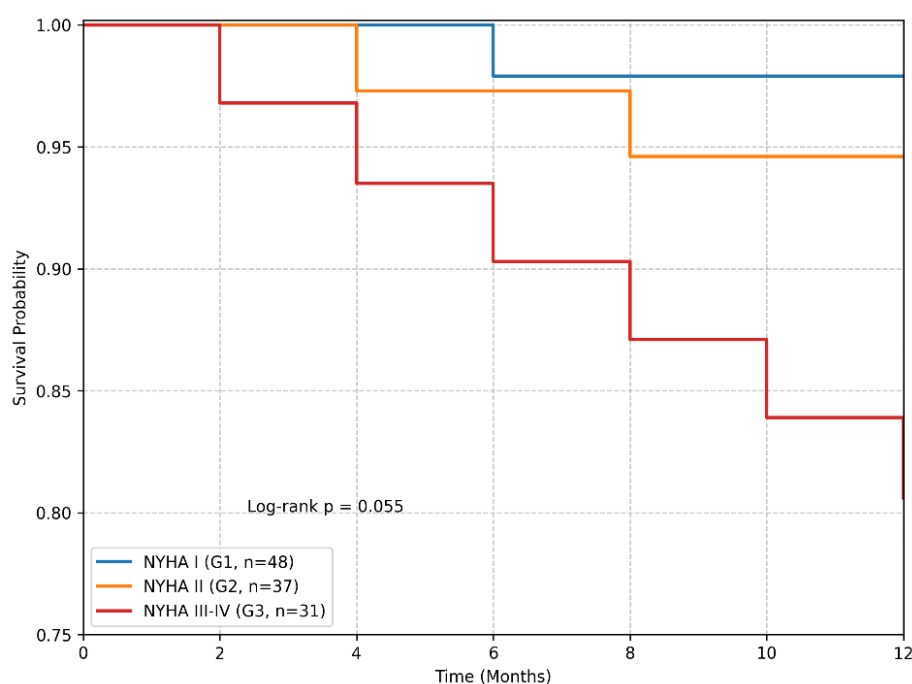


Figure 3. Kaplan-Meier Curves by NYHA Class in HFpEF Patients.

Table 5
Univariate and Multivariate Cox Models for TETRA and All-Cause Mortality

Outcome	Model	HR (per 10 ng/mL decrease)	95% CI	p-value
All-Cause Mortality	Univariable	1.38	1.06-1.81	0.045
	Multivariable	1.22	0.94-1.86	0.112

DISCUSSION

This prospective cohort study set out to evaluate tetranectin (TETRA) as a prognostic biomarker for clinical outcomes in patients with heart failure (HF) with left ventricular ejection fraction (LVEF) > 45%. Our specific aims were threefold: first, to measure baseline TETRA levels in a well-characterized HFpEF cohort stratified by New York Heart Association (NYHA) class; second, to assess TETRA's association with echocardiographic parameters and the classic heart failure biomarker, NT-proBNP; and third, to determine whether TETRA could predict all-cause mortality over a 12-month follow-up period, independent of traditional risk factors like age and NT-proBNP. By exploring these objectives, we hoped to understand the potential role and usefulness of Tetranectin as a biomarker in patients with heart failure. We specifically focused on HF with EF > 45% rather than heart failure in general because this subtype, which accounts for approximately half of all HF cases [4, 10], remains poorly understood with fewer validated biomarkers compared to heart failure with reduced ejection fraction (HFrEF), where natriuretic peptides (NT-proBNP) are more reliable predictors [20]. The pre-served ejection fraction in HF with EF > 45% patients often masks underlying diastolic dysfunction, making novel biomarkers like TETRA critical for identifying at-risk individuals who may benefit from targeted interventions. While established prognostic biomarkers like NT-proBNP remain valuable for assessing outcomes in HF management, this study focuses on exploring TETRA's novel role in disease severity and prognosis, aiming to expand the biomarker landscape rather than replace existing tools.

Our findings provide interesting insights into TETRA's potential role in HF with EF > 45%. We observed a clear stepwise decline in TETRA levels with increasing HF severity, from a median of 48.9 ng/mL in NYHA class I patients (G1) to 27.6 ng/mL in NYHA class III–IV patients (G3), with significant differences across groups

(Kruskal-Wallis $H = 62.847$, $p < 0.001$). This gradient suggests TETRA reflects disease progression, a finding further supported by its strong negative correlations with markers of diastolic dysfunction, including E/e' ratio ($\rho = -0.58$, $p = 0.003$) and LAVI ($\rho = -0.52$, $p = 0.010$), as well as NT-proBNP ($\rho = -0.62$, $p < 0.001$), indicating that lower TETRA levels are tied to worse cardiac stress and impaired diastolic function. Regarding prognosis, TETRA showed a promising association with all-cause mortality in univariable analysis (HR = 1.38 per 10 ng/mL decrease, 95% CI 1.06–1.81, $p = 0.045$), though this effect was attenuated in multivariable models adjusting for age and NT-proBNP (adjusted HR = 1.22, 95% CI 0.94–1.86, $p = 0.112$), likely due to the limited number of events ($n=9$).

These findings resonate with prior research on TETRA in heart failure. For instance, Iram *et al.* (2023) [27] and McDonald *et al.* (2020) [24] have demonstrated reduced TETRA levels in HF patients, correlating with cardiac fibrosis and disease severity, supporting our observation of decreasing TETRA levels with worsening NYHA class in HF with EF > 45%. This reinforces TETRA's potential as a biomarker reflecting heart failure progression across its spectrum.

Our study also provides novel insights by exploring TETRA's prognostic potential in HFpEF and HF with EF > 45%, an area less examined in the literature. Kopeva *et al.* (2023) investigated TETRA in anthracycline-related cardiac dysfunction, reporting that lower TETRA levels predicted worse cardiac function, but their focus on a specific chemotherapy-induced etiology differs from the heterogeneous HFpEF and HF with EF > 45% population we studied [23]. Additionally, while Tanase *et al.* (2019) emphasized the limitations of natriuretic peptides in HFpEF due to their modest elevation compared to HFrEF, our findings position TETRA as a complementary biomarker that may capture distinct aspects of HF with EF > 45% pathology, such as fibrosis, not fully addressed by traditional markers [20]. Tetranectin remains

underexplored in comparison to other markers. Studies like Ho *et al.* (2018) [28] and Wang *et al.* (2012) [29] identified biomarkers such as growth differentiation factor-15 and soluble ST2 as predictors of cardiovascular outcomes in community cohorts, yet TETRA was not evaluated. Similarly, Gilstrap and Wang (2012) [30] reviewed biomarkers like C-reactive protein and B-type natriuretic peptide for cardio-vascular risk assessment in primary prevention, without mentioning TETRA. These studies highlight the need for novel biomarkers in HFpEF and HFmrEF, where conventional markers often lack specificity or sensitivity. Our focus on TETRA's association with diastolic dysfunction and mortality risk distinguishes it from these established markers and suggests a unique role in capturing the complex pathophysiology of heart failure. These differences underscore our study's contribution to establishing TETRA as an important marker in heart failure with mildly reduced and with preserved ejection fraction.

Our study's inclusion of patients with LVEF $\geq 45\%$ encompasses both heart failure with preserved ejection fraction (HFpEF, LVEF $\geq 50\%$) and heart failure with mildly reduced ejection fraction (HFmrEF, LVEF 40–49%), with approximately 72% of the cohort having HFpEF and 28% having HFmrEF. This 45% threshold was chosen to reflect real-world clinical practice at 'Centrul Medical Sf. Luca al Crimeei,' where LVEF cut-offs vary to accommodate local diagnostic approaches, and to capture a broader spectrum of heart failure phenotypes for assessing tetranectin's biomarker potential [8]. HFmrEF, representing 10–25% of heart failure patients, shares characteristics with both HFpEF and HFrEF, notably a higher prevalence of ischemic heart disease similar to HFrEF [8, 31, 32].

This study should be interpreted in light of several constraints that may impact its findings and generalizability. First, the single-center design and modest sample size pose notable limitations. Although we included every eligible patient during the enrollment window from January 1, 2023, to March 31, 2024, the relatively small sample size and lack of diversity in patient demographics and healthcare settings limit the generalizability of our findings to broader populations. Multicenter studies with more diverse cohorts are needed to confirm the applicability of our results across different

regions and clinical contexts. Second, the scarcity of events during the 12-month follow-up period significantly constrained our statistical power. With only 9 deaths and 3 predictors in the multivariable model, the event rate (~ 3 events per predictor) is below the recommended 10 events per predictor, limiting statistical power. Consequently, the hazard ratio estimates for TETRA's association with mortality are imprecise and should be viewed as exploratory rather than definitive. Additionally, the lack of data regarding the length of time from diagnosis to study enrollment prevented adjustment for disease duration, a potential confounder in prognostic analyses. From a methodological perspective, the absence of an a-priori power calculation is a notable limitation. Because recruitment relied on consecutive sampling over a fixed period, and we sought to enroll as many patients as possible in this fixed period of time, we did not perform a formal sample-size estimation.

Taken together, these limitations underscore the need for multicenter studies with larger, prospectively powered cohorts, serial biomarker assessments, and comprehensive adjustment for potential confounders to validate tetranectin's prognostic utility in HFpEF. Future research should also incorporate longer follow-up periods to capture more events, improving statistical power, and explore the impact of temporal changes in tetranectin levels on clinical outcomes.

CONCLUSIONS

This prospective cohort study demonstrates that serum tetranectin (TETRA) levels decrease significantly with increasing HF with EF $> 45\%$ severity, as shown by a stepwise decline across NYHA classes and strong negative correlations with NT-proBNP, E/e' ratio, and left atrial volume index (LAVI). Lower TETRA levels were associated with increased all-cause mortality in univariable analysis (HR = 1.38 per 10 ng/mL decrease, $p = 0.045$), though this effect disappeared in multivariable models, likely due to the limited number of events (9 deaths) or a potential spurious univariable association influenced by confounding factors. These findings suggest TETRA's potential as a biomarker for HF with EF $> 45\%$ progression and prognosis, highlighting the need for larger, multicenter studies to confirm its clinical utility.

Introducere și Obiective: Insuficiența cardiacă (IC) cu fracție de ejeție a ventriculului stâng (FEVS) >45% nu dispune de biomarkeri fiabili pentru stratificarea riscului, ceea ce îngreunează managementul acesteia, întrucât include atât insuficiența cardiacă cu fracție de ejeție păstrată (HFpEF, FEVS $\geq 50\%$), cât și pe cea cu fracție de ejeție ușor redusă (HFmrEF, FEVS 45–49,9%). Acest studiu a avut ca scop evaluarea tetranectinei serice (TETRA) ca biomarker nou pentru aprecierea severității bolii și predicția mortalității la pacienții cu IC cu FEVS >45%.

Materiale și Metode: Într-un studiu de cohortă prospectivă, desfășurat într-un singur centru din Arad, România, au fost incluși 116 pacienți cu IC și FEVS >45%, stratificați conform clasei NYHA (G1: NYHA I, n=48; G2: NYHA II, n=37; G3: NYHA III–IV, n=31). Nivelurile serice de TETRA au fost determinate prin tehnica ELISA. Parametrii ecocardiografici (raportul E/e', LAVI, LAS, GLS, FEVS) și NT-proBNP au fost evaluați la momentul inițial, iar mortalitatea prin orice cauză (9 decese) a fost urmărită pe o perioadă de 12 luni.

Rezultate: Valorile mediane ale TETRA au scăzut progresiv cu agravarea clasei NYHA (G1: 48,9 ng/mL, G2: 33,2 ng/mL, G3: 27,6 ng/mL; $p < 0,001$) și s-au corelat negativ cu NT-proBNP ($\rho = -0,66$, $p < 0,001$), raportul E/e' ($\rho = -0,58$, $p = 0,003$) și LAVI ($\rho = -0,52$, $p = 0,010$), respectiv pozitiv cu LAS ($\rho = 0,55$, $p = 0,005$). În analiza Cox univariată, nivelurile scăzute de TETRA s-au asociat cu o mortalitate mai mare prin orice cauză (HR = 1,38 per 10 ng/mL scădere, IC 95%: 1,06–1,81, $p = 0,045$), însă această asociere nu a mai fost semnificativă după ajustarea pentru vârstă și NT-proBNP (HR = 1,22, IC 95%: 0,94–1,86, $p = 0,112$).

Concluzii: Nivelurile de TETRA sunt invers corelate cu severitatea insuficienței cardiace la pacienții cu FEVS >45% și pot reflecta progresia bolii.

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Acknowledgments

During the preparation of this manuscript, the authors used ChatGPT (OpenAI, OpenAI, San Francisco, CA, USA) for sole purpose of grammar checking and language editing. The collected data, statistical analysis, interpretations, and conclusions, belong exclusively to the authors.

The tables and figures in our study have not been published before, nor will be published elsewhere.

This manuscript has been submitted to the Romanian Journal of Internal Medicine (RJIM) on the 5th of May, 2025. After the peer-review process, a ‘Reject and Resubmit’ decision was communicated to the authors on the 16th of May, 2025. The manuscript was reviewed and resubmitted on the 22nd of May, 2025.

The study was conducted in accordance with the Declaration of Helsinki. All patients signed an informed consent statement in order to be enrolled in this study. Ethical approvals were obtained from the Review Board of the “Centrul Medical Sf. Luca Al Crimeei” medical center, where data collection took place (4th of January 2023), and the Ethics Committee of the “Vasile Goldiș” Western University of Arad, Romania (reference number 75/29.04.2024, 29th of April 2024).

This study received no external funding.

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