NT-proBNP reflects right ventricular structure and function in pulmonary hypertension

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Running title: NT-proBNP, right ventricular structure and function in PH
Abstract (151)

Aim: To investigate whether alterations in NT-proBNP reflect changes in right ventricular structure and function in pulmonary hypertension patients during treatment.

Methods and Results: 30 pulmonary hypertension patients were studied, 15 newly diagnosed and 15 on long-term treatment. NT-proBNP, right heart catheterization and cardiac MRI measurements were performed, at baseline and follow-up. There were no significant differences between newly diagnosed and on treatment patients at baseline and follow-up with respect to NT-proBNP, hemodynamics and right ventricular parameters. Relative changes in NT-proBNP during treatment were correlated to the relative changes in right ventricular end-diastolic volume index ($r = 0.59, p < 0.001$), right ventricular mass index ($r = 0.62, p < 0.001$) and right ventricular ejection fraction ($r = -0.81, p < 0.001$)

Conclusion: NT-proBNP measurements reflect changes in MRI measured right ventricular structure and function in pulmonary hypertension patients. An increased NT-proBNP over time reflects right ventricular dilatation concomitant to hypertrophy and deterioration of systolic function.
Keywords:
Magnetic resonance imaging; N-terminal pro brain natriuretic peptide; pulmonary hypertension; Right ventricle
Introduction
Pulmonary hypertension (PH) is a disease characterized by increased pulmonary vascular resistance leading to chronic right ventricular (RV) pressure overload. Without treatment patients have a poor prognosis and die of right heart failure. Right atrial pressure, measured by right heart catheterization, has been shown an important prognostic parameter in pulmonary arterial hypertension (PAH)(1). Recently, the search for a less invasive prognostic marker was successful with the detection of the cardiac hormone brain natriuretic peptide (BNP) and its biologically inactive alternative N-terminal pro brain natriuretic peptide (NT-proBNP). Research performed in patients with a diseased left ventricle, i.e. ischemia, pressure or volume overload, showed the clinical and prognostic value of this hormone (2-5). Nagaya et al. showed in idiopathic PAH patients that a high BNP level at baseline and a further increase in BNP at follow-up was associated with a poor prognosis (6). In patients with increased RV load it has been shown that BNP is related to functional status; initial hemodynamics; RV systolic function and structure (7-9). In PAH patients BNP parallels alterations in hemodynamics and functional status during treatment (10). Although these findings emphasize the clinical significance of the change in BNP after long-term therapy in PAH patients, insight into changes in RV structure and systolic function in relation to BNP are lacking. Furthermore, in previous reports BNP was measured, while it is unclear whether measurements of NT-proBNP show similar relations. Therefore the aim of this study was, to assess the temporal relationships of NT-proBNP with RV structure and systolic function in PH patients.
Methods

Study design
A total of 30 PH patients with an average age of 48 [21; 80] years and a male to female ratio of 8/22 were studied. Fifteen patients were newly diagnosed and 15 patients were on long-term treatment (27 ± 16 months) at the start of the study and were being reevaluated for treatment effect and/or add on therapy. All patients had a mean pulmonary artery pressure > 25 mmHg, pulmonary capillary wedge pressure < 15 mmHg at right heart catheterization and normal systemic blood pressure and renal function (serum creatinine < 120µml/l).

PH due to left sided heart diseases, interstitial lung disease or hypoxemia, was excluded by further diagnostic work-up (echocardiography, high resolution CT-scan and lung function test) following the 2003 Venice consensus guidelines on the diagnosis and treatment of pulmonary arterial hypertension (11). The different etiologies of PH were distributed as follows, idiopathic PAH (n = 19), PAH related to the limited type of systemic sclerosis (n = 7), PAH related to HIV (n = 1), PAH related to porto-pulmonary shunts (n = 1) and PH due to chronic thrombo-embolic disease (n = 2). Measurements were repeated after an average follow-up period of 10.5 months (range 3.7 to 15.6). Patients were treated with; bosentan (11), nifidipine (1), epoprostenol (6), sitaxentan (2), and combination therapy consisting of; epoprostenol-sildenafil (5) or bosentan-sildenafil (5). The study was part of the project that evaluates and monitors cardiac function in PAH by MRI, which was approved by the Committee on Research Involving Human Subjects of the VU University medical center. Written informed consent was obtained from all patients.
Cardiac catheterization
Diagnostic right heart catheterization was performed with a balloon tipped, flow directed 7F Swan-Ganz catheter (131HF7; Baxter Healthcare Corp; Irvine, CA). The patient was in a stable condition lying supine breathing room air. Right atrial, right ventricular, pulmonary artery and pulmonary capillary wedge pressures were measured. Blood was sampled, with the catheter positioned in the main pulmonary artery to assess mixed venous oxygen saturation. Left ventricular pressure measurements and coronary angiogram were performed in two patients with PAH associated with systemic sclerosis and old age (> 70 years) to exclude coronary artery disease. Arterial oxygen saturation was measured from blood sampled from the radial or femoral artery. Cardiac output was assessed by the Fick method and pulmonary vascular resistance was calculated using the standard formula.

MRI measurements
Magnetic resonance imaging was performed on a Siemens 1.5T Sonata scanner (Siemens Medical Solutions, Erlangen, Germany), using a four-element phased array cardiac receiver coil, according to the MRI protocol described previously (12). Perpendicular to the four chamber end-diastolic image, a stack of consecutive short-axis breath hold cine images were made with temporal resolution of 34 ms, and slice distance of 10 mm. From the stack of parallel short-axis cine images, quantitative analysis of volumes and geometry was performed by manual detection of endocardial and epicardial borders on each slice, using the MR Analytical Software System (Medis, Leiden, The Netherlands). The MRI data was analyzed by C.T.G., whom was unaware of the NT-proBNP levels at the time of analysis. The interventricular septum was considered part of the left ventricle. Right ventricular end-diastolic volume (RVEDV) and myocardial mass (RVM) were calculated an indexed (indicated with suffix I). Stroke volume was measured using MR phase-contrast flow quantification (velocity sensitivity was 150 cm/s, and temporal resolution 22 ms), ejection fraction was calculated as follows: right (RVEF = 100 * stroke volume/RV end-diastolic volume) and left ventricular ejection fraction (LVEF = 100*stroke volume/LV end-diastolic volume).

Plasma NT-proBNP levels
Blood was sampled from a peripheral vein with the patient at rest, within 24 hours of MRI measurements and right heart catheterization. N-terminal pro brain natriuretic peptide (NT-proBNP) plasma levels were analyzed on an ELECSYS 1010 bench top analyzer (Roche Diagnostics Netherlands). Temporal changes were expressed as percentage of the baseline values.

Statistical analyses
SPSS 11.0 software package was used for statistical analyses and p < 0.05 was considered statistically significant. Results are reported as median and interquartile range, unless otherwise indicated. Wilcoxon signed rank test was performed for comparison of baseline and follow-up values. Baseline NT-proBNP was correlated to hemodynamics and MRI measurements. Similar correlations were assessed for the relative changes in NT-proBNP, hemodynamics and MRI measurements. Correlation analyses were performed with a Spearman rank correlation test.
Results
At the start of the study six-minute walk distance, hemodynamics and MRI measurements were not different between newly diagnosed and on treatment patients. Furthermore, there was no clinically relevant difference in functional class among the newly diagnosed and on treatment patients. The coronary angiogram performed in the two patients with PAH associated with systemic sclerosis did not reveal coronary artery disease. Furthermore, left ventricular ejection fraction measured by MRI was greater than 60% in all patients.

Hemodynamics and MRI measurements
The functional status, hemodynamics and MRI measurements at baseline and follow-up are summarized in Table I. At baseline the most patients were in NYHA functional class III. Hemodynamic measurements showed that all patients had significant pulmonary hypertension, which is also reflected by the MRI measured RV parameters; i.e. RV dilatation, hypertrophy and impairment of systolic function. A small but significant hemodynamic improvement in pulmonary artery pressure and pulmonary vascular resistance index was observed during follow-up. However this improvement was not reflected by RV parameters or a decrease of NT-proBNP (normal values between 68-112 pg/ml) (13).

# Insert Table I
Correlations of NT-proBNP with hemodynamics and MRI measurements at baseline
At baseline serum NT-proBNP was related to invasively measured hemodynamic variables such as right atrial pressure, mixed venous saturation and cardiac index but not to pulmonary artery pressure or pulmonary vascular resistance index. Furthermore, NT-proBNP was positively related to RV end-diastolic volume index, and inversely related to RVEF, but not related to RV mass index. (Table II).

Correlation of the relative change in NT-proBNP with RV parameters during follow-up
Although for the whole group most of the variables did not change significantly during follow up, there was a considerable individual variation in the relative change of RV parameters, as is shown in Figure 1. The relative changes in NT-proBNP were closely related to the relative changes in RV end-diastolic volume index (r = 0.59, p = 0.001), mass index (r = 0.62, p< 0.001) and inversely to RVEF (r = -0.81, p< 0.001) (Figure 1). The patient with the significant increase of NT-proBNP at follow-up was a patient with an unsuccessful transition on bosentan after being stable on epoprostenol therapy for more than four years.
Discussion

This study showed that in PH patients, NT-proBNP parallels changes in RV structure and systolic function. BNP is a cardiac hormone synthesized and cleaved together with NT-proBNP from a pro-hormone in ventricular and atrial cardiomyocytes. The potential advantages of NT-proBNP measurements above BNP are a longer plasma half-life, biological inactivity and lower biological variability (14, 15). However, head-to-head comparison of BNP and NT-proBNP in patients with left ventricular dysfunction could not confirm a clinical significant advantage of NT-proBNP over BNP (16, 17). Under normal physiological conditions the left side of the heart determines BNP and NT-proBNP levels (18). Elevated NT-proBNP in PH patients presumably results from augmented synthesis and release by the overloaded RV.

In this study, baseline NT-proBNP was elevated related to hemodynamics characterizing RV pressure overload. MRI measurements showed RV dilatation, hypertrophy and impaired function. Compared to earlier studies (19-21), this study showed similar, but modest, correlations of NT-proBNP and hemodynamics. In addition, NT-proBNP was related to RV end-diastolic volume index and showed a strong inversed relation with RV ejection fraction.

The relation of NT-proBNP and RV mass index was not significant. In the study by Nagaya et al. BNP was correlated to RV mass and systolic function, but not to RV volume (22). The different findings between this study and the earlier studies might be explained by the small variation on the hemodynamic data at baseline in our study. Furthermore, RV hypertrophy is a compensatory physiological mechanism aimed at normalizing wall stress. In case of RV volume and/or pressure overload, an increase in RV mass decreases wall stress and as a consequence may reduce NT-proBNP. This may explain the absence of a significant correlation between NT-proBNP and RV mass index at baseline.

In our study, long-term follow-up showed that changes in NT-proBNP paralleled changes in RV structure and function. The long-term follow-up study by Leuchte et al (23) has shown that in PH patients there are parallel changes in hemodynamics and BNP confirming the work of a three months follow-up study and studies performed in an acute setting (24-26). In addition our study shows, that temporal changes in NT-proBNP are associated with alterations in RV structure and particularly systolic function.

Ventricular dilatation has been suggested to be a pathological state of cardiac remodeling. However data on RV dilatation and outcome in PH patients are lacking. Although an echocardiography study showed that absolute RV end-diastolic area measured as a singular parameter was not a significant predictor of outcome(27), our finding does not exclude the prognostic importance of a change in RV end-diastolic volume over time and should be investigated in future studies.

The relative change in NT-proBNP showed a positive correlation with the change in RV myocardial mass. Since NT-proBNP is produced by the cardiomyocyte, this correlation suggests that hypertrophied RV cardiomyocytes are associated with increased transcription of the proBNP gene and NT-proBNP release.

Impaired RV systolic function predisposes to right heart failure which is associated with an increased risk of death (28) in idiopathic PAH patients. Our data shows that NT-proBNP is valuable parameter marking alterations in RV systolic function.

The correlations of the change in RV parameters and NT-proBNP were stronger than between baseline RV parameters and NT-proBNP. This supports the idea that serial measurements have higher sensitivity than baseline values. Taking the current results and previous research(29), one may hypothesize that the importance of an increasing NT-proBNP level, despite treatment, is directly related to failing RV systolic function.

The recent work by Fijalkowska(30) et al. has shown that NT-proBNP is related to RV structure and function, and serves as a prognostic parameter in PH patients. Our study showed that serial changes in NT-proBNP closely reflect alterations in RV end diastolic volume and ejection fraction which are
likely to be important prognostic markers in PH (31). Since impaired RV ejection fraction predisposes to right heart failure, serial measurements might be valuable in monitoring disease. Although it has been shown that singular NT-proBNP measurements are of prognostic value in PH patients (32), longitudinal NT-proBNP measurements might potentially be superior with respect to prognosis, as was shown for BNP (33). In addition, serial NT-proBNP measurements may be valuable in clinical decisions when there is limited access to MRI or under the circumstance where the patient’s condition does not allow invasive procedures.

We included newly diagnosed patients and patients being initially treated at the start of this study. Although there were no clinically relevant differences between the two patient groups, our study group was too small to draw any conclusion on the influence of treatment on NT-proBNP. The small study group and the limited changes in hemodynamic variables are important limitations of this study, which might explain the absence of significant correlations of several hemodynamic variables with NT-proBNP. An assumption in this study was that PAH treatment does not directly affect NT-proBNP production. However, there is some evidence from experimental animal research that, phosphodiesterase-5 inhibitor, sildenafil might act on the cardiac myocytes directly (34), therefore a direct effect on the myocardial production of NT-proBNP cannot be excluded.

**Conclusion**

The data from this study showed that the change in NT-proBNP over time provides indirect information on RV remodeling and the change in RV systolic function, measured by MRI. Elevated NT-proBNP levels over time reflect progressive RV dilatation and impaired systolic function, and thus should be interpreted as a sign of RV failure.

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| Table I |
|-----------------|-----------------|-----------------|
| **Functional status:** | **Baseline** | **Follow-up** |
| NYHA (II : III : IV) | 5:22:3 | 13:15:2 |
| 6 MWD (m) | 425 [342; 509] | 461 [370; 591] |
| **Hemodynamics :** | | |
| Pra (mmHg) | 6 [4; 10] | 6 [3; 10] |
| Prv syst (mmHg) | 72 [56; 93] | 65 [56; 82]* |
| Prv diast (mmHg) | 9 [5; 13] | 9 [6; 13] |
| mPap (mmHg) | 47 [38; 57] | 43 [32; 52]* |
| SvO₂ (%) | 67 [58; 71] | 66 [61; 74] |
| CI (l/min·m²) | 2.8 [2.0; 3.6] | 3.1 [2.6; 4.0] |
| PVRI (dynes·s·cm⁻⁵·m⁻²) | 384 [236; 528] | 324 [160; 430]* |
| PCWP (mmHg) | 6 [5; 9] | 7 [5; 11] |
| **MRI measurements:** | | |
| RVEDVI (ml/m²) | 92 [76; 111] | 98 [76; 109] |
| RVMI (g/m²) | 38 [29; 51] | 45 [31; 53] |
| RVEF (%) | 34 [23; 50] | 38 [31; 53] |
| NT-proBNP (pg/ml) | 333 [192; 1363] | 541 [99; 1027] |
Table II. Correlation coefficients with NT-proBNP at baseline

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation Coefficient (r)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pra (mmHg)</td>
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<td>0.008</td>
</tr>
<tr>
<td>mPap (mmHg)</td>
<td>0.28</td>
<td>0.143</td>
</tr>
<tr>
<td>S\textsubscript{O}_2 (%)</td>
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<td>&lt;0.001</td>
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<tr>
<td>CI (l/min·m\textsuperscript{2})</td>
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<td>0.019</td>
</tr>
<tr>
<td>PVRI (dynes·s·cm\textsuperscript{5}·m\textsuperscript{2})</td>
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<td>0.122</td>
</tr>
<tr>
<td>RVEDVI (ml/m\textsuperscript{2})</td>
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<td>0.004</td>
</tr>
<tr>
<td>RVMI (g/m\textsuperscript{2})</td>
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<tr>
<td>RVEF (%)</td>
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</tr>
<tr>
<td>6MWD (m)</td>
<td>-0.51</td>
<td>0.008</td>
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</tbody>
</table>
Reference list


Figure Legends

Figure 1 Correlation of the relative change in NT-proBNP with the relative change in right ventricular mass index (RVMI) (top); right ventricular end-diastolic volume index (RVEDVI) (middle); and right ventricular ejection fraction (RVEF) (bottom) in patients with pulmonary hypertension. Gray circles represent newly diagnosed patients (n = 15) and black triangles represent on treatment patients (n = 15).

Table I. NYHA = New York heart association class; 6 MWD = six-minute walk distance; Pra = right atrial pressure; Prv syst = systolic right ventricular pressure; Prv diast = diastolic right ventricular pressure; mPap = mean pulmonary artery pressure; we; SvO2 = mixed venous saturation; CI = cardiac index; PVRI= pulmonary vascular resistance index; Pcwp = pulmonary capillary wedge pressure; RVEDVI = right ventricular end-diastolic volume index; RVMI = right ventricular mass index; RVEF = right ventricular ejection fraction; NT-proBNP = N-terminal pro brain natriuretic peptide. Values are expressed as median and interquartile range.
* p<0.05, between baseline and follow-up

Table II. Correlation of NT-proBNP with hemodynamics, right ventricular parameters and six-minute walk distance at baseline. Abbreviations as mentioned earlier