Characterization of Brain Natriuretic Peptide in Long-term Follow-up of Pulmonary Arterial Hypertension

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Study objectives: Pulmonary arterial hypertension (PAH) leads to substantial morbidity and mortality. Noninvasive parameters in the follow-up assessment of PAH could be helpful in clinical decision making. The brain natriuretic peptide (BNP) has been shown to correlate with the functional status and prognosis of these patients and could be a valuable parameter in this respect. The aim of our study was to investigate whether BNP levels could reflect clinical and hemodynamic changes, including the response to therapy during long-term follow-up in patients with PAH.

Study design: We measured pulmonary hemodynamics, functional parameters including the 6-min walk distance (6MWD), and plasma BNP levels at baseline and after a mean (± SEM) follow-up period of 12.6 ± 1.5 months in patients with PAH.

Results: In group A (n = 18), with decreasing BNP levels mean pulmonary artery pressure (PAP) and pulmonary vascular resistance (PVR) decreased (PAP, 60.89 ± 3.44 to 53.47 ± 3.24 mm Hg; PVR, 1,207.47 ± 115.35 to 942.35 ± 103.15 dyne · s · cm⁻²; p < 0.01) and 6MWD increased (408.24 ± 29.57 to 470 ± 25.54 m; p < 0.01). In group B (n = 12), with increasing BNP levels mean PAP and PVR increased (PAP, 52 ± 3.31 to 60.17 ± 5.03 mm Hg; PVR, 946.13 ± 115.35 to 1,236.6 ± 180.23 dyne · s · cm⁻²; p < 0.01) and mean 6MWD decreased from 463.64 ± 27.77 to 367.27 ± 38.87 m (p < 0.05). Comparing groups revealed statistically significant differences regarding changes in PAP (group A, −11.58 ± 3.57%; group B, +13.29 ± 5.44%; p = 0.001) and PVR (group A, −19.21 ± 5.87%; group B, +30.35 ± 7.72%; p < 0.001). Correlations existed between the changes in BNP levels and pulmonary hemodynamics.

Conclusion: We concluded that BNP levels parallel changes in pulmonary hemodynamics and functional parameters, including the 6MWD, in PAH patients. Consequently, we suggest BNP as a parameter for the follow-up assessment of PAH patients. (CHEST 2005; 128:2368–2374)

Key words: exercise; hypertension; natriuretic peptides; pulmonary

Abbreviations: BNP = brain natriuretic peptide; CI = cardiac index; CO = cardiac output; 6MWD = 6-min walk distance; PAH = pulmonary arterial hypertension; PAP = pulmonary arterial pressure; PVR = pulmonary vascular resistance; RAP = right atrial pressure; SvO₂ = mixed venous oxygen saturation; WHO = World Health Organization

Pulmonary arterial hypertension (PAH) is a progressive disease that leads to substantial morbidity and mortality without adequate treatment. New treatment options have been shown to positively influence pulmonary hemodynamics and to improve exercise capacity. Nevertheless, these new treatments are not unequivocally effective in every patient. In patients with progressive disease, the escalation of medical therapy and even a combination therapy with different vasodilators have been suggested. In this context, it seems to be of crucial importance to recognize disease progression. A number of surrogate markers of disease progression have been proposed such as pulmonary hemodynamics measured by repetitive right heart catheterization, the determination of the World Health Organization (WHO) functional class, and the 6-min walk distance (6MWD). However, there is a need for sensitive

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biological markers that are objective, independent of examiner and patient, and noninvasive.

The natriuretic peptide system may be a candidate in this respect. The atrial natriuretic peptide and brain natriuretic peptide (BNP) have been shown to significantly correlate with the degree of right ventricular overload in patients with pulmonary hypertension. BNP seems to be of special interest as it is secreted by the cardiac ventricles and has been shown to be inversely related to prognosis in patients with acute pulmonary thromboembolism. However, variations in secretion patterns among individuals have been observed and may be at least partially explained by genetic factors. In addition, there is a need for further long-term follow-up data in patients with PAH.

The aim of our study was to investigate whether BNP levels could reflect clinical and hemodynamic changes and the response to therapy during long-term follow-up in patients with PAH. We performed a prospective longitudinal study in patients with PAH, investigating plasma BNP levels, 6MWDs, and WHO functional class as indicators of changes in pulmonary hemodynamics.

**Materials and Methods**

**Subjects**

Thirty patients with PAH (12 men and 18 women; mean [± SEM] age, 46.93 ± 2.8 years), as established according to the WHO classification, were included in the study. Twenty-five subjects received diagnoses of idiopathic PAH. PAH was associated with HIV infection (n = 1), appetite suppressants (n = 1), Gaucher disease (n = 1), capillary hemangiomatosis (n = 1), and corrected atrial septal defect (n = 1).

Exclusion criteria were impaired renal function (serum creatinine level > 1.3 mg/dL; and/or creatinine clearance rate, < 50 ml/min), age > 65 years, decreased left heart function, and atrial fibrillation. Also, patients were excluded from the study if they showed signs of acute right heart decompensation or volume overload.

The study protocol was approved by the institutional ethics committee. Written informed consent was obtained from every patient.

**Right Heart Catheterization**

All patients underwent routine right heart catheterization at the beginning of the study and after a mean observation period of 12.6 ± 1.5 months. Patients received no medications by mouth, and the inhalation therapy was paused on the morning of the procedure, resulting in a discontinuation of treatment with medication of about 12 h. Right heart catheterization was performed as described previously.

**6-Min Walk Test**

Patients performed the 6-min walk test, and the distance was recorded using a standardized protocol in accordance with the American Thoracic Society 2002 guidelines. All patients walked along an enclosed level corridor and were told to proceed at their own pace but to cover as much ground as possible in 6 min. The distance to the first turnaround point was 40 m. The preceptors of the test were blinded to the hemodynamic results.

**Blood Sampling and Assay**

Blood samples were drawn before right heart catheterization and the 6-min walk test were performed. Samples were analyzed for routine laboratory parameters (including renal function) and BNP levels in all patients (n = 30), as described previously. In general, blood samples were drawn in the morning hours. The normal value obtained during a 7-month period and 31 analytical series was 5.2 pmoL/mL. A coefficient of variation of 7.7% was found for a low-concentration quality control sample (mean, 5.55 pmoL/mL); 4.0% for a high-concentration sample (mean, 85.53 pmoL/mL). The BNP data were blinded until after the 6-min walk test results and hemodynamic data were recorded.

**Statistical Analysis**

The data were presented as the mean ± SEM. A statistical software package (SPSS, version 11.0 for Windows; SPSS; Chicago, IL) was used for the analysis. Patients were assigned to two different groups regarding the development of BNP concentration during follow-up. Group A consisted of 18 patients with decreasing BNP levels during follow-up. Group B included 12 patients who showed an increase in BNP concentrations. The measured variables were compared in both groups using the Student t test for unpaired probes. For analysis of the follow-up data within one group, the Student paired t test was used.

The relative changes during the follow-up period compared to baseline values were calculated for BNP levels, pulmonary hemodynamic variables, and the 6MWD. To compare the absolute and relative changes, correlation analysis was performed using the Pearson correlation index. Correlation analysis was performed only in those patients who underwent the respective tests.

All results were tested for two-sided significance. In general, p values < 0.05 were considered to be statistically significant.

**Results**

**Patients Characteristics**

Vasodilative treatment included calcium channel blockers (n = 5), bosentan (Tracleer; Actelion; Allschwil, Switzerland) [n = 12], beraprost sodium (Dorner; Yamanouchi-Pharma; Tokyo, Japan) [n = 2], and iloprost-aerosol (Ilomedin; Schering; Berlin, Germany) [n = 6]. Five patients were treated with a combination therapy of either iloprost-aerosol plus calcium channel blockers (n = 3) or beraprost plus calcium channel blockers (n = 2). No additional specific therapy was added, and the medication dosage was kept constant during the study period. The mean follow-up time was 12.6 ± 1.5 months.

**Hemodynamics, Functional Parameters, and BNP Concentrations at Baseline**

All patients showed severe PAH (ie, mean pulmonary artery pressure [PAP], 57.33 ± 2.54 mm Hg;
mean pulmonary vascular resistance [PVR], 1.102.93 ± 83.52 dyne · s · cm⁻²) with normal mean wedge pressures (7.9 ± 0.6 mm Hg), impaired mean cardiac index (CI) [2.12 ± 0.08 L/min/m²], and reduced mean mixed venous oxygen saturation (SvO₂) [56.03 ± 1.7%]. The mean right atrial pressure (RAP) was slightly elevated in all patients (7.8 ± 0.84 mm Hg) [Table 1].

The overall mean WHO functional class was 2.6 ± 0.1. Thirteen patients were assigned to WHO functional class II, 16 patients to WHO functional class III, and 1 to WHO class IV. The 6MWD ranged from 100 to 600 m (mean 6MWD, 430 ± 21.31 m).

At baseline, the mean BNP concentration was 7.52 pmol/mL, which is elevated. Eight patients had nonelevated BNP levels.

Evolution of Hemodynamic and Functional Parameters in Patients With Increasing or Decreasing BNP

During the follow-up period, overall repetitive measurements of BNP concentration, pulmonary hemodynamics, and 6MWD showed no significant changes (Table 1). In group A, BNP levels decreased in 18 patients compared to baseline (mean change, −28.54 ± 7.27 pmol/mL; p < 0.001), and 6 of these patients who had an elevated BNP level during the initial measurement converted to normal BNP levels during the follow-up period. Moreover, a BNP reduction of at least 20% was observed in all of these patients. In this group, mean PAP (mean change, −7.6 ± 2.32 mm Hg; p < 0.01), mean PVR (mean change, −206.31 ± 103.97 dyne · s · cm⁻²), and mean RAP (mean change, −2 ± 0.88 mm Hg; p < 0.05) declined significantly compared to baseline levels. Moreover, CI increased significantly (mean change, 0.27 ± 0.13 L/min/m²). Other hemodynamic parameters did not differ significantly compared to baseline values. The 6MWD increased significantly during the follow-up period (mean change, 61.75 ± 16.16 m; p < 0.01). The mean WHO functional class decreased from 2.72 ± 0.14 to 2.33 ± 0.11 (p < 0.05).

In group B, the BNP concentrations increased in 12 patients (mean change, 54.27 ± 17.2 pmol/mL; p < 0.01) during the follow-up period, and abnormal BNP values developed in 3 patients after normal baseline levels. All other patients had elevated BNP levels at baseline. The BNP increase in this group was at least 25%. In those patients, PAP (mean change, 6.64 ± 2.38 mm Hg; p < 0.05), PVR (mean change, 223.78 ± 59.68 dyne · s · cm⁻²; p < 0.01), and RAP (mean change, 3.25 ± 1.4 mm Hg; p < 0.05) increased, whereas CI (mean change, −0.22 ± 0.11 L/min/m²), cardiac output (CO) [mean change, −0.37 ± 0.19 L/min], and SvO₂ (mean change, −5.18 ± 1.99) decreased significantly (p < 0.05 for each variable).

The 6MWD decreased significantly in these subjects (mean change, −96.36 ± 59.68 m), whereas mean WHO functional class increased from 2.42 ± 0.15 to 2.75 ± 0.18 (each p < 0.05) during the follow-up period in this group. The baseline values for groups A and B were not significantly different. However, during the follow-up period, group A patients had significantly higher CIs and longer 6MWDs than group B patients. In addition,

<table>
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<th>Variables</th>
<th>All</th>
<th>Group A</th>
<th>Group B</th>
</tr>
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<tr>
<td>Mean PAP, mm Hg</td>
<td>57.33 ± 2.54</td>
<td>56.03 ± 2.74</td>
<td>60.89 ± 3.44</td>
</tr>
<tr>
<td>PVR, dyne · s · cm⁻²</td>
<td>1,102.93 ± 83.52</td>
<td>1,060.05 ± 96.89</td>
<td>1,207.47 ± 111.75</td>
</tr>
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<td>CO, L/min</td>
<td>3.77 ± 0.17</td>
<td>3.81 ± 0.18</td>
<td>3.71 ± 0.24</td>
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<td>CI, L/min/m²</td>
<td>2.12 ± 0.84</td>
<td>2.19 ± 0.1</td>
<td>2.09 ± 0.12</td>
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<tr>
<td>SvO₂, %</td>
<td>56.03 ± 1.7</td>
<td>53.03 ± 1.88</td>
<td>53.83 ± 2.32</td>
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<tr>
<td>RAP, mm Hg</td>
<td>7.8 ± 0.84</td>
<td>7.9 ± 0.78</td>
<td>9.17 ± 1.25</td>
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<tr>
<td>6 MWD, m</td>
<td>430 ± 23.31</td>
<td>429.64 ± 23.4</td>
<td>408.24 ± 29.57</td>
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<td>WHO class</td>
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<td>II</td>
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<td>IV</td>
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<tr>
<td>BNP, pmol/mL</td>
<td>45.51 ± 7.52</td>
<td>58.2 ± 11.44</td>
<td>55.32 ± 10.76</td>
</tr>
</tbody>
</table>

*Values given as mean ± SEM.
†p < 0.01 vs preceding baseline value.
‡p < 0.05 vs preceding baseline values.
§p < 0.05 vs group A.
‖p < 0.05 vs group A.
§§p < 0.01 vs preceding baseline values.
BNP levels were significantly lower in group A during the follow-up period.

**Correlation of Changes in Hemodynamics, 6MWD, and BNP Levels During Follow-up**

Analysis revealed a positive correlation of absolute changes of BNP with PVR \((r = 0.55; p < 0.01)\), mean PAP \((r = 0.54; p < 0.01)\), and RAP \((r = 0.78; p < 0.001)\). A negative correlation existed between absolute changes in BNP with CI \((r = -0.49; p < 0.01)\), CO \((r = -0.48; p < 0.01)\), \(\overline{SvO_2}\) \((r = -0.6; p = 0.001)\), and 6MWD \((r = -0.74; p < 0.001)\) [Fig 1, 2].

**Evaluation of Relative Changes of Hemodynamic and Functional Parameters**

In the overall study population, there were no significant changes in the percentages of hemodynamic parameters, 6MWD, and WHO functional class during follow-up (Table 2, Fig 3).

In group A patients with decreasing BNP levels,
mean PAP (mean change, 1.58 ± 3.57%; p = 0.001) and mean PVR (mean change, -19.21 ± 5.87%; p < 0.001) were reduced, whereas CO (mean change, 10.42 ± 5.49%; p < 0.01), CI (mean change, 14.26 ± 5.81%; p < 0.01), and SvO₂ (mean change, 5.18 ± 5.6%; p < 0.05) increased significantly (group A vs group B).

In group B patients with increasing BNP levels, mean PAP (mean change, 13.29 ± 5.44%; p = 0.001) and mean PVR (mean change, 30.35 ± 7.72%; p < 0.001) increased, whereas mean CO (mean change, -10.3 ± 4.17%; p < 0.01), mean CI (mean change, -10.15 ± 4.13%; p < 0.01), and mean SvO₂ (mean change, -10 ± 3.18%; p < 0.05) declined significantly (group A vs group B).

The comparison of the 6MWDs revealed a significant difference between group A (mean change, 31.2 ± 17.14%) and group B (mean change, -13.01 ± 9.34%; p < 0.05). WHO functional class did not differ significantly between the two groups.

**Comparison of Demographic Factors and Different Treatments**

There were no significant differences regarding BNP levels, pulmonary hemodynamics, or functional parameters between patients receiving different treatments (data not shown).

**Discussion**

We performed a prospective longitudinal study in PAH patients to investigate the relationship of plasma BNP levels and changes in pulmonary hemodynamics and functional parameters during long-term follow-up. The main finding of our study was that progression of the disease was paralleled by an increase in BNP levels, whereas improvement of pulmonary hemodynamics and functional status was accompanied by a significant decline in plasma BNP concentrations. Additionally, we observed that the progression of pulmonary hypertension led to increasing exercise limitation, as shown in the decline of the 6MWD and a further increase of the WHO functional class. From our data, we concluded that plasma BNP concentrations parallel the development of pulmonary hemodynamics and exercise capacity in patients with PAH and may serve as an indicator of disease progression or improvement.

Different therapies have been suggested for the treatment of PAH.2–7 Nevertheless, treatment is not unequivocally effective in these patients. Serial right heart catheterization, determination of the WHO functional class, and measurement of the 6MWD are useful tools for the assessment of PAH and for clinical decision making.10 However, additional non-invasive and examiner-independent markers could contribute significantly to the follow-up assessment of these patients. Once disease progression is obvious, the escalation of medical treatment4 or the addition of interventional or surgical therapies5,9 may be indicated. Therefore, it might be beneficial for patients with PAH to identify disease progression early in the course of the disease.

Neurohormonal activation has been observed before as a sign of right ventricular failure in different etiologies of pulmonary hypertension.11,14,23 More specifically, the elevated secretion of the atrial natriuretic peptide and BNP in patients with chronic right ventricular overload13 or acute right ventricular overload16 have been reported. Almost all of these cross-sectional studies observed a strong correlation...
between hemodynamic impairment and the elevation of natriuretic peptide levels. Moreover, it has been shown that BNP elevation beyond a certain level is associated with a poor prognosis\textsuperscript{12,16,17} in patients with pulmonary hypertension.

Nevertheless, singular measurements may not be adequate because genetic factors seem to lead to strong interindividual variations in BNP secretion.\textsuperscript{18} Longitudinal studies of serial measurements would be helpful, as BNP changes could be calculated to facilitate interpretation. One follow-up study\textsuperscript{12} investigated the influence of treatment with continuous prostacyclin on hemodynamics and BNP levels after a 3-month treatment period. Interestingly, during this short period changes in BNP levels were able to reflect a successful treatment of the subjects. However, the interpretation of these data is complicated as repetitive measurements were performed under permanent prostacyclin infusion or an oral prostacyclin analog. Moreover, long-term observations are missing.

It is widely accepted that WHO functional class and the 6MWD should be determined in the follow-up assessment of patients with PAH.\textsuperscript{2–7,24} Interestingly, improvement in the 6MWD is not necessarily accompanied by significant alleviation of pulmonary hemodynamics, and there have been conflicting results on this issue.\textsuperscript{2,5} Consequently, the role of the 6-min walk test in the follow-up of patients with cardiopulmonary disease needs further evaluation.\textsuperscript{21} A good correlation among WHO functional class and the 6MWD, pulmonary hemodynamics,\textsuperscript{10} and BNP levels\textsuperscript{11} has been demonstrated. However, the potential advantages of repetitive BNP measurements to follow hemodynamic changes have not been evaluated so far (to our knowledge).

In our investigation, we observed a significant improvement of the 6MWD and the WHO functional class, and a decline of plasma BNP levels of at least 20% in patients with improved pulmonary hemodynamics \textit{(i.e., reduced mean PAP and PVb, and increased CI)} during a mean follow-up period of 13 months. Consequently, repetitive BNP measurements could contribute to the clinical assessment of PAH patients during follow-up and may guide therapeutic decisions.

We are aware of the limitations of our study. The most important limitations are the small study size and the overall small changes in the parameters measured in some subjects, although statistically significant. In addition, the study was not designed to evaluate the impact of different vasodilator therapies on BNP production. Although we allowed a therapy-free interval before measurements were made, we cannot exclude the possibility that these therapies might have influenced BNP secretion. From our data, we cannot make conclusions about the natural spread of BNP levels noted in our study. However, we observed a change in BNP concentration of at least 20 to 25% over time that was associated with significant changes in the clinical status of the patients. Of note, significant renal failure has to be
excluded as a potentially disturbing factor. Despite these limitations, we think that our observations justify additional studies in a larger cohort to overcome these limitations.

Our data suggest that BNP development during long-term follow-up reflects changes in hemodynamic and exercise parameters, and that repetitive measurements of plasma BNP levels serve as potentially useful markers in the assessment of disease progression and the response to treatment in patients with PAH.

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REFERENCES

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