The intracardiac concentrations of the N-terminal-pro B-type natriuretic peptide (NT-proBNP) and the determinants of its secretion in patients with atrial fibrillation

Witold Streb, Katarzyna Mitręga, Magdalena Szymała, Aleksandra Woźniak, Tomasz Podolecki, Zbigniew Kalarus

Department of Cardiology, Congenital Heart Diseases, and Electrotherapy, Medical University of Silesia, Silesian Centre for Heart Diseases, Zabrze, Poland

Abstract

Background: N-terminal-pro B-type natriuretic peptide (NT-proBNP) is elevated not only in heart failure (HF) but also in atrial fibrillation (AF). The role and secretion pattern of NT-proBNP in AF is still undetermined.

Aim: The study aimed to assess NT-proBNP concentrations in patients with and without preserved left ventricular ejection fraction (LVEF) depending on the type of AF. It was also intended to define the main source of NT-proBNP production within the heart. In addition, it aimed to study the relation of NT-proBNP with some echocardiographic parameters reflecting the stretch of heart chambers as well as with the chosen parameters of physical capacity.

Methods: Blood samples were collected from the right atrium (RA), left atrium (LA), and femoral artery (FA) in 53 patients referred for occlusion of the LA appendage. Thirty patients were assigned into Group I (LVEF ≥ 50%, no HF symptoms) and the remaining 23 patients to Group II (LVEF < 50%, HF symptoms). NT-proBNP concentrations were determined using the ELISA test.

Results: In Group I, the lowest NT-proBNP level was found in RA (460.47 ± 723.15 pg/mL and 1097.72 ± 851.42 pg/mL for paroxysmal and permanent AF, respectively), higher in LA (481.5 ± 724.56 pg/mL and 1188.06 ± 851.42 pg/mL for paroxysmal and permanent AF), and the highest values in FA (537.77 ± 808.49 pg/mL and 1188.04 ± 798.28 pg/mL for paroxysmal and permanent AF). In Group II the NT-proBNP values were significantly higher compared to Group I (p < 0.01), but similarly values in RA were the lowest (183.47 ± 1826.08 pg/mL and 2141.68 ± 1801.69 pg/mL for paroxysmal and permanent AF), intermediate values were observed in LA (1857.57 ± 2221.39 pg/mL and 2386.81 ± 2067.2 pg/mL for paroxysmal and permanent AF), and the highest were seen in FA (1936.27 ± 2149.85 and 2437.33 ± 1999.37 pg/mL for paroxysmal and permanent AF, respectively). In Group I, the NT-proBNP from LA best correlated with LA area (r = 0.56) and RA area (r = 0.56). In Group II, the strongest correlations were found between NT-proBNP from LA and left ventricular end-systolic dimension (r = 0.57) and volume (r = 0.6).

Conclusions: NT-proBNP is markedly elevated in the majority of patients with AF even in the absence of HF. LA secretion of NT-proBNP is an important contributor to the overall increase of NT-proBNP also in HF patients. In AF patients, the concentration of NT-proBNP correlates with the remodelling of heart chambers, but not with physical capacity.

Key words: NT-proBNP, atrial fibrillation, heart failure
INTRODUCTION
The role and importance of B-type natriuretic peptide (BNP) and N-terminal-pro B-type natriuretic peptide (NT-proBNP) in heart failure (HF) are well known [1, 2]. These biomarkers help to diagnose and manage patients with HF, but their role in atrial fibrillation (AF) is still not established. In several studies, it was shown that the mean level of NT-proBNP in AF ranged between 800 pg/mL and 1100 pg/mL [3].

NT-proBNP is released in response to volume overload of heart chambers and subsequent stretch of cardiac myocytes. Wall stress induces rapid gene transcription in cardiac myocytes and fibroblasts that form the ventricles and atria [4–6].

Similarly to other natriuretic peptides, NT-proBNP is produced from a pro-precursor molecule, which can be processed in different ways to obtain various peptides. One of these processes results in simultaneous production of the following peptides: C-terminal fragment (which is active) and N-terminal chain (which is inactive). Both fragments are secreted in equimolar amounts and are known, respectively, as BNP and NT-proBNP [7].

Various data have been published regarding the main source of NT-proBNP. Some authors have claimed that over 60% of natriuretic peptides are secreted in the ventricles, whereas others have found their higher concentration in the atria. It is also postulated that the main source of secretion depends on the underlying aetiology of a particular cardiac disease [8].

The current study aimed to assess NT-proBNP concentrations in patients with and without preserved left ventricular ejection fraction (LVEF) depending on the type of concomitant AF. It was also intended to define the main source of the peptide within the heart that particularly contributes to the overall increase in the plasma concentration of NT-proBNP in AF patients. In addition, we studied the relation of the NT-proBNP concentration with some echocardiographic parameters reflecting the stretch of heart chambers as well as with the chosen parameters of physical capacity.

METHODS

Study population
The study population consisted of 53 consecutive patients with permanent or paroxysmal AF, who were referred for percutaneous left atrial appendage (LAA) closure. All patients had either a high risk of both stroke (CHA\textsubscript{DS}\textsubscript{2},VASC score ≥ 2) and bleeding (HAS-BLED score > 3) or lower HAS-BLED score but at least one contraindication for oral anticoagulation (e.g. severe thrombocytopenia, recurrent bleeding, fragility syndrome) or recurrent stroke despite adequate oral anticoagulation.

The study population was divided into two groups: Group I with LVEF ≥ 50% and lack of HF symptoms (n = 30) and Group II with LVEF < 50% and clinical symptoms of HF (n = 23).

Pre-procedural assessments
Prior to LAA closure procedures, medical history was taken from all patients, then they were examined, and had transthoracic and transoesophageal echocardiograms performed using VIVID 9 (GE Healthcare, Wauwatosa, WI, USA). Patients with significant valvular disease or LAA anatomy unsuitable for percutaneous occlusion were excluded from the study. LVEF was automatically calculated from two- and four-chamber apical views based on the modified Simpson formula.

Cardiopulmonary exercise tests were performed using a motorised treadmill (Mac\textsuperscript{®} 5000, GE Healthcare). Each test was preceded by a calibration of the gas concentrations with a 3 L syringe, using primary standard gases and flow. All patients were familiarised with cardiopulmonary exercise testing on the treadmill, for approximately 10 min, followed by 10 min of rest. Twelve-lead electrocardiogram (ECG) monitoring was used during the entire test. During the test, the grade was increased every 2 min and participants were encouraged to walk for as long as possible, in order to achieve anaerobic threshold and respiratory exchange ratio > 1.1. Metabolic gas exchange was measured continuously during exercise and it was averaged over 30-s intervals. Peak VO\textsubscript{2} (oxygen consumption) was defined as the highest oxygen uptake for a given 30-s interval, within the last 60 s of exercise.

The six-minute walk test (6MWMT) was performed according to the American Thoracic Society guidelines. The heart rate, blood pressure, and oxygen saturation were measured prior to and after the test. During 6MWMT the patients were asked to walk as far as possible in 6 min along a 30 m-long hospital corridor. Patients were allowed to stop if necessary, but they were encouraged to resume walking if possible.

Collection of blood samples
In all patients participating in the study the concentration of NT-proBNP was assessed in blood samples collected from three different sites, i.e. right atrium (RA), left atrium (LA), and femoral artery (FA) during the percutaneous procedure of LAA occlusion.

Right atrium was accessed via the right femoral vein. Blood from RA was drawn through the transseptal sheath located in the RA at the level of oval fossa before the puncture of the interatrial septum. The proper location of the sheath’s distal tip was confirmed by fluoroscopy.

Access to the LA was achieved by a transseptal puncture of the fossa ovalis in its inferior-posterior part. After the puncture and withdrawal of the Brockenbrough needle a blood sample was taken from the LA. At this point in the procedure a third blood sample was also obtained from the FA.

All blood samples were collected in tubes containing EDTA. Blood was then centrifuged at 3000 r/min within 1 h of collection, and plasma NT-proBNP levels were determined with the use of enzyme-linked immunosorbent assay (ELISA) technique in the hospital laboratory (Roche Diagnostic Co-
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The upper reference range of plasma NT-proBNP concentration was 125 pg/mL.

**Ethics**

The study protocol was approved by the local Ethics Committee (approval no. KNW/0022/KBi/202/I/11/12 dated 07.02.2012 and KNW/0022/KBi/202/II/11/1/14 dated 01.07.2014). Prior to any procedures patients were informed about the aim and design of the study and written, informed consent was obtained from all participants.

**Statistical analysis**

Quantitative parameters were presented as mean and standard deviation. The Mann-Whitney test was used to reveal statistically significant differences in quantitative variables between the analysed groups. Qualitative variables were presented as frequencies and percentages. The χ² test was used to test the significance of differences between both groups with respect to the qualitative variables. To test the significance of differences between NT-proBNP concentrations from different collection sites, the repeated measurements analysis of variances (ANOVA) test was used. The Spearman’s rank correlation coefficient was used when searching for the relation between variables.

**RESULTS**

**Patients characteristics**

In Group I, men were insignificantly less prevalent and younger than women compared to Group II. There were no statistically significant differences between both groups in the distribution of diabetes mellitus, coronary artery disease, and hypertension, whereas history of stroke was considerably more frequent in Group I. The CHA₂DS₂VASC score was 3.97 ± 1.27 and 4.78 ± 1.68 in Group I and II, respectively. Also, no significant differences were found regarding the HAS-BLED score, which was 3.28 ± 0.65 in Group I and 3.49 ± 1.2 in Group II. Clinical characteristics of the study population are presented in Table 1.

Patients in Group I had significantly higher LVEF (58.00 ± 5.20% vs. 36.39 ± 10.77%; p < 0.01). Despite similar left atrial dimension, patients in Group II had significantly enlarged left ventricle, which was demonstrated by increased systolic and diastolic left ventricular dimensions and volumes. The details regarding differences in the echocardiographic parameters between both groups are shown in Table 2.

In Group I, permanent AF was present in nine (30%) patients and paroxysmal AF in 21 (70%) patients, whereas in Group II, in 12 (52%) and 11 (48%) patients, respectively (p > 0.05).

**NT-proBNP values**

In the present study as few as nine out of 53 patients had NT-proBNP values within the normal range. These patients represented solely Group I, and all of them had paroxysmal AF.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I</th>
<th>Group II</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>70.87 ± 9.77</td>
<td>71.52 ± 8.96</td>
<td>NS</td>
</tr>
<tr>
<td>Male</td>
<td>13 (43%)</td>
<td>16 (70%)</td>
<td>NS</td>
</tr>
<tr>
<td>Permanent AF</td>
<td>9 (30%)</td>
<td>12 (52%)</td>
<td>NS</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>28 (93%)</td>
<td>18 (78%)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes</td>
<td>15 (50%)</td>
<td>8 (35%)</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>8 (27%)</td>
<td>5 (22%)</td>
<td>NS</td>
</tr>
<tr>
<td>CKD stage 1–2</td>
<td>21 (70%)</td>
<td>15 (65%)</td>
<td>NS</td>
</tr>
<tr>
<td>CKD stage 3–4</td>
<td>9 (30%)</td>
<td>8 (35%)</td>
<td>NS</td>
</tr>
<tr>
<td>Stroke</td>
<td>27 (90%)</td>
<td>13 (56%)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

6MWT [m] 284.11 ± 93.39 307.63 ± 76.59 NS

Peak VO₂ [mL/kg/min] 14.96 ± 3.45 12.96 ± 3.08 NS

HAS-BLED 3.28 ± 0.65 3.49 ± 1.2 NS

CHAD₂SVASC 3.97 ± 1.27 4.78 ± 1.68 NS

Data are presented as mean ± standard deviation or number (percentage). P value of < 0.05 is considered statistically significant; AF — atrial fibrillation; 6MWT — six-minute walk test; Peak VO₂ — peak oxygen consumption; CKD — chronic kidney disease

The inter-group comparison for NT-proBNP assessment of blood taken from the RA, LA, and FA demonstrated significantly higher NT-proBNP values in Group II than in Group I independently of the sampling site (Fig. 1). The highest concentrations of NT-proBNP in both groups were found in the FA, and the lowest values of NT-proBNP were observed in samples from RA (Table 3). The type of AF had similar influence on the secretion of NT-proBNP in Group I and Group II. The patients with permanent AF had significantly higher NT-proBNP levels independently of the blood collection site (Fig. 2).

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The analysis of variance (ANOVA) performed separately for the patients with permanent and paroxysmal AF in each group did not show any significant differences in NT-proBNP taken from various blood collection sites. The results are presented in Table 4.

In Group I, the NT-proBNP values correlated most strongly with the LA and RA area. In contrast to these positive correlations, there was also a statistically significant negative correlation with LVEF. In Group II the strongest correlation of NT-proBNP was found for LVEF, which was negative. Positive correlations were revealed for the left ventricular systolic and diastolic dimensions and volumes and RA area (Table 5).

DISCUSSION

Until now, the assessment of NT-proBNP was mainly performed for HF patients, but there are data showing that this peptide is also elevated in the serum of patients suffering from other cardiac diseases. One of the diseases that alters
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The levels of NT-proBNP is AF. NT-proBNP concentrations are elevated in AF patients with normal left ventricular function with or without underling heart diseases [9]. The increase of NT-proBNP in AF patients may result from both atrial and ventricular production. It is postulated that an increased ventricular rate during AF leads to the oxygen mismatch, myocardial ischaemia, volume and pressure overload, and changes in microvascular blood flow, thus resulting in the ventricular production of natriuretic peptides [10]. It was also demonstrated that strict rate control yielded a prominent decrease in BNP values in AF patients [11].

Atrial peptide secretion has received less attention. proBNP secretion may be stimulated in response to increased atrial afterload. However, inflammatory cytokine-induced BNP gene expression, which is one of pathomechanisms responsible for the development of AF, may by itself also participate in this process independently from the increase in atrial pressure [12].

It was shown that BNP and NT-proBNP are robust predictors of incident AF [13–16]. It was also reported that both NT-proBNP and BNP values decrease after conversion to sinus rhythm in patients with persistent AF [16, 17]. Moreover, natriuretic peptides are predictive of recurrent AF after electrical cardioversion. Lewicka et al. [18] showed that the lack of BNP decrease below 700 fmol/mL seven days after electrical cardioversion is an independent predictor of AF recurrence during 12 months of follow-up. A meta-analysis reviewing the results of 22 studies also proved that low periprocedural BNP and NT-proBNP levels are associated with maintenance of sinus rhythm [19]. Some recent studies also suggest that NT-proBNP may help to identify AF in cryptogenic stroke patients. The exact cut-off values of 265.5 pg/mL for stroke of known aetiology and 912.0 pg/mL for cryptogenic stroke had high sensitivity and specificity for the diagnosis of paroxysmal AF in patients after stroke [20]. The same diagnostic utility for the detection of paroxysmal AF was confirmed for BNP in patients with cerebral ischaemia [21].

In a sub-study of the ARISTOTLE trial (Apixaban for the Prevention of Stroke in Subjects With Atrial Fibrillation),

<table>
<thead>
<tr>
<th>NT-proBNP [pg/mL]</th>
<th>Group I</th>
<th>Group II</th>
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<tbody>
<tr>
<td></td>
<td>Left atrium</td>
<td>Femoral artery</td>
</tr>
<tr>
<td>EDD</td>
<td>0.25</td>
<td>0.21</td>
</tr>
<tr>
<td>ESD</td>
<td>0.33</td>
<td>0.28</td>
</tr>
<tr>
<td>ESV</td>
<td>0.15</td>
<td>0.08</td>
</tr>
<tr>
<td>EDV</td>
<td>0.13</td>
<td>0.01</td>
</tr>
<tr>
<td>LAD</td>
<td>0.36</td>
<td>0.32</td>
</tr>
<tr>
<td>LAA</td>
<td>0.57*</td>
<td>0.53*</td>
</tr>
<tr>
<td>RAA</td>
<td>0.56*</td>
<td>0.56*</td>
</tr>
<tr>
<td>LVEF</td>
<td>−0.55*</td>
<td>−0.48*</td>
</tr>
<tr>
<td>6MWT</td>
<td>−0.28</td>
<td>−0.22</td>
</tr>
<tr>
<td>Peak VO_2</td>
<td>−0.33</td>
<td>−0.26</td>
</tr>
</tbody>
</table>

*p value of < 0.05 is considered statistically significant; EDD — left ventricular end-diastolic dimension; EDV — left ventricular end-diastolic volume; LVEF — left ventricular ejection fraction; ESD — left ventricular end-systolic dimension; ESV — left ventricular end-systolic volume; LAD — left atrial dimension; LAA — left atrial area; RAA — right atrial area; 6MWT — six-minute walk test; Peak VO_2 — peak oxygen consumption

![Figure 3](image-url). Test of between groups effects taking into account the type of atrial fibrillation (AF); NT-proBNP — N-terminal-pro B-type natriuretic peptide
Hijazi et al. [22] tested plasma samples for the concentration of NT-proBNP from 14,892 patients suffering from AF. The study showed that measuring NT-proBNP levels, in addition to the established risk factors included in the CHA₂DS₂-VASc score, further improved the risk stratification for stroke or systemic embolism in AF [22]. NT-proBNP was elevated in three-quarters of patients with AF and at least one risk factor for stroke, and it correlated most strongly with the type of AF. These data clearly show that AF affects the NT-proBNP production; however, less is known about the pathophysiology of this phenomenon.

It was shown that in a healthy subject, an important portion of natriuretic peptides is released in the atria, whereas in chronic HF there is an upregulation of ventricular NT-proBNP production [23, 24]. Studies of BNP in HF patients with AF are sparse. A study conducted by Corell et al. [25] documented higher levels of NT-proBNP in patients with AF compared to those with sinus rhythm in outpatients with HF due to left ventricular dysfunction, irrespective of LVEF, New York Heart Association classification, and heart rate [25].

It was previously noted that AF is an additional, independent cause of elevated NT-proBNP and that the latter may also serve as a useful surrogate for assessing AF burden when attempting to optimise AF therapy [26]. Also in our study, we confirmed that NT-proBNP is markedly elevated in the majority of patients with AF, even in the absence of HF. Moreover, the values of NT-proBNP were markedly higher in permanent AF than paroxysmal AF. These results are consistent with the results from the ARISTOTLE sub-study in which higher values of NT-proBNP were found in persistent/permanent AF and lower in paroxysmal AF [22]. Furthermore, NT-proBNP levels are increased in AF independently from the reduced LVEF; however, the secretion of NT-proBNP in HF overwhelmed the production secondary only to AF.

In our study, we gathered information concerning the regional production of NT-proBNP in patients with impaired LVEF and AF. The results showed also that NT-proBNP concentrations in the samples taken from the FA are insignificantly higher compared to those from LA, both in the patients with preserved and reduced LVEF; whereas the lowest NT-proBNP levels were found in blood returning to RA. It may reflect the importance of LA secretion of NT-proBNP in AF patients independently of left ventricular function. The differences in NT-proBNP concentration in particular heart chambers were rather small, which can result from the relatively long half-life of this peptide reaching 60–120 min.

The present study shows also that in AF patients, despite the decreased LVEF, the NT-proBNP corresponds better with the remodelling of heart chambers than with the physical capacity. There were no significant correlations with the distance in 6MWT nor with the oxygen consumption on the ergospirometry. However, the poorer mobility of patients in Group I resulting from a significantly higher percentage of strokes might have an influence on functional tests. Whereas, in the patients with AF and preserved LVEF the NT-proBNP levels correlated with the LA and the RA area. In patients with impaired LVEF a rise of NT-proBNP was associated with an enlargement of the left ventricle. The data obtained by Lam et al. [27] in patients with HF and preserved LVEF showed that after adjusting for pulmonary capillary wedge pressure or left ventricular end-diastolic pressure, AF was associated with both NT-proBNP level and left atrial remodelling. The remodelling of LA and the increase of NT-proBNP in patients solely with AF are similar to those observed in patients with HF and preserved LVEF without AF. Thus, our findings show that whenever AF is present, the diagnosis of HF with preserved LVEF based on these criteria cannot be certain.

In the current study, for the first time, evaluation of the importance of atrial secretion of NT-proBNP in AF patients was attempted. It was shown that the atrial release of NT-proBNP remains an important component of overall NT-proBNP secretion in patients with AF independently of concomitant HF with reduced ejection fraction. Another important finding is that atrial concentration of NT-proBNP correlates with atrial remodelling.

**Limitations of the study**

In the present study we assessed NT-proBNP in samples collected from regions representing different heart chambers. Samples reflecting the left ventricle were in fact drawn from the FA, so NT-proBNP levels could be slightly underestimated. Secondly, the regional secretion of natriuretic peptides is better reflected by appropriate gene activation than by assessment of the peptides themselves. Also, BNP, which has shorter half-life (15–20 min) compared to NT-proBNP (60–120 min), might better show the regional differences in natriuretic peptide synthesis in AF patients. It would also be valuable to broaden the analysis by examining the correlation of NT-proBNP with AF burden in patients suffering from paroxysmal AF. Finally, the study population was relatively small, which might also have influenced the results of this analysis.

**CONCLUSIONS**

NT-proBNP is markedly elevated in the majority of patients with AF, even in the absence of HF. LA secretion of NT-proBNP is an important contributor to the overall increase of NT-proBNP also in HF patients. In AF patients, the concentration of NT-proBNP correlates with the remodelling of heart chambers, but not with physical capacity.

**Conflict of interest:** Witold Streb, Katarzyna Mitręga, Zbigniew Kalarus — proctors for St. Jude Medical.

**References**

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