Original Article

The Role of BNP and Dickkopf-3 in Arrhythmias: A New Perspective in Biomarkers

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Abstract

Background: **Elevated levels of type B natriuretic peptide (BNP) and Dikkopf-3-associated protein (DKK-3) are associated with a range of clinical, hematological, and physiological conditions.**

Objectives: **This study aims to evaluate the relationship between BNP and DKK-3 levels and cardiac arrhythmias, and to explore their associations with available clinical and laboratory variables.**

Methods: **This case-control study included 90 participants: 70 patients with confirmed cardiac arrhythmias and 20 healthy controls. The arrhythmia group included atrioventricular nodal reentry tachycardia (AVNRT; n=20), supraventricular tachycardia (SVT; n=20), atrial fibrillation (AF; n=20), and complete heart block (CHB; n=10). Serum BNP and DKK3 concentrations were measured using an enzyme-linked immunosorbent assay. The significance level adopted in the statistical analysis was 5%.**

Results: **DKK3 and BNP levels were significantly higher in patients with arrhythmias than in controls. BNP showed weak but statistically significant positive correlations with prothrombin time and international normalized ratio. Exploratory ROC analysis showed an AUC of 0.954 for DKK3 and 0.932 for BNP. In separate multivariable logistic models adjusted for age, sex, body mass index, and smoking, both DKK3 and BNP were associated with arrhythmia status; however, when entered together in the same model, neither biomarker remained statistically significant at the 5% level.**

Conclusion: **BNP and DKK3 were significantly elevated in patients with cardiac arrhythmias compared with controls. These findings suggest an association between these biomarkers and arrhythmia status. Because of the case-control design, limited sample size, and incomplete availability of some cardiac clinical variables, the results should be considered exploratory and associative rather than predictive or prognostic.**

Keywords: **Natriuretic Peptides; Cardiac Arrhythmias; Atrial Fibrillation; Biomarkers.**

Introduction

Cardiac arrhythmias represent a clinically heterogeneous group of rhythm and conduction disorders. Their mechanisms vary considerably across atrial fibrillation, supraventricular tachycardia, atrioventricular nodal reentry tachycardia, and complete heart block. Therefore, biomarker interpretation in arrhythmia patients requires caution and should consider the underlying cardiac condition, renal function, systemic comorbidities, and available clinical context.[1]

BNP is secreted mainly in response to myocardial wall stress and is widely used in the assessment of heart failure and cardiac dysfunction. BNP levels may also rise in atrial fibrillation and other cardiac conditions,[2] but elevated concentrations are not specific to arrhythmia alone. They may reflect atrial enlargement, ventricular dysfunction, renal impairment, age-related changes, and concomitant cardiovascular disease.[3]

DKK3 is a member of the Dickkopf family of secreted glycoproteins and is involved in cellular stress responses, Wnt-related signaling, tissue remodeling, and cardiovascular injury pathways. Because remodeling and fibrosis may participate in the pathophysiology of some arrhythmias, DKK3 has emerged as a candidate biomarker of interest. Nevertheless, its direct role in rhythm disorders remains incompletely defined.[4]

The present study investigated whether serum BNP and DKK3 levels differ between patients with cardiac arrhythmias and healthy controls. The study also explored whether these biomarkers correlate with available clinical, hematological, renal, hepatic, and coagulation parameters. The analysis was framed as an exploratory association study rather than as a prognostic or predictive investigation.

Methods

Study design and participants

This case-control study was conducted at the Center for Cardiac Surgery and Catheterization. A total of 90 participants

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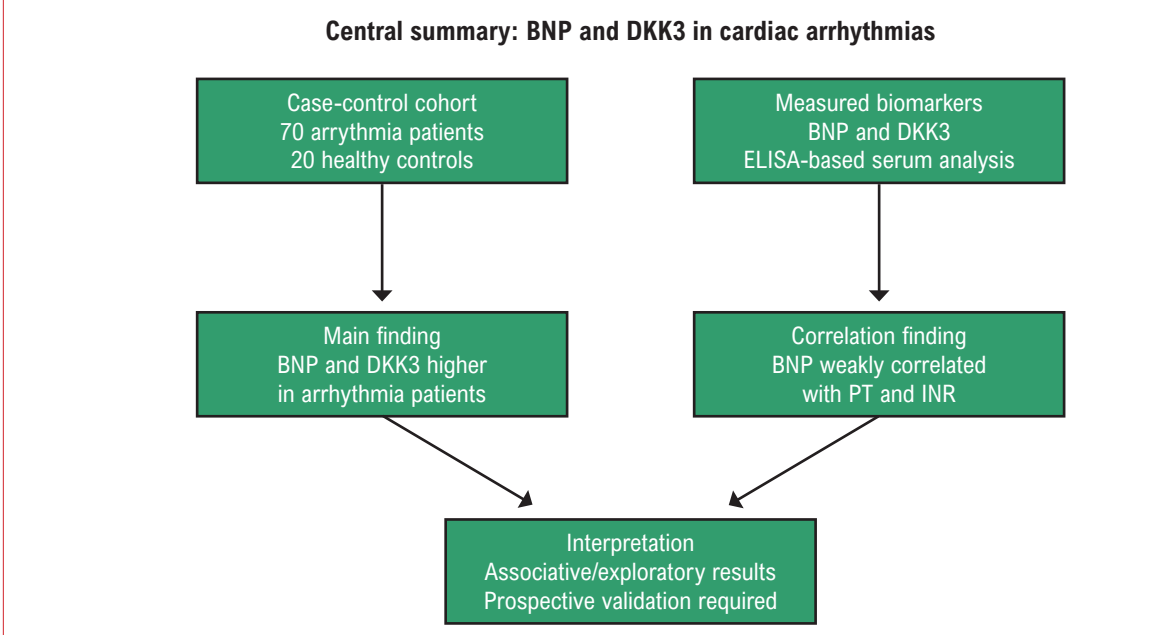
Central Illustration: The Role of BNP and Dickkopf-3 in Arrhythmias: A New Perspective in Biomarkers



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Summary of the exploratory association between BNP, DKK3, and cardiac arrhythmia status.

were enrolled, including 70 patients with confirmed cardiac arrhythmias and 20 healthy controls. The arrhythmia group included AVNRT, SVT, AF, and CHB. The sample size was based on eligible participants available during the study period and was therefore considered a convenience sample.

Patients were included after confirmation of arrhythmia by electrocardiography. Comprehensive cardiac assessment, including echocardiography and stress testing where clinically indicated, was performed as part of routine clinical evaluation. Health controls were selected from individuals without reported cardiac arrhythmia or past medical history in the available clinical sheet.

Ethical considerations

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Ethical approval was obtained from the Institutional Ethics Committee of Najaf Center for Cardiac Surgery and Catheterization, Approval No. 7728, dated December 24, 2024. Written informed consent was obtained from all participants before inclusion in the study. No animal experiments were performed.

Sample collection and processing

Peripheral venous blood samples were collected under standardized conditions. Samples were drawn into plain gel tubes and allowed to clot at room temperature for 30 minutes. They were then centrifuged at 3000 rpm for 15 minutes to separate serum. Serum aliquots were stored at -40°C until biochemical analysis.

Biochemical analysis

Serum concentrations of DKK3 and BNP were quantified using commercially available high-sensitivity enzyme-linked immunosorbent assay kits according to the manufacturers' protocols. The kits used were Human Dickkopf-3 ELISA Kit (ADL-EL-HM02031) and Human BNP ELISA Kit (ADL-EL-HM00148), Shanghai Ideal Medical Technology Co., Ltd., China.

Clinical and laboratory data

The following variables were recorded when available: age, sex, heart rate, blood pressure status, diabetes status, smoking status, oxygen saturation, respiratory rate, weight, height, body mass index, prothrombin time, partial thromboplastin time, international normalized ratio, AST, ALT, total bilirubin, platelet count, hematocrit, hemoglobin, white blood cell count, urea, creatinine, MCV, MCH, MCHC, RBC count, past medical history, and family history. Available past medical history data were used to identify ischemic heart disease, hypertension, and diabetes when recorded.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 8.0.2 and independent verification using Python

statistical libraries. The significance level adopted in the statistical analysis was 5%. The Shapiro-Wilk test was used to assess normality of continuous variables. Normally distributed continuous variables were presented as mean ± standard deviation, while non-normally distributed variables were presented as median and interquartile range. Categorical variables were presented as numbers and percentages.

Comparisons between two groups were performed using the independent samples t-test or Mann-Whitney U test, as appropriate. Comparisons among more than two groups were performed using one-way ANOVA followed by Tukey's post-hoc test for normally distributed variables, or the Kruskal-Wallis test followed by Dunn's multiple comparisons post-hoc test for non-normally distributed variables. Correlations between biomarkers and clinical or laboratory variables were assessed using Pearson's or Spearman's correlation coefficients according to data distribution.

Exploratory ROC curve analyses were performed for BNP and DKK3 to estimate discrimination between arrhythmia patients and controls. AUC, sensitivity, specificity, and optimal cutoff values based on the Youden index were calculated. Exploratory binary logistic regression was performed to examine whether BNP and DKK3 were associated with arrhythmia status after adjustment for available covariates. Because complete left ventricular ejection fraction, medication use, and structural heart disease data were not available for all participants, the regression models were restricted to age, sex, body mass index, and smoking status. Odds ratios were reported per standard deviation increase for continuous predictors.

Results

Baseline characteristics

The study included 90 participants: 70 patients with cardiac arrhythmias and 20 healthy controls. The arrhythmia subgroups were AVNRT (n=20), SVT (n=20), AF (n=20), and CHB (n=10). The sex distribution was balanced across groups, with 10 males and 10 females in each 20-participant group and 5 males and 5 females in the CHB group. Patients with arrhythmias were older and had higher body mass index, heart rate, urea, and creatinine values compared with controls. Available clinical history also showed higher frequencies of hypertension/elevated BP, ischemic heart disease, and diabetes in the arrhythmia group. The baseline characteristics of controls and arrhythmia patients are summarized in Table 1.

BNP and DKK3 levels in arrhythmia patients and controls

DKK3 and BNP were significantly higher among patients with arrhythmias compared with controls. DKK3 was 27.88 ± 3.27 ng/mL in the arrhythmia group and 19.51 ± 3.20 ng/mL in controls (p<0.0001) Figure 1). BNP was 132.74 ± 22.24 pg/mL in the arrhythmia group and 87.65 ± 18.82 pg/mL in controls (p<0.0001) (Figure 2).

Biomarker levels according to arrhythmia subtype

When biomarkers were analyzed according to arrhythmia subtype, DKK3 levels were lowest in controls and higher across all arrhythmia subgroups, with the highest mean value observed in CHB. BNP levels were also higher across

Table 1 – Baseline clinical and laboratory characteristics of the study population

Variable	Control (n=20)	Arrhythmia (n=70)	p-value
Age, years	30.30 ± 7.79	46.06 ± 17.19	<0.0001
BMI, kg/m^2	24.36 ± 2.62	27.35 ± 4.83	0.0057
Heart rate, bpm	75.25 ± 4.91	84.84 ± 16.16	<0.0001
SpO_2, %	97.15 ± 1.46	97.27 ± 1.44	0.8392
Respiratory rate/min	18.50 ± 0.95	18.79 ± 3.18	0.9482
Urea, mg/dL	22.55 ± 2.34	34.28 ± 18.88	0.0001
Creatinine, mg/dL	0.56 ± 0.10	1.01 ± 1.13	<0.0001
PT, seconds	11.87 ± 0.60	12.58 ± 2.15	0.5185
PTT, seconds	25.88 ± 1.96	24.01 ± 4.40	0.0014
INR	0.96 ± 0.55	1.01 ± 0.21	0.0040
Male sex	10 (50%)	35 (50%)	1.0000
Diabetes mellitus	0 (0%)	13 (19%)	0.0644
Smoker	3 (15%)	14 (20%)	0.7539
Elevated BP/history of hypertension	0 (0%)	32 (46%)	<0.0001
Ischemic heart disease history	0 (0%)	28 (40%)	0.0002
COVID-19 history	0 (0%)	27 (39%)	0.0005

BMI: body mass index; PT: Prothrombin time; PTT: Partial thromboplastin time.

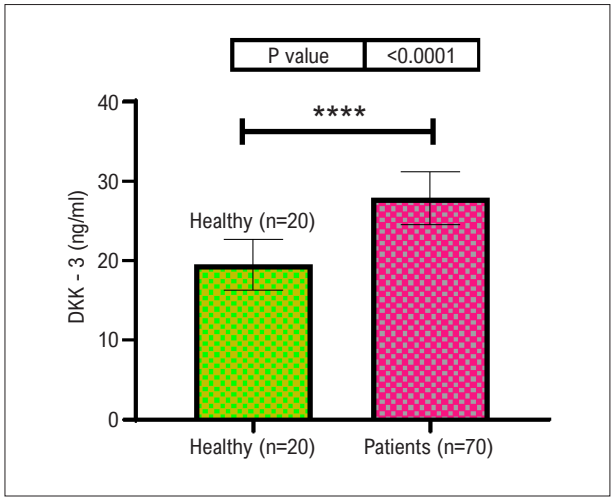
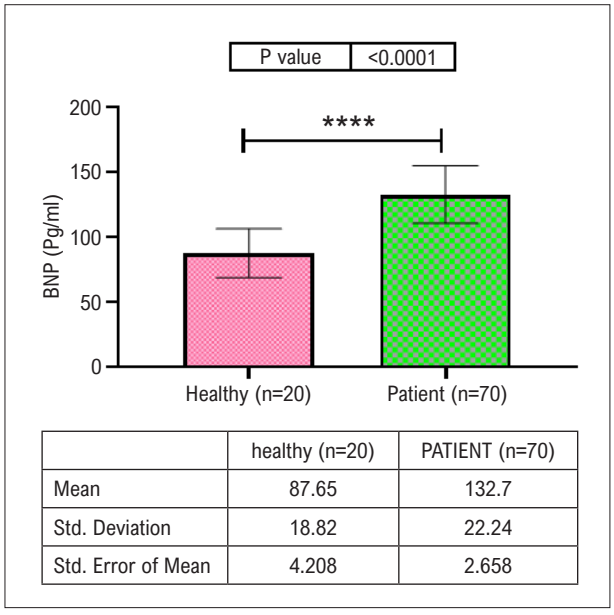


Figure 1 – *Serum DKK3 levels in healthy controls and patients with cardiac arrhythmias.*



	healthy (n=20)	PATIENT (n=70)
Mean	87.65	132.7
Std. Deviation	18.82	22.24
Std. Error of Mean	4.208	2.658

Figure 2 – *Serum BNP levels in healthy controls and patients with cardiac arrhythmias.*

arrhythmia groups, with the highest mean value observed in AF. These subgroup findings should be interpreted cautiously because the CHB subgroup included only 10 patients (Table 2 summarizes the DKK3 and BNP results by study group, as illustrated in Figures 3 and 4).

Correlation analysis

In the patient group, BNP showed weak but statistically significant positive correlations with PT ($r=0.321$, $p=0.0068$) and INR ($r=0.289$, $p=0.0153$). No significant correlations were observed between DKK3 and the evaluated clinical or laboratory variables. The correlations between BNP and total bilirubin, platelets, and MCHC did not reach the 5% significance level and should not be considered statistically significant (Table 3 summarizes the correlation analysis between DKK3 and BNP results and clinical or laboratory variables).

Exploratory ROC analysis

Exploratory ROC analysis showed high discrimination between arrhythmia patients and controls for both biomarkers. DKK3 showed an AUC of 0.954 with an optimal cutoff of 22.78 ng/mL, sensitivity of 92.9%, and specificity of 90.0%. BNP showed an AUC of 0.932 with an optimal cutoff of 120.90 pg/mL, sensitivity of 77.1%, and specificity of 100.0%. These findings should be interpreted as exploratory because the study was not prospectively designed to validate diagnostic thresholds. (The ROC analysis results are summarized in Table 4, and the ROC curves are shown in Figure 5).

Exploratory multivariable logistic regression

In separate logistic regression models adjusted for age, sex, BMI, and smoking status, both DKK3 and BNP were associated with arrhythmia status. DKK3 showed an adjusted OR of 28.27 per standard deviation increase (95% CI: 4.73-168.90; $p=0.0002$). BNP showed an adjusted OR of 26.88 per standard deviation increase (95% CI: 4.89-147.88; $p=0.0002$). In a combined model including both biomarkers together, DKK3 and BNP did not independently reach the 5% significance threshold, suggesting overlapping information and model instability due to the limited sample size. These models should therefore be interpreted as exploratory (The exploratory logistic regression models are presented in Table 5).

The main finding of this study is that serum BNP and DKK3 levels were significantly higher in patients with cardiac arrhythmias compared with healthy controls, as summarized in the Central Illustration.

Discussion

The main finding of this study is that serum BNP and DKK3 levels were significantly higher in patients with cardiac arrhythmias compared with healthy controls. This pattern was observed across several arrhythmia subtypes, although subgroup interpretation is limited by sample size and the biological heterogeneity of the included rhythm disorders. These findings support a possible association between biomarker elevation and arrhythmia status, but they do not establish causality, prediction, risk stratification, or prognosis.

BNP levels in healthy individuals are often less than 100 pg/ml, with a mean of 87.65 pg/ml for the control group, which is within the normal range. In persons with constant chronic heart failure, BNP concentrations under 100 pg/ml are considered typical. Elevated BNP indicates cardiac dysfunction or other causes and is a strong predictor of the danger of demise and heart attacks in individuals with heart disease.[5]

Table 2 – BNP and DKK3 levels according to arrhythmia subtype

Group	n	DKK3 mean ± SD (ng/mL)	DKK3 median [IQR]	BNP mean ± SD (pg/mL)	BNP median [IQR]
Control	20	19.51 ± 3.20	19.37 [17.43-20.66]	87.65 ± 18.82	89.86 [73.66-101.99]
AVNRT	20	27.44 ± 3.96	26.61 [25.18-30.83]	122.63 ± 24.72	125.21 [104.26-146.07]
SVT	20	26.50 ± 3.25	27.27 [23.68-28.44]	124.90 ± 17.54	129.34 [119.00-135.64]
AF	20	28.28 ± 2.06	28.33 [26.57-29.61]	148.02 ± 19.18	142.10 [136.41-165.03]
CHB	10	30.77 ± 1.82	30.35 [29.63-31.25]	138.10 ± 12.66	142.45 [127.19-146.24]

AVNRT: atrioventricular nodal reentry tachycardia; SVT: supraventricular tachycardia; AF: atrial fibrillation; CHB: complete heart block.

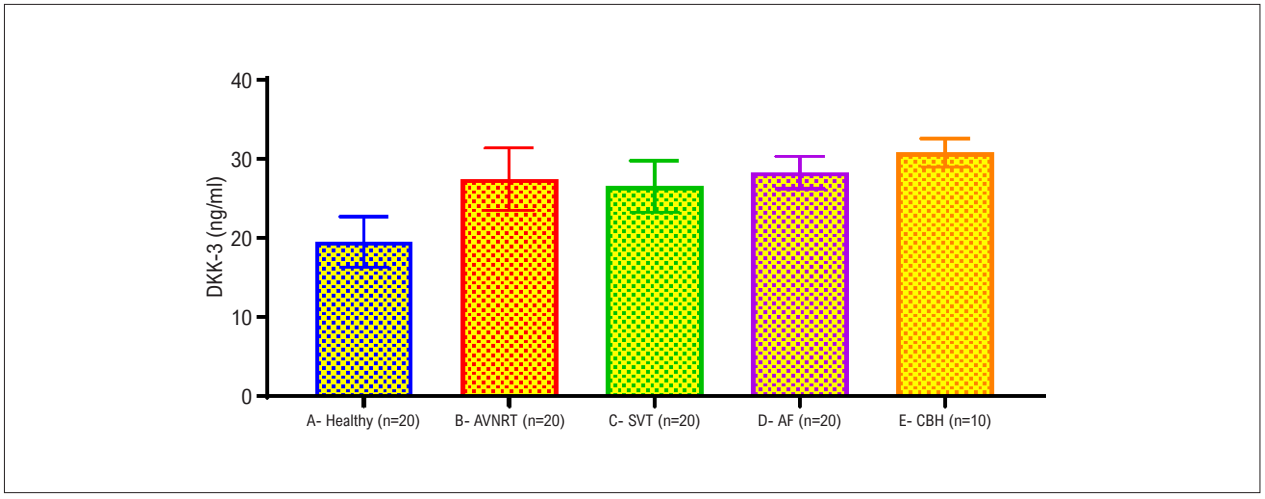


Figure 3 – *Serum DKK3 levels according to arrhythmia subtype.*

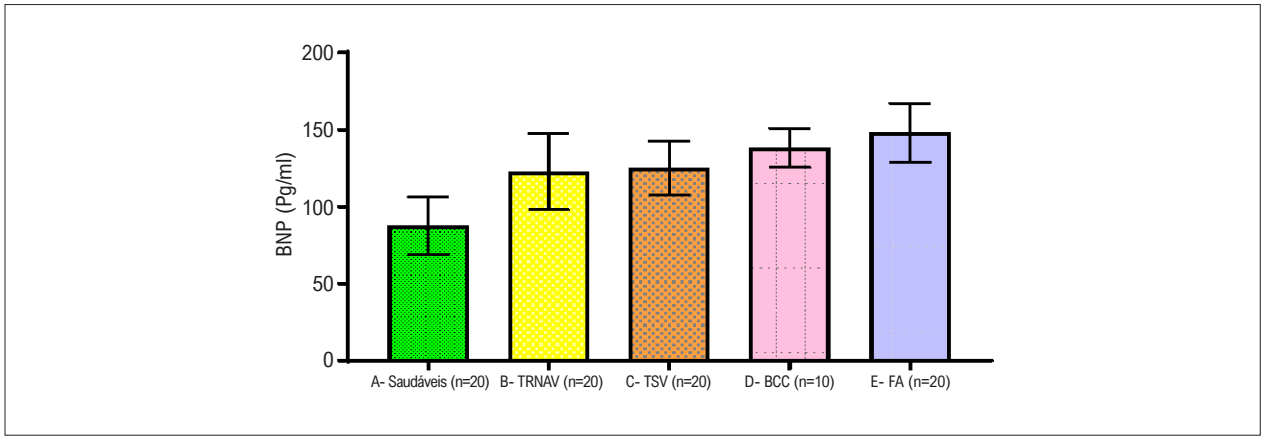


Figure 4 – *Serum BNP levels according to arrhythmia subtype.*

BNP elevation in arrhythmia patients is biologically plausible because BNP reflects myocardial wall stress and may increase in atrial dilation, ventricular dysfunction, renal impairment, older age, and concomitant cardiovascular disease.[6] In the present dataset, BNP was highest among patients with atrial fibrillation. However, because heart failure status, left ventricular ejection fraction, medication use, and detailed structural heart disease data were not available for all participants, it is not possible to determine whether BNP elevation reflects arrhythmia itself or underlying cardiac comorbidity.

Table 3 – Spearman correlation analysis between biomarkers and clinical/laboratory variables among arrhythmia patients

Variable	BNP Spearman r	BNP p-value	DKK3 Spearman r	DKK3 p-value
Age	0.057	0.6366	0.029	0.8127
Heart rate	0.087	0.4759	-0.031	0.7966
SpO_2	0.068	0.5767	0.001	0.9946
Respiratory rate	-0.012	0.9212	-0.087	0.4717
BMI	-0.071	0.5596	0.014	0.9088
PT	0.321	0.0068	0.023	0.8481
PTT	-0.107	0.3788	-0.098	0.4193
INR	0.289	0.0153	0.048	0.6916
AST	-0.113	0.3522	-0.149	0.2171
ALT	-0.073	0.5477	0.065	0.5914
Total bilirubin	0.229	0.0561	0.068	0.5774
Platelets	0.223	0.0638	0.194	0.1070
HCT	0.123	0.3104	0.137	0.2572
HB	0.070	0.5658	0.163	0.1773
WBC	0.047	0.6988	0.142	0.2409
Urea	0.166	0.1684	-0.021	0.8615
Creatinine	0.004	0.9732	-0.055	0.6507
MCV	-0.089	0.4650	-0.103	0.3974
MCH	-0.197	0.1023	-0.043	0.7231
MCHC	-0.226	0.0636	-0.051	0.6798
RBC	0.126	0.3003	0.120	0.3237

BMI: body mass index; T: Prothrombin time; PTT: Partial thromboplastin time; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; HCT: Hematocrit; HB: Hemoglobin; WBC: White blood cells; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration.

Table 4 – Exploratory ROC analysis for BNP and DKK3

Biomarker	AUC	Optimal cutoff	Sensitivity	Specificity	Youden index
DKK3	0.954	22.78 ng/mL	92.9%	90.0%	0.829
BNP	0.932	120.90 pg/mL	77.1%	100.0%	0.771

DKK3 was also higher in arrhythmia patients than in controls. DKK3 has been linked to cellular stress and cardiovascular remodeling pathways, which may be relevant to arrhythmia substrates.[7] Nevertheless, fibrosis, atrial remodeling, conduction tissue remodeling, and structural myocardial changes were not directly measured in this study. Therefore, mechanistic interpretations should remain cautious. The present findings are better interpreted as an association between circulating DKK3 and arrhythmia status rather than evidence of a direct mechanistic role.

One of the strongest pieces of evidence from the research is that the DKK3 protein has a statistically significant association with the disease. This means that elevated DKK3 levels in the context of disease enhance its value as a biomarker of chronic structural changes rather than simply an indicator of acute damage, given its involvement in cellular stress and tissue remodeling processes, reflecting profound pathological alterations.[8]

Elevated BNP concentration in patients with heart syndrome is related to an increased danger of stroke and clot formation, suggesting an indirect relationship with clotting disorders. Heart conditions, such as heart failure and high blood pressure, also result in the formation of dense, dissolvable fibrin meshes.[9]

The correlation analysis provides additional clarification. BNP showed weak but significant positive correlations with PT and INR, while DKK3 did not correlate significantly with the evaluated clinical or laboratory variables. These BNP

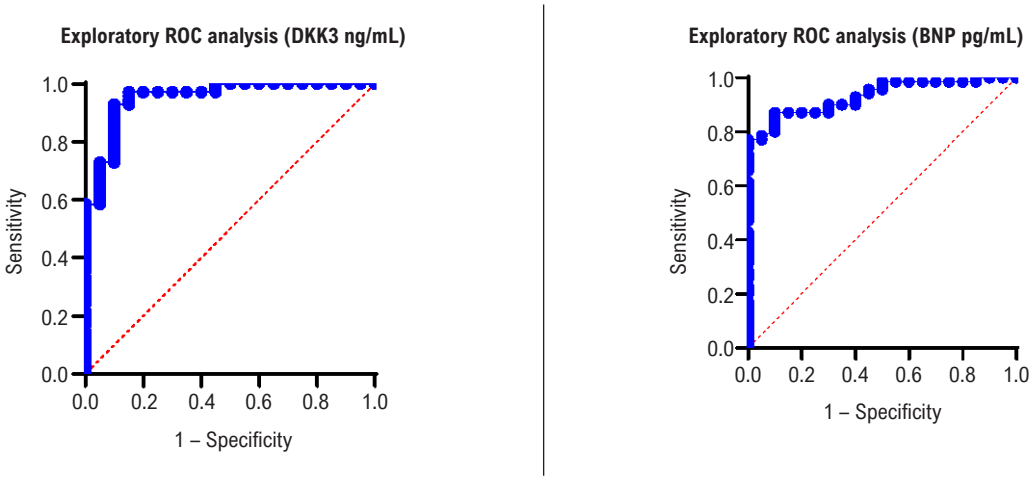


Figure 5 – *Exploratory ROC curves for BNP and DKK3 in discriminating arrhythmia patients from healthy controls.*

correlations may reflect clinical comorbidity, medication effects, or broader cardiovascular illness rather than a direct effect of BNP on coagulation. Therefore, this finding requires confirmation in larger cohorts with detailed anticoagulants and antiplatelet medication data.

The exploratory ROC analysis suggested that BNP and DKK3 can discriminate between arrhythmia patients and controls in this dataset. However, ROC results derived from a single-center case-control sample may overestimate diagnostic performance. The reported cutoffs should not be used as clinical thresholds without validation in larger prospective cohorts that include well-characterized cardiac structure, renal function, medication use, and clinical outcomes.

The logistic regression analysis was added to address confounding using available variables. In separate models adjusted for age, sex, BMI, and smoking, both biomarkers were associated with arrhythmia status. However, when BNP and DKK3 were included together, neither remained independently significant at the 5% level. This may reflect overlapping biomarker information, limited sample size, or model instability. These findings should be interpreted as hypothesis-generating.

Limitations

This study has several limitations. First, the case-control design does not allow conclusions regarding causality, prediction, prognosis, or risk stratification. Second, the sample size was limited, particularly in sex-stratified and arrhythmia subtype analyses; the CHB subgroup included only 10 patients. Third, important clinical variables such as left ventricular ejection fraction, heart failure status, structural heart disease, valvular disease, and medication use were not available for all participants; therefore, residual confounding cannot be excluded. Fourth, the study included heterogeneous arrhythmia subtypes with different pathophysiological mechanisms. Fifth, the sample was a convenience sample from a single center, which may limit generalizability. Accordingly, the findings should be considered exploratory and require validation in larger prospective studies.

Conclusion

BNP and DKK3 levels were significantly higher in patients with cardiac arrhythmias compared with healthy controls. BNP showed weak but statistically significant positive correlations with PT and INR, whereas DKK3 did not show significant correlations with the evaluated clinical or laboratory variables. These findings suggest that BNP and DKK3 may be associated with arrhythmia status; however, due to the case-control design, limited sample size, and incomplete availability of key cardiac clinical variables, the results should be interpreted as associative rather than diagnostic or prognostic. Larger prospective studies are required to determine the clinical utility of these biomarkers in patients with cardiac arrhythmia.

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Author Contributions

Conception and design of the research; Data collection; Data analysis and interpretation; Statistical analysis; Manuscript drafting and Critical revision of the manuscript for important intellectual content: Alyasiri AJ e AL-Sallami ASM.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Table 5 – Exploratory logistic regression models for arrhythmia status

Model	Variable	Adjusted OR	95% CI	p-value
Model 1: DKK3 + age + sex + BMI + smoking	DKK3 (per SD)	28.27	4.73-168.90	0.0002
Model 1: DKK3 + age + sex + BMI + smoking	Age (per SD)	2.02	0.59-6.88	0.2614
Model 1: DKK3 + age + sex + BMI + smoking	Male sex	0.12	0.01-1.65	0.1133
Model 1: DKK3 + age + sex + BMI + smoking	BMI (per SD)	1.95	0.30-12.59	0.4814
Model 1: DKK3 + age + sex + BMI + smoking	Smoking	4.28	0.32-57.17	0.2711
Model 2: BNP + age + sex + BMI + smoking	BNP (per SD)	26.88	4.89-147.88	0.0002
Model 2: BNP + age + sex + BMI + smoking	Age (per SD)	2.80	0.82-9.59	0.1012
Model 2: BNP + age + sex + BMI + smoking	Male sex	0.12	0.01-1.35	0.0859
Model 2: BNP + age + sex + BMI + smoking	BMI (per SD)	6.29	0.72-54.83	0.0963
Model 2: BNP + age + sex + BMI + smoking	Smoking	1.63	0.18-15.13	0.6667
Model 3: DKK3 + BNP + age + sex + BMI + smoking	DKK3 (per SD)	8.17	0.95-70.14	0.0555
Model 3: DKK3 + BNP + age + sex + BMI + smoking	BNP (per SD)	5.50	0.63-48.12	0.1238
Model 3: DKK3 + BNP + age + sex + BMI + smoking	Age (per SD)	2.15	0.56-8.20	0.2619
Model 3: DKK3 + BNP + age + sex + BMI + smoking	Male sex	0.07	0.00-1.41	0.0837
Model 3: DKK3 + BNP + age + sex + BMI + smoking	BMI (per SD)	2.78	0.27-28.49	0.3899
Model 3: DKK3 + BNP + age + sex + BMI + smoking	Smoking	2.86	0.23-34.98	0.4108

BMI: body mass index; BNP: type B natriuretic peptide; DKK3: Dikkopf-3.

Sources of Funding

There were no external funding sources for this study.

Study Association

This article is part of the thesis of Doctoral submitted by Ali Jawad Alyasiri, from University of Kufa.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Najaf Center for Cardiac Surgery and Catheterization under the protocol number 7728. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

Use of Artificial Intelligence

During the preparation of this work, the author(s) used ChatGPT (OpenAI) to improve the language, grammar, and clarity of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the published article.

Availability of Research Data

All datasets supporting the results of this study are available upon request from the corresponding author.

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