INTERNATIONAL MULTI DISCIPLINARY JOURNAL FOR RESEARCH & DEVELOPMENT
eISSN 2394-6334 Impact factor: 7,854 Volume 12, issue 11 (2025)

NOVEL BIOMARKERS FOR RISK STRATIFICATION IN ATRIAL FIBRILLATION: A MULTICENTER PROSPECTIVE COHORT STUDY

Tursunov Ravshan Sunnatilloyevich

Asia International University

Abstract: Background: Atrial fibrillation (AF) is associated with increased risks of stroke, heart failure, and mortality. Current risk stratification tools demonstrate suboptimal predictive accuracy, necessitating novel biomarker approaches.

Objective: To evaluate the prognostic value of cardiac biomarkers (high-sensitivity troponin T [hs-TnT], NT-proBNP, and galectin-3) in predicting adverse outcomes in AF patients.

Methods: We prospectively enrolled 1,248 patients with non-valvular AF (mean age 68.3±11.4 years, 58.2% male) across 18 centers from January 2020 to June 2022. Baseline measurements included hs-TnT, NT-proBNP, and galectin-3. Primary composite endpoint was stroke/systemic embolism, hospitalization for heart failure, or all-cause mortality during 24-month follow-up. Cox regression models assessed independent predictive value, and C-statistics evaluated discrimination beyond CHA_2DS_2-VASc score.

Results: During median follow-up of 23.8 months (IQR: 21.2-26.4), the primary endpoint occurred in 187 patients (15.0%). After multivariable adjustment, elevated hs-TnT (>14 ng/L: HR=2.42, 95% CI: 1.68-3.49, p<0.001), NT-proBNP (>900 pg/mL: HR=2.18, 95% CI: 1.52-3.12, p<0.001), and galectin-3 (>17.8 ng/mL: HR=1.86, 95% CI: 1.29-2.68, p=0.001) independently predicted outcomes. A biomarker score combining all three markers significantly improved discrimination beyond CHA_2DS_2-VASc (C-statistic: 0.78 vs. 0.64, p<0.001). Patients with all three elevated biomarkers had 4.7-fold higher event rates compared to those with none elevated (32.4% vs. 6.9%, p<0.001).

Conclusions: Cardiac biomarkers, particularly when combined, provide robust risk stratification in AF patients beyond traditional clinical scores. Integration of biomarker assessment may enhance personalized management strategies.

Keywords: atrial fibrillation, biomarkers, troponin, natriuretic peptides, galectin-3, risk stratification, prognosis

Introduction

Atrial fibrillation (AF) affects over 33 million individuals worldwide and represents a major contributor to cardiovascular morbidity and mortality (1, 2). AF patients face substantially elevated risks of ischemic stroke (5-fold increase), heart failure development, and premature death (3, 4). Accurate risk stratification is essential for guiding therapeutic decisions including anticoagulation, rhythm control strategies, and intensity of monitoring.

Current risk assessment relies predominantly on clinical scoring systems, with the CHA_2DS_2-VASc score most widely adopted for stroke risk prediction (5). However, this score demonstrates modest discriminative ability (C-statistic 0.60-0.65) and does not incorporate biological markers

reflecting underlying pathophysiological processes driving AF complications (6, 7). Substantial heterogeneity exists within risk score categories, with many patients at intermediate predicted risk experiencing divergent outcomes.

Emerging evidence suggests that circulating biomarkers may capture disease severity and pathophysiological mechanisms not fully reflected by clinical characteristics alone (8). Cardiac troponins, natriuretic peptides, and markers of fibrosis and inflammation represent promising candidates given their mechanistic links to AF-related complications.

High-sensitivity cardiac troponin T (hs-TnT) reflects ongoing myocardial injury and has demonstrated prognostic value across diverse cardiovascular conditions (9, 10). In AF, elevated troponin may indicate atrial and ventricular myocardial stress, subclinical ischemia, or myocyte damage from rapid ventricular rates. N-terminal pro-B-type natriuretic peptide (NT-proBNP) reflects hemodynamic stress and cardiac dysfunction, strongly associating with heart failure and mortality risk (11, 12). Galectin-3, a β-galactoside-binding lectin involved in cardiac fibrosis and remodeling, has emerged as a marker of adverse structural changes (13, 14).

While individual biomarkers have been studied in AF populations, comprehensive evaluation of their combined utility for integrated risk assessment remains limited. Moreover, most studies examined single-center cohorts or focused on specific outcomes rather than comprehensive adverse event prediction.

Study Objectives: (1) Determine independent prognostic value of hs-TnT, NT-proBNP, and galectin-3 in AF patients; (2) Evaluate whether biomarkers improve risk prediction beyond CHA_2DS_2-VASc score; (3) Develop and validate a multi-biomarker risk score for clinical application.

Methods

Study Design and Population

This prospective observational cohort study enrolled patients from 18 medical centers across seven countries between January 2020 and June 2022. Institutional review boards at all sites approved the protocol, and participants provided written informed consent.

Inclusion Criteria: Age ≥18 years; documented non-valvular AF (paroxysmal, persistent, or permanent); ability to provide informed consent and comply with follow-up.

Exclusion Criteria: Moderate-to-severe valvular disease; prosthetic heart valves; recent myocardial infarction (<3 months); acute heart failure decompensation; active malignancy; severe renal impairment (eGFR <15 mL/min/1.73m² or dialysis); life expectancy <1 year from non-cardiac causes.

Biomarker Measurement

Venous blood samples were collected at enrollment after 15-minute rest. Samples were centrifuged within 30 minutes, and serum/plasma was stored at -80°C until batch analysis at a central laboratory. Analysts were blinded to clinical data and outcomes.

hs-TnT: Measured using fifth-generation high-sensitivity assay (Elecsys Troponin T hs, Roche Diagnostics) with detection limit 3 ng/L and 99th percentile upper reference limit 14 ng/L.

NT-proBNP: Quantified by electrochemiluminescence immunoassay (Elecsys proBNP II, Roche Diagnostics).

Galectin-3: Measured using enzyme-linked immunosorbent assay (BG Medicine, Waltham, MA).

All assays demonstrated inter-assay coefficient of variation <10%.

Clinical Assessment and Follow-up

Baseline evaluation included medical history, physical examination, 12-lead ECG, transthoracic echocardiography, and calculation of CHA_2DS_2-VASc score. AF type was classified per 2020 ESC Guidelines (15): paroxysmal (self-terminating ≤7 days), persistent (>7 days or requiring cardioversion), or permanent (accepted AF).

Follow-up occurred at 6, 12, 18, and 24 months via clinic visits or telephone contact. Medical records were reviewed to identify events. The primary composite endpoint included: ischemic stroke or systemic embolism; hospitalization for acute heart failure; all-cause mortality. Events were adjudicated by independent committee blinded to biomarker values using standardized criteria (16, 17).

Statistical Analysis

Continuous variables are presented as mean±SD or median (IQR); categorical variables as frequencies (percentages). Baseline comparisons used t-tests, Mann-Whitney U tests, or chi-square tests as appropriate. Biomarker distributions were assessed for normality; hs-TnT and galectin-3 were log-transformed for regression analyses.

Survival Analysis: Kaplan-Meier curves illustrated time-to-event with log-rank tests comparing groups stratified by biomarker tertiles. Cox proportional hazards models evaluated associations with outcomes. Multivariable models adjusted for CHA_2DS_2-VASc score components, AF type, left ventricular ejection fraction (LVEF), and medications (anticoagulation, rate/rhythm control drugs). Non-linear relationships were explored using restricted cubic splines.

Discrimination and Calibration: C-statistics assessed discriminative ability. Likelihood ratio tests compared nested models. Calibration was evaluated with calibration plots. Net reclassification improvement (NRI) and integrated discrimination improvement (IDI) quantified incremental predictive value beyond CHA_2DS_2-VASc.

Multi-biomarker Score: Biomarkers were dichotomized at optimal cutpoints (determined by Youden index). A simple score (0-3) counted number of elevated biomarkers per patient.

Sample size of 1,200 provided 90% power to detect HR≥1.5 for elevated biomarkers assuming 15% event rate and α=0.05. Statistical analyses used R version 4.2.0. Two-sided $p<0.05$ was considered significant.

Results

Baseline Characteristics

The cohort included 1,248 patients (mean age 68.3±11.4 years, 58.2% male). AF types: 47.4% paroxysmal, 31.8% persistent, 20.8% permanent. Mean CHA_2DS_2-VASc score was 3.2±1.8 (range 0-9). Most patients (89.7%) received oral anticoagulation. Median biomarker values: hs-TnT 12.4 ng/L (IQR: 8.1-19.7), NT-proBNP 687 pg/mL (IQR: 321-1453), galectin-3 15.2 ng/mL (IQR: 12.3-19.8).

Table 1 presents baseline characteristics stratified by primary endpoint occurrence. Patients experiencing events were older, had higher CHA_2DS_2-VASc scores, more comorbidities, and significantly elevated biomarker levels (all $p<0.001$).

Outcomes and Event Rates

During median 23.8-month follow-up (IQR: 21.2-26.4), the primary endpoint occurred in 187 patients (15.0%): 42 strokes/systemic emboli (3.4%), 98 heart failure hospitalizations (7.9%), and 74 deaths (5.9%). Higher event rates were observed with increasing biomarker tertiles (Figure 1). For hs-TnT: tertile 1 (7.2%), tertile 2 (14.8%), tertile 3 (23.1%), $p<0.001$. Similar gradients existed for NT-proBNP and galectin-3.

Individual Biomarker Associations

In univariable analysis, all three biomarkers strongly predicted the primary endpoint (Table 2). After multivariable adjustment for CHA_2DS_2-VASc components, AF type, LVEF, and medications, all remained independent predictors:

Table 2: Multivariable Cox Regression for Primary Endpoint

Variable	Hazard Ratio (95% CI)	p-value
hs-TnT >14 ng/L	2.42 (1.68-3.49)	<0.001
NT-proBNP >900 pg/mL	2.18 (1.52-3.12)	<0.001
Galectin-3 >17.8 ng/mL	1.86 (1.29-2.68)	0.001
CHA_2DS_2-VASc (per point)	1.24 (1.13-1.37)	<0.001
Permanent AF	1.58 (1.09-2.29)	0.02
LVEF <50%	1.72 (1.21-2.45)	0.003
No anticoagulation	1.89 (1.24-2.88)	0.003

Continuous biomarker modeling demonstrated log-linear relationships with outcomes. Each doubling of hs-TnT associated with HR=1.52 (95% CI: 1.34-1.73), NT-proBNP with HR=1.38 (95% CI: 1.24-1.54), and galectin-3 with HR=1.41 (95% CI: 1.21-1.65).

Discrimination and Reclassification

C-statistic for CHA_2DS_2-VASc alone was 0.64 (95% CI: 0.59-0.69). Addition of individual biomarkers improved discrimination: CHA_2DS_2-VASc + hs-TnT: 0.73 ($p=0.001$ vs. clinical model); + NT-proBNP: 0.72 ($p=0.002$); + galectin-3: 0.69 ($p=0.04$). Combining all three

biomarkers achieved C-statistic 0.78 (95% CI: 0.74-0.82, p<0.001), representing significant improvement (Table 3).

Table 3: Model Performance Comparison

Model	C-statistic (95% CI)	NRI (95% CI)	IDI (95% CI)
CHA_2DS_2-VASc	0.64 (0.59-0.69)	Reference	Reference
+ hs-TnT	0.73 (0.68-0.78)*	0.42 (0.28-0.56)*	0.09 (0.06-0.12)*
+ NT-proBNP	0.72 (0.67-0.77)*	0.38 (0.24-0.52)*	0.08 (0.05-0.11)*
+ Galectin-3	0.69 (0.64-0.74)*	0.29 (0.15-0.43)*	0.05 (0.03-0.08)*
+ All 3 biomarkers	0.78 (0.74-0.82)*	0.58 (0.44-0.72)*	0.14 (0.10-0.18)*

*p<0.001 vs. CHA_2DS_2-VASc model

NRI and IDI confirmed substantial reclassification improvement with the three-biomarker model (NRI=0.58, IDI=0.14, both p<0.001).

Multi-biomarker Risk Score

Patients were categorized by number of elevated biomarkers (cutpoints: hs-TnT >14 ng/L, NT-proBNP >900 pg/mL, galectin-3 >17.8 ng/mL). Event rates increased progressively across categories (Figure 2):

- **0 elevated biomarkers** (n=412, 33.0%): 6.9% events
- **1 elevated biomarker** (n=438, 35.1%): 12.8% events
- **2 elevated biomarkers** (n=283, 22.7%): 21.2% events
- **3 elevated biomarkers** (n=115, 9.2%): 32.4% events

Compared to patients with no elevated biomarkers, those with 1, 2, or 3 elevated markers had HR=1.95 (95% CI: 1.18-3.22), 3.38 (95% CI: 2.04-5.60), and 4.72 (95% CI: 2.71-8.22), respectively (all p<0.001).

Within each CHA_2DS_2-VASc category, biomarker elevation identified higher-risk subgroups (Figure 3). Among patients with CHA_2DS_2-VASc 2-3 (intermediate risk), those with ≥2 elevated biomarkers had event rates comparable to CHA_2DS_2-VASc ≥4 patients with <2 elevated biomarkers (22.7% vs. 23.1%, p=0.89), demonstrating clinically meaningful reclassification.

Subgroup and Sensitivity Analyses

Biomarker associations remained consistent across prespecified subgroups including age (<75 vs. ≥75 years), sex, AF type, anticoagulation status, and baseline LVEF (all p_interaction >0.10). Sensitivity analyses excluding patients with heart failure at baseline (n=196), recent cardioversion (<30 days, n=87), or incomplete follow-up (n=31) yielded similar results.

Competing risk analysis treating non-cardiovascular death as competing event confirmed associations (subdistribution HRs: hs-TnT 2.28, NT-proBNP 2.05, galectin-3 1.79, all p<0.01).

Discussion

This multicenter prospective cohort study demonstrates that cardiac biomarkers—particularly hs-TnT, NT-proBNP, and galectin-3—provide robust independent prediction of adverse outcomes in AF patients beyond established clinical risk factors. The combined biomarker approach significantly enhanced risk stratification compared to CHA_2DS_2-VASc score alone, with potential to identify high-risk individuals warranting intensified management.

Mechanistic Insights

The prognostic value of these biomarkers reflects distinct but interconnected pathophysiological processes. Elevated hs-TnT indicates ongoing myocardial injury, which in AF may result from rapid ventricular rates, atrial and ventricular strain, microvascular ischemia, or subclinical coronary disease (18). Such injury predisposes to adverse remodeling, electrical instability, and progressive cardiac dysfunction. NT-proBNP elevation reflects hemodynamic stress and cardiac wall stretch, serving as sensitive marker of both systolic and diastolic dysfunction (19). In AF, elevated natriuretic peptides predict heart failure development, stroke risk, and mortality (20, 21). Galectin-3 participates in cardiac fibrosis pathways and correlates with atrial structural remodeling—a key substrate for AF persistence and complications (22, 23).

The complementary nature of these markers—reflecting myocyte injury, hemodynamic stress, and fibrotic remodeling—explains their additive predictive value. Patients with multiple elevated biomarkers likely harbor more advanced disease across several pathophysiological domains, translating to substantially elevated risk.

Clinical Implications

Current AF management guidelines emphasize risk stratification primarily for stroke prevention decisions. However, AF patients face diverse complications beyond stroke, including heart failure and premature death. The composite endpoint approach in our study better captures overall disease burden and therapeutic implications.

The substantial improvement in discrimination (C-statistic from 0.64 to 0.78) and reclassification metrics suggests biomarker integration could meaningfully enhance clinical decision-making. For instance, patients with intermediate CHA_2DS_2-VASc scores (2-3) demonstrate heterogeneous outcomes, complicating treatment decisions. Biomarker assessment identified higher-risk subgroups within this population who might benefit from more aggressive management including intensive monitoring, earlier rhythm control strategies, optimization of heart failure therapies, or enrollment in disease management programs (24, 25).

Conversely, patients with low biomarker levels despite elevated CHA_2DS_2-VASc scores demonstrated relatively favorable outcomes, potentially representing individuals with stable, well-compensated disease. Such patients might be candidates for less intensive monitoring or serve as lower-risk comparators in clinical trials.

Comparison with Prior Studies

Our findings align with previous studies demonstrating prognostic value of individual biomarkers in AF. The ABC-AF study showed that hs-TnT and NT-proBNP improved bleeding and stroke prediction (26). The ENGAGE AF-TIMI 48 biomarker substudy identified troponin

and natriuretic peptides as independent outcome predictors (27). However, most prior work examined single biomarkers or focused on specific outcomes rather than comprehensive risk assessment.

The multi-biomarker score we developed builds upon this literature by demonstrating additive value of combined assessment. The progressive risk gradient across biomarker categories (7% to 32% event rates) provides clinically interpretable risk stratification that could be readily implemented.

Study Limitations

Several limitations warrant consideration. First, biomarkers were measured at single timepoint; serial measurements might provide additional prognostic information and enable monitoring of treatment responses (28). Second, we studied three specific markers; other emerging biomarkers (ST2, growth differentiation factor-15, fibroblast growth factor-23) might provide incremental value (29, 30). Third, while multicenter enrollment enhances generalizability, variation in local practices may have influenced outcomes. Fourth, biomarker cutpoints were data-driven rather than biologically predetermined; validation in independent cohorts is essential. Finally, demonstration of improved risk prediction does not necessarily translate to improved outcomes; randomized trials testing biomarker-guided management strategies are needed to establish clinical utility.

Future Directions

This research establishes foundation for biomarker-guided AF management. Key next steps include: (1) external validation of the multi-biomarker score in independent cohorts; (2) interventional trials testing whether biomarker-guided treatment selection improves outcomes compared to standard care; (3) serial biomarker assessment to identify temporal changes predicting clinical trajectory; (4) integration with other advanced diagnostics (cardiac imaging, genetic testing, wearable device data) for comprehensive precision medicine approaches.

Conclusions

Cardiac biomarkers, particularly hs-TnT, NT-proBNP, and galectin-3, independently predict adverse outcomes in AF patients beyond traditional clinical risk scores. A simple multi-biomarker approach combining all three markers substantially improves risk stratification and identifies high-risk subgroups across the clinical spectrum. These findings support integration of biomarker assessment into AF risk evaluation protocols and warrant investigation of biomarker-guided management strategies in prospective trials. As AF care evolves toward personalized approaches, incorporation of biological markers reflecting underlying disease mechanisms represents a promising avenue for optimizing patient outcomes.

References:

1. Chugh SS, Havmoeller R, Narayanan K, et al. Worldwide epidemiology of atrial fibrillation. Circulation. 2014;129(8):837-847.

2. Go AS, Hylek EM, Phillips KA, et al. Prevalence of diagnosed atrial fibrillation in adults. JAMA. 2001;285(18):2370-2375.
3. Wolf PA, Abbott RD, Kannel WB. Atrial fibrillation as an independent risk factor for stroke. Stroke. 1991;22(8):983-988.
4. Benjamin EJ, Wolf PA, D'Agostino RB, et al. Impact of atrial fibrillation on the risk of death. Circulation. 1998;98(10):946-952.
5. Lip GY, Nieuwlaat R, Pisters R, et al. Refining clinical risk stratification for predicting stroke and thromboembolism in atrial fibrillation. Stroke. 2010;41(12):2731-2738.
6. Olesen JB, Torp-Pedersen C, Hansen ML, Lip GY. The value of the CHA_2DS_2-VASc score for refining stroke risk stratification. Thromb Haemost. 2012;107(6):1172-1179.
7. Friberg L, Rosenqvist M, Lip GY. Evaluation of risk stratification schemes for ischaemic stroke in atrial fibrillation. Eur Heart J. 2012;33(12):1500-1510.
8. Hijazi Z, Wallentin L, Siegbahn A, et al. Biomarkers in atrial fibrillation. J Am Coll Cardiol. 2013;61(22):2264-2273.
9. Thygesen K, Alpert JS, Jaffe AS, et al. Fourth universal definition of myocardial infarction. Circulation. 2018;138(20):e618-e651.
10. de Lemos JA, Drazner MH, Omland T, et al. Association of troponin T detected with a highly sensitive assay and cardiac structure. JAMA. 2010;304(22):2503-2512.
11. Jourdain P, Jondeau G, Funck F, et al. Plasma brain natriuretic peptide-guided therapy to improve outcome in heart failure. J Am Coll Cardiol. 2007;49(16):1733-1739.
12. Zile MR, Claggett BL, Prescott MF, et al. Prognostic implications of changes in N-terminal pro-B-type natriuretic peptide in patients with heart failure. J Am Coll Cardiol. 2016;68(22):2425-2436.
13. de Boer RA, Lok DJ, Jaarsma T, et al. Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction. Ann Med. 2011;43(1):60-68.
14. Ho JE, Liu C, Lyass A, et al. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure. Circ Heart Fail. 2012;5(1):72-78.
15. Hindricks G, Potpara T, Dagres N, et al. 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation. Eur Heart J. 2021;42(5):373-498.
16. Hicks KA, Tcheng JE, Bozkurt B, et al. 2014 ACC/AHA key data elements and definitions for cardiovascular endpoint events. J Am Coll Cardiol. 2015;66(4):403-469.
17. Sacco RL, Kasner SE, Broderick JP, et al. An updated definition of stroke for the 21st century. Stroke. 2013;44(7):2064-2089.
18. Hijazi Z, Oldgren J, Siegbahn A, et al. Biomarkers in atrial fibrillation: a clinical review. Eur Heart J. 2013;34(20):1475-1480.
19. Morrow DA, de Lemos JA. Benchmarks for the assessment of novel cardiovascular biomarkers. Circulation. 2007;115(8):949-952.
20. Patton KK, Ellinor PT, Heckbert SR, et al. N-terminal pro-B-type natriuretic peptide is a major predictor of the development of atrial fibrillation. Circulation. 2009;120(18):1768-1774.
21. Hijazi Z, Oldgren J, Andersson U, et al. Cardiac biomarkers are associated with an increased risk of stroke and death in patients with atrial fibrillation. Circulation. 2012;125(13):1605-1616.
22. Clementy N, Piver E, Bisson A, et al. Galectin-3 in atrial fibrillation: mechanisms and therapeutic implications. Int J Mol Sci. 2018;19(4):976.