Chapter 3

Biological serum markers in the management of pediatric pulmonary arterial hypertension

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Submitted
Abstract

Rationale: Appropriate markers are needed for the monitoring of children with pulmonary arterial hypertension. Various biological serum markers have been suggested to be of use in adults with pulmonary arterial hypertension. No data are available on the value of these markers in children with pulmonary arterial hypertension.

Objectives: To study the relation between NT-proBNP, uric acid, norepinephrine and epinephrine and functional parameters and hemodynamic variables in pediatric pulmonary hypertension and to determine the ability of these serum markers to predict survival.

Methods: Serum NT-proBNP, uric acid and cathecholamines were measured and correlated with invasive hemodynamics, functional parameters and outcome in 30 pediatric patients with pulmonary arterial hypertension that visited a tertiary reference center for pediatric pulmonary arterial hypertension between 1997 and 2005.

Results: Serum NT-proBNP correlated with WHO-class \( (R = 0.36; p = 0.03) \) and 6-minute walking distance \( (R = -0.53; p < 0.001) \). Serum uric acid correlated with mean pulmonary arterial pressure, pulmonary vascular resistance and cardiac index \( (R = 0.63, p = 0.01; R = 0.71, p = 0.03 \) and \( R = -0.65, p = 0.007 \) respectively). After initiation of treatment, NT-proBNP levels decreased and this decrease correlated with an increase in 6-minute walking distance. Finally, norepinephrine and NT-proBNP levels were highly predictive for mortality.

Conclusions: In this series of children with pulmonary arterial hypertension, biological serum markers were correlated with hemodynamics and functional capacity, as parameters of disease severity. Furthermore, the data indicate that these markers can be used to monitor treatment effects and predict mortality in pediatric pulmonary arterial hypertension.
Introduction

Pulmonary arterial hypertension (PAH) is a chronic, progressive and usually fatal disease. It is characterized by proliferation of pulmonary vascular cells and obliteration of small pulmonary arteries, leading to increased pulmonary vascular resistance and eventually to right heart failure and death. PAH may present at any age, from infancy into high age. In the last decade, new pharmacological therapies, although not curative, have been demonstrated to improve hemodynamics, exercise capacity and survival in these patients. For optimal clinical decision making, it has therefore become of growing importance to accurately assess disease severity, effectiveness of therapy and prognosis in the individual patient with PAH.

Several correlates of disease severity and survival have been described in adults with PAH. Hemodynamic and functional capacity parameters are currently the cornerstones in characterizing disease progression. Invasively obtainable hemodynamic parameters have been shown to represent disease severity and to predict survival. Non-invasive parameters for functional capacity are used for assessing clinical condition, severity of disease and effectiveness of therapy. Six-minute walking distance (6MWD) has been shown to correlate well with WHO-class, and less well with hemodynamic parameters. Maximal cardiopulmonary exercise testing can also be safely performed in these patients using specific guidelines, and is used with increasing frequency.

In pediatric patients with PAH these parameters have specific drawbacks in their use. As in adults, cardiac catheterization is invasive and associated with specific risks. In the pediatric age group, however, cardiac catheterization is mostly performed under general anesthesia, making repetitive catheterizations for follow-up unattractive. Exercise capacity tests as the 6MWD or maximal exercise tests are often not feasible and less validated in young childhood. Therefore additional parameters to monitor disease severity, prognosis and efficacy of treatment are highly needed in pediatric patients with PAH.

In PAH the neurohumoral axis is activated, as evidenced by elevated circulating levels of brain natriuretic peptide (BNP), N-terminal pro-brain natriuretic peptide (NT-proBNP), catecholamines and other neurohumoral markers. Also uric acid levels have shown to be increased in adults with PAH, both idiopathic and secondary. Studies in adult patients with PAH have suggested that NT-proBNP, norepinephrine and uric acid are correlated with hemodynamic and functional parameters and could be used for monitoring therapy effects and prognosis in these patients. Although appropriate reference values are lacking in children, preliminary data suggest that BNP and NT-proBNP levels are useful in diagnosing and managing pediatric heart failure, congenital heart disease and cardiac transplantation.

In this study we aimed to investigate the value of uric acid, NT-proBNP, epinephrine and norepinephrine in a cohort of children with PAH, with respect to predicting prognosis and monitoring disease severity and effectiveness of therapy as assessed by hemodynamic and functional parameters.
Methods

Patients
Thirty consecutive pediatric patients in whom pulmonary hypertension was diagnosed in a tertiary reference center for pediatric pulmonary hypertension between 1997 and 2005 were followed using a standardized protocol, in which WHO functional class, physical examination, blood withdrawal for biological markers and a 6-minute walking test were performed every 3-6 months. Informed consent was obtained from the parents of all children.

Cardiac catheterization
Cardiac catheterization was performed under general anesthesia at presentation to confirm the diagnosis pulmonary hypertension. In 17 patients concomitant blood samples were collected prior to the catheterization procedure. Complete hemodynamic data were obtained, including systemic and pulmonary blood flows using the Fick method and vascular resistances, indexed for body surface.

Blood sampling and assays
Peripheral venous blood samples were further obtained at each outpatient visit. For prognostic value, serum uric acid was determined at presentation, whereas the other serum markers were collected from 2003, when the role of the neurohumoral axis in pulmonary hypertension became more obvious. Blood samples drawn at start of new therapy (either as first line or add-on therapy) and three months later were used to determine the value of the different serum markers in the assessment of therapeutic efficacy.
To determine NT-proBNP levels, blood was collected in EDTA tubes, transported on ice and stored at -20°C. Plasma NT-proBNP was determined with an Elecsys proBNP assay (generously provided by Roche Diagnostics, Basel, Switzerland). Uric acid and creatinin were measured using standard clinical chemical methods with a MEGA (Merck, Darmstadt, Germany). For determination of catecholamines, samples were stored at -20°C in glass tubes containing glutathione solution and measured using chromatography. All blood samples were drawn after a 10-minute stabilization period of the patient in a horizontal position.

Statistical analysis
All data are expressed as mean value ± standard error of the mean (SEM) unless otherwise indicated. Log transformation was used to normalize the distribution of variables. Correlations between variables were measured using Pearson’s correlation coefficient or, in case of non-continuous variables, Spearman’s rho correlation coefficient. To analyze the relation between the two functional parameters WHO-class and 6MWD and simultaneously derived serum markers, we included the first three visits per patient to increase sample size, leaving 21 patients and 63 data points. The relation between WHO-class and serum markers was obtained by logistic regression analysis, after transformation of the classification scores into
a dichotomous variable (either $< 3$ or $\geq 3$). An estimated glomerular filtration rate (GFR) was calculated for each patient at the time of cardiac catheterization using the formula \((38 \times \text{height (cm)})/\text{creatinin (μmol/l)}\) and the relation between hemodynamics and uric acid was corrected for GFR and diuretic use in a multivariate regression analysis.

To determine the value of the biological markers in relation to effectiveness of therapy, serum levels at the start of treatment were compared with those three months later with a paired sample t-test or a Wilcoxon signed ranks test when data were not normally distributed.

For Kaplan-Meier survival curves, patients were divided into high, middle and low categories, with an equal number of patients in each group. A logrank test based on trends was used. Receiver operating characteristics (ROC) were generated from multiple sensitivity/specificity pairs. All analyses were performed using SPSS© version 12.02 for Windows (SPSS Inc. Chicago, IL). A p-value $< 0.05$ was considered to be significant.

**Results**

The clinical and demographic characteristics of the 30 patients included in this study are provided in table 1. Eighteen children were diagnosed with idiopathic PAH and eleven patients with PAH due to a congenital left to right shunt. Of these, 10 had Eisenmenger syndrome with a post-tricuspid defect: patent arterial duct (PDA) \(n = 2\), ventricular septal defect (VSD) \(n = 7\) (4 of which combined with a PDA), AVSD \(n = 1\). One patient had a surgically corrected truncus arteriosus. Finally, one patient had chronic thromboembolic pulmonary hypertension associated with a ventriculo-atrial drain (table 1). Median age at inclusion was 7.0 years (range 0.1 – 17.3). Girls were slightly older than boys (median age in girls 9.3 (2.9 – 17.3) and in boys 4.0 (0.1 – 15.2), \(p = 0.03\)). Median and range of all serum markers at the first sample collection was as follows: NT-proBNP 138 (27-7589) pg/ml, uric acid 0.29 (0.12-0.56) mmol/l, norepinephrine 1.46 (0.39-9.60) nmol/l, epinephrine 0.19 (0.03-1.68) nmol/l.

Patients were treated as clinically indicated. At the moment of inclusion 20 patients received anticoagulants, 9 patients received diuretics, 7 patients received a calcium-antagonist, 1 patient received digoxin, 6 patients received bosentan, 2 patients received epoprostenol and 1 patient received beraprost sodium. During follow-up, 6 patients were started on anticoagulants, 2 on diuretics, 1 on digoxin, 14 patients were started on bosentan, 4 patients were started on epoprostenol and 1 patient received treatment with sildenafil.

Median follow up was 32 months (range 8-156 months). During this follow-up, nine patients (30%) died as a result of circulatory insufficiency due to progressive right ventricular failure, one of these during massive hemoptysis.
Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>IPAH</th>
<th>PAH associated with congenital systemic-to-pulmonary shunt</th>
<th>Chronic thromboembolic pulmonary hypertension</th>
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<td>11</td>
<td>1</td>
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<td><strong>Median age at inclusion (range)</strong></td>
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<td>5.3 (0.1 – 15.4)</td>
<td>11.8 (1.6 – 17.3)</td>
<td>16.1</td>
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<td><strong>WHO</strong></td>
<td></td>
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<td></td>
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<tr>
<td>I</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
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<td>5</td>
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<td>17</td>
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<tr>
<td>IV</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><strong>Deceased</strong></td>
<td>9 (30%)</td>
<td>6 (33%)</td>
<td>3 (27%)</td>
<td>0 (0%)</td>
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</tbody>
</table>

**Biological serum markers and demographic data**

Uric acid was correlated with age ($R = 0.431$, $p = 0.03$) and sex (males $0.25 \pm 0.02$ mmol/l vs. females $0.32 \pm 0.03$ mmol/l, $p = 0.04$). Uric acid did not differ between patients with idiopathic or other type of PAH. NT-proBNP, norepinephrine and epinephrine did not correlate with sex, age or diagnosis.

**Biological serum markers and disease severity**

**Hemodynamics**

In 17 patients concomitant biological markers and invasive hemodynamic data were available. Serum uric acid correlated with mean pulmonary arterial pressure ($R = 0.63$, $p = 0.01$, figure 2A) and pulmonary vascular resistance ($R = 0.71$, $p = 0.03$, figure 2B). A negative relation with cardiac index was demonstrated ($R = -0.65$, $p = 0.007$, figure 2C). After correction for GFR, these correlations remained basically unchanged ($R = 0.66$, $p = 0.08$; $R = 0.69$, $p = 0.03$ and $R = -0.65$, $p = 0.04$. respectively). Also, after correction for diuretic use, the correlations remained unchanged. Further, a positive correlation between right atrial pressure and norepinephrine was suggested ($R = 0.62$, $p = 0.06$). NT-proBNP and epinephrine levels did not correlate with hemodynamic variables. No correlation could be demonstrated between any of the serum markers and arterial or venous oxygen saturation or hemoglobin levels at cardiac catheterization.
Functional capacity

A higher WHO-classification was associated with a decreased 6-minute walking distance ($R = -0.57$, $p = 0.001$). A higher NT-proBNP was also associated with a higher WHO-classification (logistic regression $R = 0.36$, $p = 0.03$; figure 1A). NT-proBNP correlated with 6-minute walking distance as tested on the day of blood sampling ($R = -0.527$, $p < 0.001$, figure 1B). No correlations could be demonstrated between the other biological markers and these functional parameters.

**Biological serum markers and treatment effect**

In 13 patients, biological markers, WHO-class and 6MWD were compared at initiation of therapy with either bosentan ($n = 10$), epoprostenol ($n = 2$) or sildena-
Figure 2. Relation between serum markers and hemodynamics. Serum uric acid and 
A) mean pulmonary arterial pressure,  
B) pulmonary vascular resistance,  
C) cardiac output.
Figure 3. Kaplan-Meier survival curves for the different biochemical markers. Cumulative survival estimated by Kaplan-Meier curves for NT-proBNP, A) p = 0.004) for uric acid, B) p = 0.01) and for norepinephrine, C) p = 0.04) . P-values are derived from overall log rank testing for trends.
Figure 4. ROC-analysis. ROC-analysis for serum NT-proBNP A), uric acid B) and norepinephrine C) in the prediction of death in pediatric patients with PAH.
fil (n = 1) and three months later. NT-proBNP levels were significantly decreased after three months of therapy compared to levels prior to therapy (mean ± SEM 631 ± 228 pg/ml vs 1484 ± 600 pg/ml respectively, p = 0.016). In these patients 6MWD increased more than 50 meters (302 ± 32 m prior to therapy vs. 367 ± 30 after three months of treatment, p = 0.03) and WHO-class improved (3.0 ± 0.1 prior to therapy vs. 2.5 ± 0.1 after three months of treatment, p = 0.006). The size of the change in NT-proBNP was related to the size of the change in 6MWD (R = -0.63, p = 0.04) and in WHO class (R = 0.72, p = 0.02). No changes in the levels of uric acid, norepinephrine and epinephrine could be demonstrated after three months of treatment.

**Biological serum marker and prognosis**

Of the 30 patients studied, 9 (30%) died during follow-up, 3 boys and 6 girls (table 1). The Kaplan-Meier survival curves of the different serum markers are shown in figure 3. High NT-proBNP, uric acid and norepinephrine levels all predicted increased mortality. ROC-curves for NT-proBNP, uric acid and norepinephrine predicting mortality are provided in figure 4. NT-proBNP and norepinephrine both had an area under the curve > 0.85. When using a cut-off value of 1664 pg/ml for NT-proBNP, the test would have a 100% sensitivity and 94% specificity in predicting mortality. Norepinephrine with a cut-off of 1.63 nmol/l had a 80% sensitivity and 77% specificity in predicting mortality. The area under the ROC curve for uric acid was 0.65 and did not differ significantly from 0.5.

**Discussion**

This study demonstrates that serum levels of NT-proBNP, uric acid and norepinephrine can be used for the assessment of disease severity, prognosis and effectiveness of therapy in children with PAH. Serum uric acid levels correlated with invasively obtained hemodynamic data, NT-proBNP levels correlated with functional outcome parameters as WHO-class and 6-minute walking distance. NT-proBNP levels decreased after initiation of therapy and this decrease correlated with the functional response to the initiation of this treatment. Furthermore, serum levels of NT-proBNP and norepinephrine were highly predictive for mortality in the individual patient.

The neurohumoral axis is known to be activated in conditions characterized by abnormal loading conditions of the cardiovascular system, including heart failure and congenital heart disease. Therefore, it has been suggested that serum levels of neurohormones may function as biological markers for disease progression and prognosis. NT-pro-BNP is generated in case of ventricular stretch from its precursor proBNP by cleavage. Sympathetic activation occurs in different types of cardiovascular disease and is evidenced by increased epinephrine and norepinephrine serum levels. These markers have been shown to be useful in the detection and diagnosis of heart failure.
and to predict morbidity and mortality in different cardiovascular conditions \[13-17\]. Furthermore, an increased serum level of uric acid has been demonstrated to be associated with morbidity and mortality in heart failure and in congenital heart disease \[18-21\]. The mechanism behind the increased uric acid in heart failure patients warrants further discussion. Increased serum uric acid in heart failure seems to be caused by the activity of xantine oxidase. Xantine oxidase is the enzyme that catalyzes the oxidation of xanthine to uric acid. In patients with heart failure, inhibition of xantine oxidase activity with allopurinol improved vascular function \[12\], indicating that xantine oxidase activity is directly contributing to the pathogenetic process in heart failure patients. Since uric acid has been shown to be increased in pulmonary hypertensive patients \[11\], xantine oxidase activity may also be a mediator in the pathobiological mechanisms of pulmonary hypertension.

### Disease severity

Disease severity in PAH is characterized by hemodynamics and functional capacity. Hemodynamic parameters, including right atrial pressure, pulmonary arterial pressure, pulmonary vascular resistance and cardiac index, are considered indicators for the severity of disease in PAH and have been associated with prognosis \[1;23;24\]. In the present study, of all biological markers investigated, uric acid level displayed the strongest correlations with invasive hemodynamics. It correlated with mean pulmonary arterial pressure, pulmonary vascular resistance and cardiac index, but not with right atrial pressure. To exclude the possibility that hyperuricemia was caused by impaired kidney function or diuretic use, a multivariate analysis was performed to correct for these variables. However, the correlations appeared to be independent of these factors in our pediatric series. In adult PAH patients, uric acid has been previously reported to be correlated with right atrial pressure \[10;25\], pulmonary vascular resistance \[11\] and cardiac index \[11;25\].

Norepinephrine levels have also been described to correlate with hemodynamic parameters in adult PAH, although reported data were not always consistent. Nootens et al found a significant correlation between norepinephrine levels and pulmonary arterial pressures, pulmonary vascular resistance and cardiac index in 21 adult patients with idiopathic PAH, whereas Nagaya et al could demonstrate a correlation only with cardiac index \[6;9\]. In the current pediatric study, we found that norepinephrine tended to correlate with right atrial pressure, but not with other hemodynamic parameters.

No correlations between serum levels of NT-proBNP and hemodynamic parameters could be demonstrated in the current study. This is in contrast with studies in adult patients with PAH and systemic sclerosis, in which NT-proBNP was found to correlate with pulmonary arterial pressure \[26;27\]. Similarly to our data, Fijalkowska et al could not demonstrate a relation with pulmonary arterial pressure in patients with idiopathic PAH \[7\].

Correlations of NT-proBNP levels with right atrial pressure, pulmonary vascular resistance and cardiac index have been described in adults with PAH \[7;27;28\].

### Functional capacity

We found NT-proBNP to correlate well with the functional status of
pediatric PAH patients, characterized by WHO functional class and 6-minute walking distance. This is in concordance with findings in adult patients, in whom also correlations with these parameters have been described \(^7\). At present, 6MWD is generally accepted as a valuable parameter for clinical status and a predictor for outcome in PAH patients. In pediatric patients, the 6MWD is not always feasible because of young age or lack of co-operation. Furthermore, 6MWD has not been validated in children younger than 8 years of age. Our findings indicate that NT-proBNP serum levels may form a substitute for the 6MWD in pediatric patients with PAH. Serum levels of uric acid and norepinephrine did not correlate with parameters of functional class in our study. In contrast, both uric acid and norepinephrine have been reported to be correlated with WHO functional class in adult patients with PAH \(^9\).

**Prognosis**

Increased levels of NT-proBNP have been associated with poor long-term prognosis in adults with pulmonary hypertension. Fijalkovska et al reported that a serum NT-proBNP higher than 1400 pg/ml identified patients with a poor long-term prognosis with a 88% specificity and a 53% specificity \(^7\). In this pediatric study, 5 of 6 patients (83%) with a NT-proBNP level higher than this level died within two years, while all patients with a level below 1400 pg/ml survived. When we used a cut-off value of 1664 pg/ml, sensitivity and specificity for predicting mortality could even be improved to 100% and 94% respectively. In other words, in this study NT-proBNP showed to be an excellent predictor of mortality that can be used in the individual pediatric patient with PAH.

Norepinephrine serum levels also correlated significantly with mortality in our study. Although not as strong as NT-proBNP, the area under the ROC curve was 0.85, indicating that norepinephrine serum level was a good predictor of mortality in this population. These findings are in congruency with data in adult patients with iPAH as reported by Nagaya and coworkers. These authors also found increased norepinephrine levels to be correlated with mortality \(^6\). In contrast, no correlation could be demonstrated between epinephrine levels and mortality. This is in congruency with findings in adults with PAH \(^8\).

Serum uric acid level has also been described as a prognostic factor in adult PAH \(^29\). In the current pediatric series, serum uric acid levels also appeared to be correlated with mortality. However, the ROC-curve showed that uric acid level was less valuable in predicting mortality in the individual child.

It should be noted that, in the current study, the different biological markers predicted mortality, irrespective of the received treatment.

**Treatment effect**

Additionally, NT-proBNP appeared to be useful in monitoring treatment effects in pediatric patients with PAH, since treatment was associated with a decrease in serum NT-proBNP levels and this decrease correlated inversely with improvement in 6MWD. The number of patients and observations in this study did not allow to answer the question if the magnitude of changes in NT-proBNP serum levels after
initiation of therapy was predictive for outcome or affected the value of baseline NT-proBNP level in predicting mortality in the individual patient. Our data indicate that NT-proBNP may be used to evaluate the effect of treatment in children with PAH. This is of especially great importance in the youngest patients in whom the accepted clinical endpoints applied for PAH are often not feasible to obtain.

**Limitations of this study**

No healthy control group was used in this study. Normal values for serum levels of the described biological markers in children are not fully established. Therefore, the value of biological markers in diagnosing the presence of PAH in children could not be determined in this study. However, the value of these markers as correlates for disease severity, prognosis and treatment effect in the specific condition of pediatric PAH could be established.

Since the levels of the investigated biological serum markers did not correlate with diagnosis and no differences in demographic variables and survival could be demonstrated between patients with iPAH or congenital shunts, we chose to analyze all patients as one group. Therefore, potential differences in serum marker correlates between these diagnosis groups could have been missed in the current study.

The 6-minute walking test has not been validated in children younger than 8 years of age. Lack of co-operation at younger age may hamper its results. Seven of our patients underwent at least one 6-minute walking test between 5 and 7 years of age. In our experience, familiarizing the young child with the test by training can result in reproducible 6MWD assessments from the age of 5 years.

**Conclusion**

This study demonstrates that serum levels of NT-proBNP, uric acid and norepinephrine can be used for the assessment of disease severity, prognosis and effectiveness of therapy in children with PAH. These biological serum markers may therefore allow to support clinical decision making in the management of the individual child with PAH, especially in those in whom obtaining accepted endpoint parameters is not feasible (exercise capacity tests) or unattractive (repeated invasive hemodynamic evaluation). Further prospective studies are warranted to assess the predictive value of treatment induced changes in the serum levels of these markers.
References


