Prognostic factors, treatment goals and clinical endpoints in pediatric pulmonary arterial hypertension
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Chapter 6

Serially measured uric acid levels predict disease severity and outcome in pediatric pulmonary arterial hypertension

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ABSTRACT

Background
Optimal clinical decision-making in the treatment of paediatric pulmonary arterial hypertension (PAH) requires a reliable and non-invasive biomarker. Uric acid has been suggested to be such a candidate. We aimed to evaluate the association of serum uric acid with disease severity and outcome during the full disease course of paediatric PAH.

Methods and results
Eighty-one consecutive children from the Dutch National Network for Paediatric Pulmonary Hypertension underwent prospective follow-up in accordance with a standardized protocol. During a median follow-up of 3.9 years, 860 serum uric acid measurements were collected. Uric acid levels differed significantly from healthy controls (p<0.001). Taking into account all measurements during follow-up, higher uric acid levels were associated with higher WHO functional class, N-terminal pro brain natriuretic peptide, lower tricuspid annular plane systolic excursion and higher mortality (p=0.007, p<0.001, p<0.001 and p<0.001, respectively). Non-survivors showed a significantly steeper increase in uric acid levels during follow-up (p=0.001) and increases of ≥50% were associated with a 3.9 times higher risk of death or lung-transplantation (p=0.005).

Conclusion
This study demonstrates that higher serum uric acid levels are associated with disease severity and mortality in children with PAH, throughout the full disease course. Monitoring absolute values and changes of uric acid levels provides valuable information and could help guide decisions in the management of paediatric PAH.
INTRODUCTION

Pulmonary arterial hypertension (PAH) is a serious disease, ultimately causing an early death of affected individuals. As a consequence of vascular remodelling processes, the pulmonary vascular resistance increases and pulmonary hypertension develops. Over time, the attempt to compensate for the increased resistance overwhelms the capacity of the right ventricle, resulting in right heart failure and premature death.2,3

To ensure optimal monitoring and clinical decision-making, reliable markers of disease severity and outcome are needed. For a delicate patient population such as children, there is a preference for non-invasive and easily obtainable biomarkers.4,5 Previous observations have provided clues that serum uric acid could fulfil such a role.6-8 Uric acid is the end product of purine degradation by xanthine oxidase and high levels have been implicated in many cardiovascular diseases.9,10 In clinical states of tissue hypoxia, such as chronic heart failure,11 and cyanotic congenital heart disease,12 high levels of uric acid have been observed. Hyperuricemia is likely to reflect impairment in energy generation, which might be linked to the severity of the disease.6,11 Besides its value as a disease marker, uric acid might also provide a contributing factor to the progression of cardiovascular diseases.13 In high-risk patients with acute coronary syndrome and heart failure, hyperuricemia is a known risk factor for mortality, through its incitement of endothelial dysfunction and impaired vasodilation.14-16 In adult PAH studies, it has been confirmed that baseline acid levels correlate with disease severity and outcome.6,7,17,18 Also in cross-sectional observations in children with PAH, baseline levels of uric acid have previously been shown to correlate with outcome.8,19,20 However, associations with serial uric acid values collected during long-time follow-up to evaluate the time-dependent relationship with outcome are lacking.

A reliable biomarker should reflect the disease process and is expected to fluctuate according to the severity of the disease. Candidate biomarkers require extensive longitudinal evaluation in well-defined cohorts prior to incorporation in clinical practice. Therefore, the aim of this study was to evaluate the association of uric acid levels, measured both at baseline and longitudinally during the course of the disease, with disease severity and outcome in children with PAH.

METHODS

Study design and population

This is a longitudinal study of data from a prospective clinical registry. In the Netherlands, all children with PAH are referred to the University Medical Center Groningen (UMCG), the expert center of the Dutch National Network for Paediatric Pulmonary
Hypertension. All patients are followed and registered prospectively according to a standardized protocol. Ethical approval for this ongoing registry was obtained from the Institutional Review Board and informed consent was obtained from the patients and/or their guardians. In the current study, all children with PAH who were enrolled between July 1997 and March 2015 and who had at least one uric acid value available were included. Diagnosis of PAH was confirmed with heart catheterization and defined as mean pulmonary artery pressure (mPAP) ≥25 mmHg, pulmonary vascular resistance index (PVRi) ≥3 WU*m² and mean pulmonary capillary wedge pressure ≤15 mmHg. In cases of clinical instability, diagnosis was made by echocardiography, defined as either the presence of right-to-left shunting in the case of congenital heart defects or a maximum systolic tricuspid regurgitant velocity >2.8 m/s accompanied by septal flattening and/or right ventricular hypertrophy.

Data collection
Diagnostic work-up included full hemodynamic evaluation with vasoreactivity testing. Diagnostic evaluation and standardized clinical follow-up every 3-12 months, further included assessment of WHO functional class (WHO-FC), 6-minute walking distance (6MWD), echocardiographic evaluation including measurement of tricuspid annular plane systolic excursion (TAPSE), and blood sampling including uric acid, creatinine and NT-proBNP.

As part of standardized clinical practice, venous blood samples were collected in lithium- and heparin-containing tubes. Serum uric acid concentrations were measured using the MEGA clinical chemistry analyzer (Merck, Darmstadt, Germany), the VITROS chemistry system (Ortho-Clinical Diagnostics, Inc., Johnson & Johnson Co., Raritan, NJ, USA) or the Modular analyzer (Roche Diagnostics, Mannheim, Germany). All three types of analyzers use the indirect equilibrium uricase method. This colorimetric uricase-catalyzed reaction causes uric acid to transform into hydrogen peroxide (amongst other products), which can then be quantified by measuring its absorbance with spectrophotometry.

Outcome was defined as death/lung-transplantation. The observation time was calculated from the first measurement of uric acid until death/lung-transplantation or until the last follow-up visit before March 2015.

Data analysis
Statistical analysis was conducted using IBM SPSS 20.0 (Armonk, NY, USA), STATA 11.0 (StataCorp., College Station, Texas, USA) and RStudio (www.rstudio.com). Normally distributed continuous variables are presented as mean ± standard deviation. Continuous non-normally distributed variables were normalized using log-transformation. Categorical variables are presented as absolute numbers (percentage). P-values of ≤0.05 were
considered statistically significant. As uric acid levels have been shown to depend on age and sex,\textsuperscript{24} and renal excretion,\textsuperscript{9} analyses were repeated with adjustment for age, sex and creatinine.

\textit{Disease severity analyses.}
To determine the association of baseline uric acid levels with disease severity markers, correlation coefficients were calculated. To evaluate this association during the course of the disease, including all longitudinal data, the predictive value of uric acid for three clinical markers (WHO-FC, NT-proBNP or TAPSE Z-score), which have an important prognostic value in PAH,\textsuperscript{25} was separately analysed using linear mixed effects modelling with random intercept and slope.

\textit{Outcome analyses.}
To determine the association of baseline uric acid levels with outcome, Cox regression analysis was performed. Kaplan Meier analysis with log-rank testing was performed to compare transplantation-free survival of patients with high or low baseline uric acid levels. To evaluate this association during the course of the disease, the predictive value of time-varying uric acid levels was analysed using time-dependent Cox regression. Two outcome models were created: one including all follow-up levels uric acid as time-varying covariate, and another one with the occurrence of a ≥50\% increase in uric acid since baseline as a dichotomous time-varying covariate. As a ceiling effect could distort the latter analysis, with uric acid barely being able to increase further because of already high levels, all hazard ratios derived from this model were adjusted for baseline uric acid.

Furthermore, the linear development of uric acid over time was compared between survivors and non-survivors using linear mixed effects modelling. A model was created with random patient intercepts, with uric acid as dependent variable and observation time, transplantation-free survival and the interaction of observation time with survival status at last visit as independent variables. This yielded separate intercepts and slope estimates for the survivors and non-survivors, with the ability to evaluate the statistical significance of the difference of the intercepts and slopes between these respective groups.

\section*{RESULTS}

\textbf{Patient characteristics at baseline}
Eighty-one patients (35 males and 46 females) diagnosed with PAH were included in this study. 46 patients (57\%) had either idiopathic or heritable PAH, while 27 (33\%) had PAH associated with congenital heart disease and 8 (10\%) other types of PAH (Table 1). Uric
Acid levels were significantly higher compared to levels healthy controls (mean Z-score deviation +1.36, 95% CI 0.91-1.82, one-sample T-test p<0.001). 41

Association of uric acid levels with disease severity

Table 2 shows the correlations between disease severity markers and baseline uric acid values. Serum uric acid levels measured at baseline showed significant positive correlations with age (r=0.29; p=0.009), creatinine (r=0.45; p<0.001), WHO-FC (r=0.31; p=0.005), NT-proBNP (r=0.31; p=0.014), mean right atrial pressure (r=0.41; p=0.003), mean pulmonary arterial pressure (r=0.31; p=0.025), the ratio between mean pulmonary and systemic arterial pressure (r=0.39; p=0.004) and pulmonary vascular resistance index.
(r=0.41; p=0.003) and negative correlations with TAPSE Z-score (r=-0.30; p=0.027) and mixed venous saturation (r=-0.37; p=0.008). With the exception of mPAP, these associations tended to remain significant after adjusting for age, sex and creatinine.

During a median (IQR) follow-up of 3.9 (0.8-7.5) years, serum uric acid levels were measured repeatedly, yielding a total of 860 uric acid measurements from 81 patients. The results of linear mixed effects modelling involving all follow-up time points are shown as three disease severity models in Table 3, with WHO-FC, NT-proBNP and TAPSE Z-scores as the respective dependent outcome variables. Higher uric acid levels were significantly associated with higher WHO-FC (p=0.007), higher NT-proBNP (p<0.001) and lower TAPSE Z-scores (p<0.001). These associations tended to remain significant after adjusting for age, sex and creatinine.

Table 2. Correlations of Uric Acid With Disease Severity Markers at Baseline

<table>
<thead>
<tr>
<th></th>
<th>Univariable Analysis</th>
<th>Adjusted for Age, Sex and Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n  r       p value</td>
<td>n  r       p value</td>
</tr>
<tr>
<td><strong>Clinical Characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPAH/HPAH (vs.associated PAH)</td>
<td>81  0.04  0.714</td>
<td>71  0.05  0.660</td>
</tr>
<tr>
<td>Female*</td>
<td>81 -0.10  0.381</td>
<td>N.A.</td>
</tr>
<tr>
<td>Age at baseline</td>
<td>81  0.29  0.009</td>
<td>N.A.</td>
</tr>
<tr>
<td>WHO-FC§</td>
<td>80  0.31  0.005</td>
<td>70  0.36  0.002</td>
</tr>
<tr>
<td>Creatinine</td>
<td>71  0.45 &lt;0.001</td>
<td>N.A.</td>
</tr>
<tr>
<td>Log NT-proBNP</td>
<td>61  0.31  0.014</td>
<td>54  0.27  0.050</td>
</tr>
<tr>
<td>TAPSE Z-score</td>
<td>54 -0.30  0.027</td>
<td>52 -0.26  0.067</td>
</tr>
<tr>
<td><strong>Hemodynamic Characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed venous oxygen saturation</td>
<td>50 -0.37  0.008</td>
<td>46 -0.41  0.006</td>
</tr>
<tr>
<td>Mean right atrial pressure</td>
<td>52  0.41  0.003</td>
<td>48  0.40  0.006</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure</td>
<td>52  0.31  0.025</td>
<td>48  0.21  0.159</td>
</tr>
<tr>
<td>mPAP/mSAP</td>
<td>52  0.39  0.004</td>
<td>48  0.34  0.020</td>
</tr>
<tr>
<td>PVR index</td>
<td>51  0.41  0.003</td>
<td>47  0.29  0.055</td>
</tr>
<tr>
<td>PVR/SVR</td>
<td>51  0.25  0.072</td>
<td>47  0.22  0.146</td>
</tr>
<tr>
<td>Cardiac index</td>
<td>51 -0.02  0.875</td>
<td>47  0.00  0.999</td>
</tr>
<tr>
<td>Qp/Qs</td>
<td>51 -0.22  0.128</td>
<td>47 -0.16  0.306</td>
</tr>
</tbody>
</table>

Data presented as Pearson correlation coefficient r (univariable analysis) or partial correlation coefficient r (adjusted analysis), unless otherwise indicated. Definition of abbreviations: PAH = pulmonary arterial hypertension; IPAH = idiopathic PAH; HPAH = hereditary PAH; WHO-FC = World Health Organization functional class; NT-proBNP = N-terminal pro brain natriuretic peptide; TAPSE = tricuspid annular plane systolic excursion; mPAP/mSAP = pulmonary-to-systemic arterial pressure ratio; PVR/SVR = pulmonary-to-systemic vascular resistance ratio; Qp/Qs = pulmonary-to-systemic flow ratio; N.A. = not applicable. *Point-Biserial Correlation, §Spearman correlation coefficient.
Table 3. Association of Serially Measured Uric Acid with Disease Severity Markers and Outcome.

<table>
<thead>
<tr>
<th>Disease severity markers</th>
<th>Univariable Analysis</th>
<th>Adjusted for Age, Sex, Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n* β/HR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>WHO-FC†</td>
<td>785/80 0.09 (0.03 to 0.16)</td>
<td>0.007</td>
</tr>
<tr>
<td>NT-proBNP, log-transformed†</td>
<td>751/61 0.19 (0.14 to 0.24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TAPSE, Z-score†</td>
<td>563/54 -0.46 (-0.69 to -0.23)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Outcome

| Predictive value of time-varying UA for death/LTx‡ | 860/81 1.78 (1.40 to 2.26) | <0.001 | 831/79 1.52 (1.13 to 2.04) | 0.006 |
| Predictive value of ≥ 50% UA increase for death/LTx§ | 860/81 3.94 (1.51 to 10.27) | 0.005 | 831/71 3.63 (1.22 to 10.77) | 0.020 |

Definition of abbreviations: HR = hazard ratio; CI = confidence interval; UA = uric acid; WHO-FC = World Health Organization functional class; NT-proBNP = N-terminal pro B-type natriuretic peptide; TAPSE = tricuspid annular plane systolic excursion; LTx = lung-transplantation. Data presented as β or HR (hazard ratio) with respective 95% confidence intervals per 0.1 unit change of uric acid. P-values ≤0.05 were considered statistically significant. *Number of uric acid measurements involved in analysis/ number of patients involved in analysis. †All follow-up measurements of the patients were analyzed using linear mixed effects modeling with correcting for repeated measurements. Uric acid was added as predictor variable to separate starting models consisting of disease severity markers as dependent variable, with random intercepts. ‡Time-dependent Cox regression analysis with longitudinally collected uric acid as time-varying predictor of outcome. §Segmented time-dependent Cox regression with the occurrence of a ≥ 50% increase in uric acid since baseline tested as time-varying predictor of outcome, adjusted for level of uric acid at baseline.
Association of uric acid levels with outcome

Baseline uric acid was significantly associated with death or lung-transplantation (HR=1.81, 95% CI 1.35-2.43, p<0.001), also after adjusting for age, sex and creatinine (HR 1.63, 95% CI 1.17-2.28, p=0.004). Figure 1 panel A illustrates that patients with higher uric acid levels at baseline (≥0.41 mmol/L) had a significantly worse transplantation-free survival rate when compared to patients with lower uric acid levels at baseline (log rank, p<0.001). The cut-off of 0.41 mmol/L was identified as a threshold with high specificity.

Figure 1. (A) Lung transplantation-free survival of patients with baseline uric acid levels above or below 0.41 mmol/L. (B) Time-dependent receiver operating characteristics analysis of uric acid levels for survival status at 10-year follow-up (analyzed with the TimeROC package in R). Area under the curve: 0.78 (SE 0.07). The optimal threshold value when maximizing specificity and positive predictive value was estimated at 0.41 mmol/L. Sensitivity: 0.34 (SE 0.08), Specificity: 0.92 (SE 0.07), Positive predictive value: 0.85 (SE 0.13), Negative predictive value: 0.53 (SE 0.08). (C and D) Development of uric acid levels over time stratified by survival status at end of follow-up. Estimates of intercepts and slopes are derived from random linear mixed effects modelling of uric acid over time, with transplantation-free survival tested as time-interaction. Uric acid intercept and slope were significantly higher in non-survivors compared with survivors (P=0.003 and P=0.035, respectively).
for distinguishing survivors from non-survivors at 10 year follow-up (determined using receiver operating characteristics analysis, see Figure 1 panel B).

Table 3 additionally shows the results of the time-dependent Cox regression analysis, with all uric acid measurements collected during follow-up as time-varying covariate. The serially measured uric acid values were significantly associated with death or lung-transplantation (HR=1.78, p≤0.001), also after adjusting for age, sex and creatinine. Increases of ≥50% in uric acid compared to baseline, occurred in 18 of 81 (22%) cases after a median follow-up time of 2.99 (1.44 - 7.21) years. An event of ≥50% increase, independent of time point of occurrence, was associated with an almost 4-times higher chance of adverse outcome (HR= 3.94, p=0.005), also when adjusted for age, sex and creatinine.

Based on the results from linear mixed effects modelling, there was a significant positive linear trend in uric acid levels over time in the total cohort during the full observation period (0.0081mmol/L per year increase, p=0.003). Panels C and D of Figure 1 depict the intercepts and regression coefficient derived from the linear mixed effect modelling of uric acid development over time, stratified by survival status at end of follow-up. The longitudinal development of uric acid levels differed significantly between transplant-free survivors and non-survivors (p=0.035). Non-survivors had higher baseline values and a steeper increase (0.3377 mmol/L at baseline and +0.0148 mmol/L per year increase) compared to survivors (0.2689 mmol/L at baseline and +0.0043 mmol/L per year increase).

Effects of treatment
An exploratory before-after comparison was conducted in a subgroup of 53 children in whom therapy was started or escalated during the study period. Uric acid measured at a median (IQR) duration of 1.7 (1.2-3.1) months after therapy onset, was significantly lower than uric acid levels measured just before therapy onset (0.29±0.09 mmol/L compared to 0.31±0.10 mmol/L, paired samples T-test p=0.035).

DISCUSSION

This study demonstrates that in children with PAH, uric acid serum levels correlate with disease severity markers (including WHO-FC, NT-proBNP levels and TAPSE Z-score), both at baseline as well as throughout the course of the disease. Moreover, absolute serum levels of uric acid at baseline and, importantly, also changes in uric acid serum levels during the disease course are associated with transplant-free survival.

Previous studies have demonstrated that hyperuricemia is common in pulmonary hypertension. Increases of serum uric acid levels can result from several mecha-
nisms, with increased activation of xanthine oxidase caused by oxidative stress or local or systemic tissue hypoxia being the most likely in the setting of vascular and cardiac dysfunction. Interestingly, it has been speculated that elevated uric acid levels are not only a consequence of pulmonary hypertension, but may also have a secondary role in the pathogenesis, contributing to progression of the disease. Chronic hyperuricemia has been shown to directly induce endothelial dysfunction, thereby facilitating vascular remodeling, and to stimulate vasoconstrictive, and proinflammatory effects. Its role in the pathophysiology, irrespective of whether or not being pathogenic, makes uric acid a suitable candidate in the quest for prognostic biomarkers in PAH.

A cross-sectional association of uric acid levels with disease severity and outcome has been demonstrated in various PAH populations including children. Recently, Wagner et al. evaluated the prognostic value of 27 candidate biomarkers in children with PAH, and found uric acid among the strongest predictors of outcome. The current longitudinal analysis confirms the previously reported correlations and extends these by revealing the prognostic value of serially measured uric acid levels throughout the course of the disease. This is the first study to provide evidence that the rate of increases of uric acid levels over time, obtained by repeated measurements, is predictive for the disease severity status and predictive for disease outcome. The potential of uric acid as a prognostic biomarker is further reinforced by our observation that patients in whom uric acid values increased by more than 50% during follow-up had an almost 4-times higher risk of adverse outcome compared to those who did not show such increases.

Uric acid has been suggested to be a potentially modifiable risk factor in pulmonary hypertension. Our preliminary finding that treatment may influence uric acid levels is in line with adult reports showing that PAH-targeted therapies may reduce serum uric acid levels. In chronic heart failure, but also in PAH, treatment-induced reduction of serum uric acid values have been demonstrated to improve endothelial function and correlate with better outcome. Our data, showing that deterioration of uric acid serum levels were associated with worse outcome, support this concept of uric acid as a treatable prognostic factor or marker in PAH.

The fact that not only absolute values, at any time during the disease, but also changes in serum levels of uric acid carry prognostic value, indicates that uric acid is not only a useful prognostic biomarker, but may also qualify as a treatment target for the guidance of clinical management or as a surrogate endpoint in clinical trials.

Clinical implications

The available data provides evidence that serum uric acid is a valuable marker for estimating disease severity and prediction of outcome in the management of children with PAH. Uric acid values are predictive at baseline and at any time point of measurement throughout the disease course. Moreover, higher rates of increase of uric acid levels
over time correlate with PAH disease progression and increased risk for death or lung-transplantation. Closely monitoring the uric acid levels and especially their course over time in patients with PAH provides clues to understanding and predicting the state of the disease. Therefore, uric acid level monitoring may form a valuable addition to the current management of children with PAH and could improve clinical decision-making.

**Strengths and limitations**

A strong feature of this study is the standardized collection of data in this cohort during a substantial period of time, as well as the consistent and complete follow-up. Also, the prospective collection of the data allowed evaluating changes over time, giving it a robust explanatory power. In the adult population serum uric acid levels are influenced by various comorbidities such as metabolic syndrome or renal disease.\(^{16,30}\) These comorbidities are much less likely to play a role in children, making uric acid levels less vulnerable to bias in the paediatric population.

The sample size of the current study hampered extensive multivariable modeling. However, the number of included patients is not small for a rare disease such as paediatric PAH. In view of the invasiveness and associated risks of heart catheterization,\(^{40}\) the collection of hemodynamic data, additional to those collected at diagnosis, was limited to follow up catheterizations that were regarded to be clinically indicated in the individual patients. Therefore the relationship of uric acid with hemodynamics could not be evaluated in a longitudinal fashion.

**CONCLUSION**

This study demonstrates that the longitudinal development of serial serum uric acid levels is consistently associated with disease severity markers and clinical outcome in children with PAH. Higher serum uric acid levels at baseline, as well as increases during the course of the disease correspond to more serious disease severity and poorer prognosis in children with PAH. Closely monitoring uric acid levels and especially their course over time provides important information on the state of the disease and may aid in clinical decision-making in the management of children with PAH.
REFERENCES


SUPPLEMENTARY MATERIAL

To the Editor:

Pediatric pulmonary arterial hypertension (PAH) is a serious disease, ultimately causing an early death of affected individuals. To ensure optimal monitoring and clinical decision-making, reliable markers of disease severity and outcome are highly needed. Previous observations have suggested that serum uric acid, a degradation product of purine metabolism, has potential as a non-invasive, inexpensive and easily obtainable biomarker in PAH. Uric acid is increased in oxidative stress conditions such as vascular and cardiac dysfunction, including chronic heart failure, cyanotic congenital heart disease, and also PAH. In cross-sectional observations in children with PAH, baseline levels of uric acid have previously been shown to correlate with outcome in two independent pediatric cohorts. Since a reliable biomarker should reflect the disease process and fluctuate according to the course of the disease, additional long-term follow-up data on longitudinal trends and time-dependent associations are required. In this study, we therefore evaluated the association of serially measured uric acid levels, with disease severity and outcome in children with PAH.

We analyzed longitudinal uric acid data from 81 children who were consecutively enrolled in the prospective clinical registry of the National Referral Center for Pediatric Pulmonary Hypertension in The Netherlands. In this ongoing registry, all Dutch children with PAH are followed and registered prospectively according to a standardized protocol, with ethical approval from the Institutional Review Board of the University Medical Center Groningen and informed consent obtained from the children and/or their guardians. The inclusion criteria for this study were: confirmation of PAH diagnosis by heart catheterization (n=74) or echocardiography (n=7) according to current international guidelines, availability of a minimum of one uric acid measurement within three months of diagnosis and enrollment between July 1997 and March 2015.

Baseline characteristics of the cohort are shown in Table 1. During a median (IQR) follow-up of 3.9 (0.8-7.5) years, serum uric acid levels were measured repeatedly, yielding a total of 860 uric acid measurements. World Health Organization functional class (WHO-FC), N-terminal Pro-B-type natriuretic peptide (NT-proBNP) and tricuspid annular plane systolic excursion (TAPSE) were serially and concurrently collected and were defined as markers of disease severity on the basis of previous research. The outcome endpoint was defined as death or lung-transplantation, which occurred in 41 children (32 [40%] died, 9 [11%] underwent lung-transplantation). As uric acid levels have been shown to depend on age, sex and renal excretion, we adjusted significant results for age, sex and creatinine.

Uric acid levels differed significantly from healthy controls (mean Z-score deviation +1.36, 95% CI 0.91-1.82, one-sample T-test p<0.001). Higher baseline levels of uric
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acid correlated with higher WHO-FC (n=80, r=0.31, p=0.005), higher NT-proBNP (n=61, r=0.31, p=0.014), and lower TAPSE Z-scores (n=54, r=-0.30, p=0.027), and were predictive of adverse outcome (HR=1.81, p<0.001, and see Kaplan Meier analysis Figure 1A). The adjustment analysis yielded similar results. Uric acid at baseline also correlated with hemodynamic characteristics. To evaluate whether uric acid also correlated with disease severity markers throughout the course of the disease, associations with WHO-FC, NT-proBNP or TAPSE Z-score were analyzed separately using linear mixed effects modeling with random intercept and slope. These analyses, involving all longitudinally collected uric acid and disease severity data, for the first time confirm the association of uric acid with disease severity throughout the disease course (Table 3). In order to evaluate the association of serially measured uric acid with outcome, time-dependent Cox regression was performed, which showed a significant association with outcome throughout the disease course, independent of age, sex and creatinine (831 measurements from 79 patients, adjusted HR 1.52, p=0.006). Time-dependent receiver operating characteristics analysis yielded an area under the curve (AUC) of 0.78 for the predictive accuracy of uric acid (compared to an AUC of 0.81 for NT-proBNP in this cohort). A level of 0.41 mmol/L was identified as a threshold with high specificity for distinguishing survivors from non-survivors at 10-year follow-up (Figure 1B). The positive and negative predictive values of this threshold were 0.85 and 0.53, indicating that mortality was observed in a very high proportion of patients above the threshold, but that patients below the threshold were not excluded from the risk of death either. Thus, uric acid ≥0.41 mmol/L could be considered as a red flag, warranting immediate attention and action of caregivers, bearing in mind that levels <0.41 mmol/L are no guarantee of survival.

To evaluate potential effects of PAH-targeted treatment on uric acid levels, an exploratory before-after comparison was conducted in a subgroup of 53 children in whom therapy was started or escalated during the study period. Uric acid measured at a median (IQR) duration of 1.7 (1.2-3.1) months after therapy onset, was significantly lower than uric acid levels measured just before therapy onset (0.29±0.09 mmol/L compared to 0.31±0.10 mmol/L, paired samples T-test p=0.035).

Of particular interest in the quest for biomarkers, is the prognostic value of longitudinal changes over time. We evaluated this in two ways. First, the prognostic value of the occurrence of a ≥50% increase in uric acid since baseline was tested as dichotomous time-varying covariate in a Cox regression model. Increases of ≥50% in uric acid, occurred in 18 of 81 (22%) cases after a median (IQR) follow-up time of 2.99 (1.44 - 7.21) years. An event of such an increase, independent of time point of occurrence, was associated with an almost 4-times higher chance of death or lung-transplantation (HR=3.94, p=0.005), and remained significant after adjustment for age, sex and creatinine. Second, the patients were stratified for survival status at the end of follow-up and then their linear development of uric acid levels over time was compared using interaction
analysis in a linear mixed effects model. Figures 1C and 1D depict the individual uric acid trajectories together with the intercepts and regression coefficient derived from this model, and show that non-survivors not only had significantly higher baseline values (intercept 0.34 mmol/L versus 0.27 mmol/L, p=0.003) but also a significantly steeper increase in uric acid over time compared to survivors (slope 0.014 mmol/L/year compared to 0.004 mmol/L/year, p=0.035). These results provide the interesting new insight that the rate of uric acid increases over time carries important prognostic information and that a gradual incline is an ominous sign associated with poor outcome.

This study demonstrates that uric acid is associated with disease severity and outcome, not only at baseline but throughout the full disease course in pediatric PAH, and that changes in serum uric acid levels correspond to changes in outcome. Our data suggest that treatment may influence uric acid levels.

The retrospective analyses of patients followed and data collected prospectively in this study, as well as the limited sample size due to the rareness of the disease form potential limitations to the study, which should be taken into account. Purine metabolism and its degradation products are interesting candidate biomarkers in PAH, in view of their suggested role in vascular remodeling and inflammation. It has been speculated that elevated uric acid levels are not only a consequence of PAH, but may also play a secondary role in the pathogenesis, contributing to the progression of the disease. As purine metabolism is involved in many other mechanisms, hyperuricemia can occur in other conditions not directly related to PAH, such as gout, renal dysfunction, and insulin resistance. The lack of specificity of uric acid in these settings may reduce its clinical value in the adult population. Nevertheless, as these comorbidities are rare in childhood, uric acid holds promise as a useful and valuable biomarker for pediatric PAH. Closely monitoring uric acid levels and especially their course over time provides important information on the state of the disease and may aid in clinical decision-making in the management of children with PAH.
SUPPLEMENTARY REFERENCES


