Association Between Apelin and Atrial Fibrillation in Patients With High Risk of Ischemic Stroke

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Background: Atrial fibrillation (AF) is associated with high risk of stroke preventable by timely initiation of anticoagulation. Currently available screening tools based on ECG are not optimal due to inconvenience and high costs. Aim of this study was to study the diagnostic value of apelin for AF in patients with high risk of stroke.

Methods: We designed a multicenter, matched-cohort study. The population consisted of three study groups: a healthy control group (34 patients) and two matched groups of 60 patients with high risk of stroke (AF and non-AF group). Apelin levels were examined from peripheral blood.

Results: Apelin was significantly lower in AF group compared to non-AF group (*0.694 ± 0.148* vs. *0.975 ± 0.458* ng/ml, *p* = 0.001) and control group (*0.982 ± 0.060* ng/ml, *p* < 0.001), respectively. Receiver operating characteristic (ROC) analysis of apelin as a predictor of AF scored area under the curve (AUC) of *0.658*. Apelin's concentration of *0.969 [ng/ml]* had sensitivity = *0.966* and specificity = *0.467*. Logistic regression based on manual feature selection showed that only apelin and NT-proBNP were independent predictors of AF. Logistic regression based on selection from bivariate analysis showed that only apelin was an independent predictor of AF. A logistic regression model using repeated stratified K-Fold cross-validation strategy scored an AUC of 0.725 ± 0.131.

Conclusions: Our results suggest that apelin might be used to rule out AF in patients with high risk of stroke.

Keywords: atrial fibrillation, apelin, biomarker, electrical atrial remodeling, ischemic stroke

INTRODUCTION

Atrial fibrillation (AF) is associated with high mortality, morbidity, and significant health care costs (1, 2). Despite substantial progress in cardiovascular prevention, constantly increasing incidence and prevalence of AF have reached dimensions of cardiovascular epidemic (3–5). As an independent factor, AF increases the risk of ischemic stroke 5-fold, as well as significantly contributes to the risk of heart failure and death (1, 6). Adequate anticoagulation therapy protects patients from these adverse events, but timely and accurate diagnosis remains a basic precondition (1, 7–11).

Currently available AF diagnostic tools are not sufficient. Standard 12-lead ECG is unreliable because of its low detection rate, especially in the setting of asymptomatic AF, and prolonged ECG monitoring is often impractical due to its high cost and inconvenience. Moreover, it is not always available due to high demand (12–14). There is an increasing need for a new, simple, cost-effective and accurate diagnostic tool, such as a biomarker detectable in peripheral blood.

Our knowledge of AF pathogenesis has evolved and emerging evidence strongly links AF with inflammation, oxidative stress and atrial fibrosis (15–20). Several plasmatic biomarkers for AF have been studied (21–25) and apelin, an endogenous regulatory peptide associated with many physiological and pathophysiological processes (26), has shown promising results (27–29). Among other effects on cardiovascular system, apelin shortens action potential duration in atrial myocytes via its effects on multiple ionic channels. It also affects the renin-angiotensin-aldosterone signaling pathway, acts as a second catalytic substrate for angiotensin-converting enzyme 2 (ACE2) and functions as an inotrope, all of which are processes directly or indirectly associated with AF (30, 31).

In our previous research that included only patients with low risk of stroke, we showed that apelin is significantly decreased in patients with AF compared to patients without AF (27, 28). Whether this result also applies to patients with cardiovascular comorbidities and high risk of stroke is unknown.

Our study sought to further investigate the relationship between apelin and atrial fibrillation and to determine apelin's predictive value for AF in patients with high risk of stroke.

MATERIALS AND METHODS
Study Population

We designed a multicenter, matched-cohort study. Four Slovak hospitals in Bratislava, Malacky, Nitra and Kosice were included. The population consisted of three study groups: A healthy control group consisting of 34 patients without AF (control group) and two matched groups of 60 patients with high risk of stroke: one with atrial fibrillation (AF group) and the other without atrial fibrillation (non-AF group). The healthy control group consisted of random blood donors. Atrial fibrillation was excluded in both control and non-AF group based on the history and 12-lead ECG at the time of enrollment. The inclusion criteria for the AF group were: Age > 17 years, documented, non-valvular paroxysmal AF in the duration of more than 30 s (ECG

documented), CHA2DS2-VASc score > 2 for males, CHA2DS2-VASc score > 3 for females and sinus rhythm at the time of inclusion. The inclusion criteria for the non-AF group were: Age > 17 years, CHA2DS2-VASc score > 2 for males, CHA2DS2-VASc score > 3 for females, sinus rhythm at the time of inclusion, no history of palpitations and 30 s AF exclusion using a continuous 7-day ECG Holter and additional 30-day ECG event recorder monitoring three times a day or when the patient felt unwell. Continuous 7-day ECG monitoring was performed using a QardioCore® device and 30-day ECG event recording was performed using a Hartmann Veroval®. The AF group and non-AF group were matched according to these parameters: age, gender, CHA2DS2-VASc parameters, left ventricular ejection fraction (LVEF): reduced (<40%), mid-range (40–49%) and preserved (≥50%), presence of diastolic dysfunction, glomerular filtration rate: (≥1.5 ml/s), (1.4–1 ml/s) and (0.9–0.5 ml/s), drugs (angiotensin-converting enzyme inhibitors and an angiotensin receptor blockers, betablockers, digoxin, amiodarone), body mass index (BMI): (<30 kg/m^2), (30–39 kg/m^2), and (≥40 kg/m^2) and smoking (>5 cigarettes per day). Exclusion criteria for both groups were: electrical cardioversion <7 days prior to inclusion, acute coronary syndrome <1 month prior to inclusion, cardiac surgery <3 months prior to inclusion, acute or decompensated heart failure at the time of inclusion, pregnancy, cardiomyopathy, alcoholism (≥8 drinks/week), thyrotoxicosis, renal disease (dialysis/transplant/CrCl < 0.5 ml/s), liver disease (cirrhosis/transaminase > 3x ULN/bilirubin > 2x ULN), mechanical prosthetic valve, severe mitral stenosis, class I and IV antiarrhythmic drugs usage in the last month, class III antiarrhythmic drugs usage in the last 3 months.

The study was approved by the Ethics Committee of the National Cardiovascular Institute, Bratislava, Slovakia and a written informed consent was obtained from all patients and donors in the control group.

Data Collection and Biochemical Analysis

In AF and non-AF groups, baseline clinical data were obtained during ambulatory visits or during a hospitalization and were recorded into an electronic online case report form. Peripheral fasting blood was taken in the morning using K3EDTA tubes. In the control group, baseline clinical data and fasting blood samples were collected at the time of blood donation. The blood was centrifuged at 2,700 g for 5 min and the obtained plasma samples were stored at −80°C. The apelin-12 concentration was measured using a commercially available ELISA kit (Phoenix Pharmaceutical, Karlsruhe, Germany) in plasma samples. Fifty microliters of plasma samples were used for measurement according to the manufacturer's protocol.

Statistical Methods

Continuous variables are presented as sample means and standard deviations. Normality of data was tested using a Shapiro–Wilk test and inspected on Q-Q plots, with homoscedasticity assessed using Levene's test. Classic or Welch ANOVA was employed to analyze the between group differences based on equality of variances, followed by post-hoc tests (Tukey-HSD or Games-Howell, respectively) in

order to study pairwise differences between groups. Between group differences for categorical variables were estimated using the χ^2 test of independence with $\lambda = -2$ (Neyman test). All correlations were computed using Spearman's correlation coefficient in order to suppress the effect of tentative outliers. All logistic regression models were fitted either in *sklearn* (with *Elastic-Net* regularization with equal L1 and L2 ratios, and *saga* solver) or *statsmodels* (with the iteratively reweighted least squares method) python libraries, and all receiver operating characteristic (ROC) curves and area under the curve (AUC) statistics were computed using the *sklearn* python library. Before entering the logistic regression, all data were scaled using the standard scaler (to

zero mean and unit variance). *P*-values < 0.05 were considered statistically significant.

Based upon our previous research, the expected mean difference in apelin concentration was 0.15 ng/ml with a standard deviation of 0.14. Assuming an alpha of 0.05 and 90% power, the minimum sample size was 24 patients in each matched group.

Data were analyzed using Python version 3.7.9 (https://www.python.org/) with appropriate libraries (for statistical analyses *pingouin* package version 0.3.8: https://pingouin-stats.org/, for

TABLE 1 | Baseline demographics of the study population.

	Non-AF group (*n* = 30)	AF group (*n* = 30)	*p*-value
Age (years)	71.83 ± 8.00	73.63 ± 7.40	0.378
Male gender (%)	19 (63.3%)	19 (63.3%)	> 0.999
Weight (kg)	83.93 ± 12.20	82.63 ± 15.89	0.728
Height (cm)	170.57 ± 9.04	171.10 ± 9.68	0.829
BMI (kg/m^2)	28.86 ± 3.53	28.10 ± 4.09	0.45
Smoking (>5 cigarettes per day) (%)	2 (6.7%)	1 (3.3%)	> 0.999
Systolic blood pressure (mmHg)	134.13 ± 12.48	131.37 ± 9.77	0.366
Diastolic blood pressure (mmHg)	79.13 ± 7.23	75.70 ± 8.36	0.214

Data in the table are presented as mean ± standard deviation or n (%).
AF, atrial fibrillation; BMI, body mass index.

TABLE 2A | Patient characteristics: echocardiography and laboratory parameters.

	Non-AF group (*n* = 30)	AF group (*n* = 30)	*p*-value
Echocardiography			
Left ventricular end-diastolic diameter (mm)	48.73 ± 4.73	48.80 ± 5.34	0.96
Diameter of left atrium in PLAX (Parasternal long axis) (mm)	42.87 ± 5.16	43.00 ± 5.12	0.922
Diastolic dysfunction	0.93 ± 0.73	1.17 ± 0.73	0.295
Left ventricular hypertrophy (%)	13 (43.3%)	14 (46.7%)	> 0.999
Laboratory parameters			
D-dimer (ug/ml)	314.22 ± 391.99	308.33 ± 443.38	> 0.999
Fibrinogen (g/l)	3.71 ± 1.24	3.56 ± 0.58	0.605
CRP (mg/l)	7.62 ± 25.83	4.94 ± 5.31	0.012
NT-proBNP (ng/l)	286.84 ± 297.27	664.82 ± 773.48	0.026
Hs-troponin (ng/l)	11.55 ± 6.77	36.02 ± 96.97	0.071
Apelin (ng/ml)	0.98 ± 0.45	0.69 ± 0.15	0.032
Creatinine (umol/l)	82.00 ± 15.80	85.42 ± 16.53	0.425
Creatinine clearance (ml/s)	1.25 ± 0.21	1.20 ± 0.22	0.376

Data in the table are presented as mean ± standard deviation or n (%).
AF, atrial fibrillation; CRP, C-reactive protein; Hs-troponin, High-sensitivity troponin; NT-proBNP, N-terminal fragment of brain natriuretic peptide.

TABLE 2B | Patient characteristics: medical history and medication.

	Non-AF group (*n* = 30)	AF group (*n* = 30)	*p*-value
Medical history			
AF burden (months)	0	29.85 ± 28.43	N/A
Ischemic stroke/TIA	1.14 ± 0.35	1.25 ± 0.43	0.677
STEMI	1.20 ± 0.40	1.00 ± 0.00	0.606
NSTEMI	1.33 ± 0.47	1.33 ± 0.47	0.792
Ventricular tachycardia/ventricular fibrillation (%)	1 (3.3%)	2 (6.7%)	> 0.999
Arterial hypertension (%)	29 (96.7%)	29 (96.7%)	> 0.999
Pulmonary embolism (%)	0 (0.0%)	1 (3.3%)	> 0.999
Deep vein thrombosis (%)	0 (0.0%)	3 (10.0%)	0.116
Peripheral arterial disease/aortic plaque (%)	10 (33.3%)	13 (43.3%)	0.594
Left ventricular hypertrophy (%)	13 (43.3%)	14 (46.7%)	> 0.999
Stable coronary artery disease (%)	8 (26.7%)	4 (13.3%)	0.32
Chronic obstructive pulmonary disease (COPD) (%)	1 (3.3%)	5 (16.7%)	0.141
Obstructive sleep apnea (OSA) (%)	1 (3.3%)	0 (0.0%)	> 0.999
Severe valvulopathy (%)	0 (0.0%)	0 (0.0%)	> 0.999
Electrical cardioversion (%)	0 (0.0%)	2 (6.7%)	0.408
Pharmacological cardioversion (%)	0 (0.0%)	3 (10.0%)	0.116
CHADS2-VASc	3.7	3.7	N/A
Medication			
ACE- inhibitor/ARB (%)	28 (93.3%)	26 (86.7%)	0.663
Spironolactone/Eplerenone (%)	1 (3.3%)	1 (3.3%)	> 0.999
Beta-blocker (%)	26 (86.7%)	26 (86.7%)	> 0.999
Digoxin (%)	1 (3.3%)	1 (3.3%)	> 0.999
Proton pump inhibitors (%)	6 (20.0%)	13 (43.3%)	0.083
Antidepressants/Antipsychotics (%)	0 (0.0%)	2 (6.7%)	0.408
Acetylsalicylic acid (%)	16 (53.3%)	8 (26.7%)	0.056
Clopidogrel (%)	7 (23.3%)	3 (10.0%)	0.279
Prasugrel (%)	1 (3.3%)	0 (0.0%)	> 0.999
Ticagrelor (%)	0 (0.0%)	0 (0.0%)	> 0.999
Warfarin (%)	1 (3.3%)	9 (30.0%)	0.001
Dabigatran etexilat (%)	0 (0.0%)	4 (13.3%)	0.021
Rivaroxaban (%)	0 (0.0%)	0 (0.0%)	> 0.999
Apixaban (%)	0 (0.0%)	8 (26.7%)	0.001
Edoxaban (%)	0 (0.0%)	1 (3.3%)	> 0.999

Data in the table are presented as mean ± standard deviation or n (%).
ACE, Angiotensin-converting enzyme; ARB, angiotensin receptor blocker; NSTEMI, Non-ST-elevation myocardial infarction; STEMI, ST-Elevation myocardial infarction; TIA, transient ischemic attack.

regression models and their statistics *statsmodels* package version 0.12.1: https://www.statsmodels.org/, *scikit-learn* package version 0.23.2: https://scikit-learn.org/, and *RStudio* 1.2.5033 (32) which was also used for sample size calculation.

RESULTS

Baseline Characteristics

A total of 94 patients were enrolled in the study: 30 in the AF group, 30 in the non-AF group and 34 in the healthy control group. Patient characteristics are presented in **Tables 1**, **2A–C**. There were statistically significant differences between the AF and non-AF groups in CRP levels [4.94 ± 5.31 vs. 7.62 ± 25.83 (mg/l), respectively, $p = 0.012$], NT-proBNP levels (664.82 ± 773.48 vs. 286.84 ± 297.27, respectively, $p = 0.026$), Apelin levels (0.69 ± 0.15 vs. 0.98 ± 0.45, respectively, $p = 0.032$) and antithrombotic therapy (see **Tables 1**, **2A,B**). Patients in the control group were significantly younger than patients in the AF and non-AF groups.

The analysis of variance test (ANOVA) for all three groups showed a significant group effect on apelin concentrations with $F_{(2, 90)} = 10.67$, $p < 0.001$, $\eta_p^2 = 0.192$ with statistical power *0.994*

given our number of participants. Subsequent analysis showed significant difference in apelin concentration between healthy controls and patients with AF (0.982 ± 0.060 vs. 0.694 ± 0.148 ng/ml, $p = 0.001$, $d = 1.044$) as well as between patients with and without AF (0.694 ± 0.148 vs. 0.975 ± 0.458 ng/ml, $p = 0.001$, $d = -1.021$), respectively. The difference between healthy controls and patients without AF was not significant (0.982 ± 0.060 vs. 0.975 ± 0.458 ng/ml, $p = 0.900$, $d = 0.023$) (**Figure 1**).
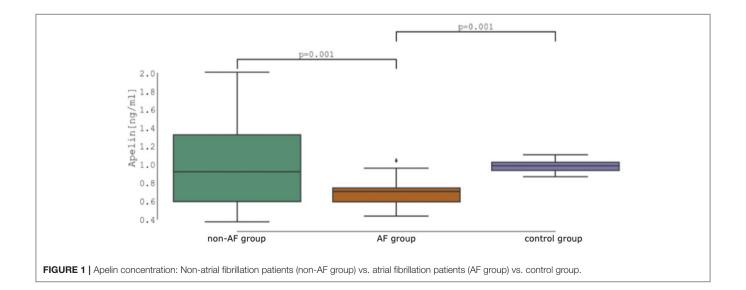
There was no significant correlation between apelin concentration and diastolic dysfunction [*Spearman's* $r = -0.126$, *CI 95% (-0.37, 0.13)*, $p = 0.341$], left atrium diameter in parasternal short axis [mm] [*Spearman's* $r = -0.097$, *CI 95% (-0.34, 0.16)*, $p = 0.466$], and NT-proBNP [ng/l] [*Spearman's* $r = -0.147$, *CI 95% (-0.39, 0.11)*, $p = 0.267$] (**Figure 2**).

ROC analysis of apelin as a predictor of AF scored AUC = 0.658. T = 0.658. The ideal threshold of *apelin concentration was 0.969 [ng/ml] with* accuracy of *0.712*, sensitivity of *0.966*, and specificity of *0.467*, respectively (**Figure 3**).

Finally, we built a logistic regression model for classifying AF using multiple predictors, including apelin. We compared two approaches to this problem, with the first being the manual feature selection based on known predictors of AF from available literature. We selected 16 predictors from our gathered data and fitted a logistic regression model using our patients' data. The model trained on all data scored *AUC = 0.875* (**Figure 4**).

The full list of predictors with their coefficients and *p*-values can be seen in **Table 3A**. Only two predictors were statistically significant with *p*-values lower than 0.05: *apelin*, and *NT-proBNP*.

The second, data-driven route was to compute bivariate analysis (significant differences in our dataset between AF and no AF patients) and include all predictors, whose differences between groups had *p*-value lower than 0.1 (based on *t*-test, Mann-Whitney *U*-test, or χ^2 test where appropriate). Differences in medication were not included in this analysis because they directly depend on the presence of AF. This landed us with four predictors (of course, including apelin) and the final model scored *AUC = 0.825* (**Figure 5**). In this model, only apelin scored

TABLE 2C | Patient characteristics: atrial fibrillation patients vs. non-atrial fibrillation patients vs. control group.

Characteristics	Non-AF group (n = 30)	AF group (n = 30)	Control group (n = 34)	p-value
Age (years)	71.83 ± 8.00	73.63 ± 7.40	41.03 ± 9.34	< 0.001
Apelin (ng/ml)	0.98 ± 0.45	0.69 ± 0.15	0.98 ± 0.06	= 0.001
Male gender (%)	19 (63.3%)	19 (63.3%)	13 (38.2%)	0.0544

Data in the table are presented as mean ± standard deviation or n (%).
AF, atrial fibrillation.

FIGURE 1 | Apelin concentration: Non-atrial fibrillation patients (non-AF group) vs. atrial fibrillation patients (AF group) vs. control group.
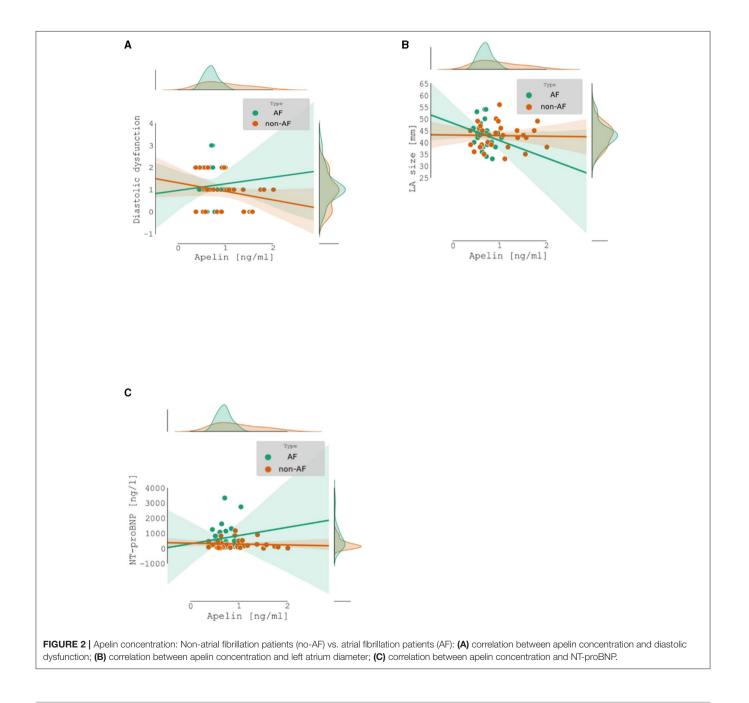
p-value lower than preselected threshold of 0.05. The full list of predictors with their coefficients and *p*-values can be seen in **Table 3B**.
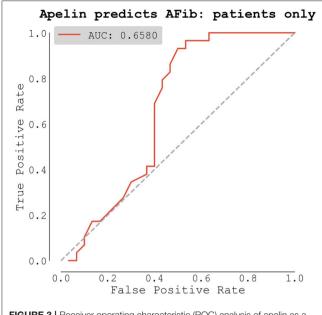
To assess the true model performance, we selected predictors from our bivariate analysis with *p*-value < 0.1, and repeatedly trained logistic regression model using repeated stratified K-Fold cross-validation strategy. The receiver operating characteristic (ROC) was computed only from testing dataset. Our final model scored *AUC = 0.725 ± 0.131, with improved sensitivity: 0.851 ± 0.209 and specificity: 0.685 ± 0.250*. Full ROC curve showed as mean ± one standard deviation can be seen in **Figure 6**.
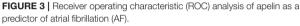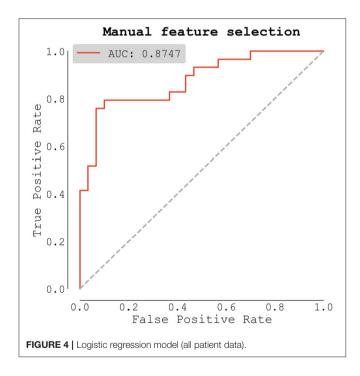
DISCUSSION

Our study demonstrated that in matched cohorts of patients with cardiovascular comorbidities and high risk of stroke, the cohort with AF had significantly lower concentration of apelin compared to the cohort without AF. Similar, there was a statistically significant difference in apelin concentration between patients with AF and the healthy control group.

Further analysis of known AF contributors (1, 33) in our dataset demonstrated that only apelin and NT-proBNP were independent predictors of AF. Increased levels of NT-proBNP in patients suffering from AF have been observed in several studies and their association is well-established (34–36). There

FIGURE 2 | Apelin concentration: Non-atrial fibrillation patients (no-AF) vs. atrial fibrillation patients (AF): **(A)** correlation between apelin concentration and diastolic dysfunction; **(B)** correlation between apelin concentration and left atrium diameter; **(C)** correlation between apelin concentration and NT-proBNP.

FIGURE 3 | Receiver operating characteristic (ROC) analysis of apelin as a predictor of atrial fibrillation (AF).

FIGURE 4 | Logistic regression model (all patient data).

are several unmeasured factors such as amount of exercise (37, 38) or dietary intake (39–41) which may alter apelin plasmatic levels. These changes may be pronounced between patient and healthy control group, however, should not be significant between matched cohorts. Additionally, it is not possible to completely eliminate the potential influence of medication on plasmatic levels of apelin. However, there was no statistically significant difference between plasmatic levels of apelin when

TABLE 3A | Logistic regression model for AF predictors.

Predictor	Coef	(95% CI)	p-value
(Intercept)	−2.875	(−5.958 to 0.209)	0.068
Signs of heart failure (%)	−0.606	(−2.856 to 1.645)	0.598
Diastolic dysfunction (Grade)	1.512	(−0.622 to 3.647)	0.165
Chronic obstructive pulmonary disease (COPD) (%)	3.745	(0.005 to 7.485)	0.05
Vascular disease (%)	−0.175	(−2.005 to 1.656)	0.852
Gender (%)	1.64	(−0.495 to 3.775)	0.132
Diabetes Mellitus (%)	1.252	(−1.073 to 3.577)	0.291
D-Dimer (ug/ml)	−0.325	(−1.19 to 0.541)	0.462
Systolic blood pressure (mmHg)	0.263	(−0.701 to 1.228)	0.593
Age (years)	−0.47	(−1.398 to 0.457)	0.320
NT-proBNP (ng/l)	1.823	(0.251 to 3.396)	0.023
Diastolic blood pressure (mmHg)	−0.45	(−1.447 to 0.548)	0.377
BMI (kg/m^2)	0.047	(−0.916 to 1.011)	0.923
Apelin (ng/ml)	−1.936	(−3.551 to −0.320)	0.019
CRP (mg/l)	−0.222	(−1.068 to 0.624)	0.607
Creatinine (umol/l)	0.464	(−0.605 to 1.534)	0.395
Diameter of left atrium in PLAX (Parasternal long axis) (mm)	−0.645	(−1.81 to 0.520)	0.278

Data in the table are presented as mean ± standard deviation or n (%).
AF, atrial fibrillation; BMI, body mass index; CRP, C-reactive protein; NT-proBNP, N-terminal fragment of brain natriuretic peptide.

TABLE 3B | Logistic regression model for AF predictors based on selection from bivariate analysis (predictors with $p < 0.1$).

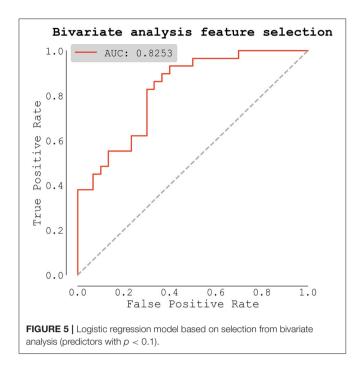| Predictor	Coef	(95% CI)	p-value
(Intercept)	0.409	(−0.653 to 1.471)	0.450
Apelin (ng/ml)	−1.019	(−1.915 to −0.123)	0.026
Hs-troponin (ng/l)	3.907	(−1.512 to 9.327)	0.158
NT-proBNP (ng/l)	0.777	(−0.142 to 1.696)	0.097
CRP (mg/l)	−0.321	(−1.061 to 0.418)	0.395

Data in the table are presented as mean ± standard deviation or n (%).
AF, atrial fibrillation; CRP, C-reactive protein; Hs-troponin, High-sensitivity troponin; NT-proBNP, N-terminal fragment of brain natriuretic peptide.

comparing high risk patients in non-AF group and healthy donors receiving no chronic medication.

Based on ROC analysis, apelin was able to predict AF with an AUC of 66%. By setting the apelin level threshold to 0.969 [ng/ml] with the aim of maximizing sensitivity we demonstrated a classification accuracy of 0.712, sensitivity of 0.966, and specificity of 0.467.

Our previous research confirmed that apelin has high sensitivity and specificity to predict and quantify AF in patients with minimal cardiovascular comorbidities and low risk of stroke (27, 28). However, this result is not sufficient for use in clinical practice where there would be more complex cases and it would not be known whether apelin would be able to provide sufficient diagnostic power in patients with multiple comorbidities who
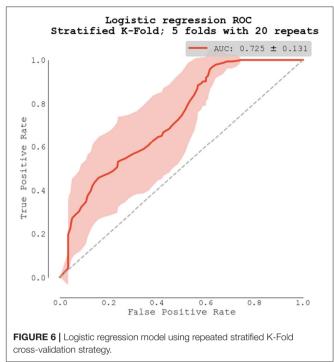
FIGURE 5 | Logistic regression model based on selection from bivariate analysis (predictors with *p* < 0.1).

FIGURE 6 | Logistic regression model using repeated stratified K-Fold cross-validation strategy.

are at high risk of stroke. Furthermore, young patients with a low risk profile and lone AF *(reflected in low CHA2DS2-VASc score)* do not meet existing criteria for anticoagulation treatment. To address these questions, we designed a study where apelin was studied in a high-risk cohort of patients with AF and multiple cardiovascular comorbidities. Patients with persistent/permanent AF were not included in this study because we wanted to study if apelin is reduced in the setting of paroxysmal AF and therefore if it potentially can be used for AF detection (e.g., in patients after cryptogenic stroke with no symptoms of arrhythmia).

We were able to confirm good sensitivity, however specificity for AF was low. The APJ receptor for apelin is detectable in many central and peripheral tissues (42, 43) and compelling evidence demonstrates that this complex is involved in a large number of physiological and pathophysiological processes (44, 45). Specificity for AF is therefore limited in patients with comorbidities. This situation could be overcome by including variables besides apelin in a classification model for AF detection.

The Stratified K-Fold cross-validation strategy was performed to ascertain the performance of logistic regression models and to compare this result with the performance of apelin alone. The overall predictive value increased from 66 to 73% *with improved sensitivity: 0.851 ± 0.209 and specificity: 0.685 ± 0.250.* These results suggest a potential improvement in the predictive value of apelin when incorporate into a multi-factor scoring system. The potential benefit of multi-factorial biomarker-based prediction models has already been described in several studies (36, 46, 47).

Optimal patient selection could improve the predictive value of apelin or apelin-based scoring systems for AF. For example, in the case of heart failure with decreased LV ejection fraction, some

studies have reported decreased, unaltered or even increased plasma levels compared to control subjects (48, 49). Therefore, patients with reduced LVEF were excluded from our study. On the other hand, patients with heart failure with preserved ejection fraction (HFpEF) were included in our study and they did not show any association with apelin levels. Our observations suggest that apelin, despite its low specificity in the presence of several comorbidities indicating high risk of stroke, could still be used to rule out AF due to its high sensitivity. This, however, should be validated further in a larger cohort of patients.

We also hypothesized about the potential cause of apelin reduction in AF. Previous studies including our findings (28, 50) showed that increased stretch might play a pathophysiological role in decreased apelin concentration. However, in the present study, apelin showed no statistically significant correlation with left atrium (LA) size, NT-proBNP and diastolic dysfunction, all of which are known and verified risk factors for AF development and continuation, and reflect elevated pressure and volume in the atrium. In the context of our present findings, we hypothesize that these previously reported correlations were not causal and that apelin more likely reflects electrical remodeling rather than structural remodeling. This theory also corresponds with experimental findings showing that apelin increases atrial conduction velocity, refractoriness, shortens action potential, affects multiple ionic currents and prevents the inducibility of atrial fibrillation (51, 52).

We believe that our results encourage further research of apelin as a biomarker that might be used to rule out atrial fibrillation.

Study Limitations

Our study had several limitations. The predictive value of apelin with multiple risk factors model was not validated on an independent cohort. However, the cross-validation strategy using repeated K-Fold was used to substitute the independent cohort validation. Secondly, although our inclusion criteria were relatively broad, there are still many unmeasured factors that could alter apelin plasmatic levels. Thirdly, apelin levels may change during the natural history of atrial fibrillation and our study did not follow changes of apelin levels over time. Lastly, a matched-cohort design cannot assess a causal relationship between apelin and AF. Thus, while our results are provocative, they need to be confirmed in future studies.

CONCLUSION

Our results showed that low level of apelin has good sensitivity for atrial fibrillation even in the setting of multiple cardiovascular comorbidities that increase the risk of ischemic stroke. Additional research is needed to verify whether apelin could be used in clinical practice to rule out atrial fibrillation and to improve AF screening in patients with increased risk of ischemic stroke.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the National Cardiovascular Institute, Bratislava, Slovakia. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AB conceived the ideas and designed the study. AB, PS, MV, MK, and TU conducted the study. AB, BB, and NJ analyzed the data. LT performed biochemical analyses. AB, BB, NJ, TH, and KP wrote the manuscript. SF, JK, VM, VK, PM, KD, and PO provided supervision. All the authors have read and approved the final version for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.742601/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.