Diagnostic and prognostic role of biomarkers for pulmonary hypertension in interstitial lung disease

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fibrin D-dimer

Summary
Background: Pulmonary hypertension (PH) is an important complication to interstitial lung disease (ILD). The aim of the present study was to investigate the relation of NT-proBNP, fibrin D-dimer, troponin-T, uric acid and exhaled nitric oxide (NO) to the presence of PH and mortality in ILD.
Methods: In a previously described cohort of 212 ILD patients of whom 29 had PH, levels of the above mentioned biomarkers were analyzed as routine tests.
Results: A value of NT-proBNP below 95 ng/l had a negative predictive value for PH of 99% (95% CI: 94–100). Values of troponin-T were higher in patients with PH (median (inter quartile range) = 9 (9–20) vs. 9(9–10) ng/l), and the odds ratio (OR) for PH was increased in patients with abnormal levels of uric acid (OR (95% CI) = 3.1(1.1–8.8)). NT-proBNP and troponin-T values above the 50th percentile, and uric acid and fibrin D-dimer values above the 90th percentile were each associated with increased mortality.
Conclusions: A value of NT-proBNP below 95 ng/l may be used as a rule-out test for PH in ILD, while an abnormal value of uric acid is a risk factor for PH. NT-proBNP, troponin-T, uric acid and fibrin D-dimer have prognostic value in ILD patients, while exhaled levels of NO do not seem to predict PH or mortality.

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Introduction

Pulmonary hypertension (PH) is an important complication to interstitial lung disease (ILD). In a recent study, we estimated that the overall prevalence in a tertiary referral centre was 14%. In accordance with previous reports, PH was associated with a worse prognosis and is therefore important to diagnose.

Gold standard for diagnosis of PH is right heart catheterisation (RHC). Echocardiography is a widely used screening tool, but may not, however, be readily available to pulmonologists. Therefore biomarkers as first line screening tools are warranted. A number of biomarkers for PH have been proposed, including NT-pro brain natriuretic peptide (NT-proBNP) and troponin-T of which detectable levels are related to higher mortality in pulmonary arterial hypertension (PAH). Furthermore, elevated levels of fibrin D-dimer and uric acid have been demonstrated in PAH, while lower levels of exhaled nitric oxide (NO) have been associated with ILD and PH in scleroderma patients.

The aim of this study was to investigate the diagnostic value for PH and the prognostic significance of the above mentioned biomarkers in ILD patients.

Methods

The present study is an analysis of a previously described cohort of 212 patients enrolled in a cross-sectional study at a tertiary referral centre for diagnosis and evaluation of ILD.

Briefly, the study was approved by The Danish Research Ethics Committee, Region Central Jutland (Issue nr: M-20080219) and conducted in accordance with the Helsinki Declaration.

All patients with a diagnosis of ILD at the centre were eligible, and participants had echocardiography performed for the purpose of the study. PH was defined as a tricuspid regurgitation pressure gradient (TR) >40 mmHg, right ventricular dilatation or a tricuspid annular plane systolic excursion <1.8 cm. Patients fulfilling one or more of these criteria were asked to undergo right heart catheterization (RHC). Patients were defined as having PH if they were screened positive by echocardiography and fulfilled the criteria for PH on RHC or if they were screened positive by echocardiography, but did not undergo RHC. Patients screened negative for PH by echocardiography, and patients who were screened positive by echocardiography but had a MPAP <25 mmHg at RHC were defined as non-PH patients.

Biomarkers

Venous blood samples were analysed routinely for NT-proBNP, troponin-T, uric acid (cobas 6000 E/C, Roche, West Succex, England) and fibrin D-dimer (Sta Cr, Stago, Asnières sur Seine, France) at the hospital’s CME-accredited laboratory. Upper limit of normal was 300 ng/l for NT-proBNP, 0.5 mg/l for fibrin D-dimer and 0.40 mmol/l for uric acid. During enrolment, the reagent for troponin-T (Troponin T, fourth generation reagent,Roche, West Succex, England) with a detection level of 20 ng/l and an upper limit of normal of 30 ng/l was replaced by a new reagent with a higher sensitivity (TNT-HS, Roche, West Succex, England). This analysis had a detection level of 9 ng/l and an upper limit of normal of 50 ng/l.

Exhaled NO was measured by the aerocrine NIOX MINO™ (Intramedic, Copenhagen, Denmark).

Data analysis

Data were analysed in Stata/IC 10 (StataCorp, College Station, Texas, USA). Normality was checked by histograms. Parametric data were analysed using Student’s t-test, non-parametric data by rank sum test.

The discriminatory power for PH for each biomarker was analysed by receiver operating characteristic (ROC) curves. Cut-off values for exclusion or identification of PH were evaluated, and sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated if the area under the ROC curve (AUC) was higher than 0.8.

Survival was analysed with time since inclusion as time scale. Follow-up was censored eight months after inclusion of the last patient. Mortality was estimated by Kaplan-Meier curves, and differences in hazard ratio for death (HR) between patients with biomarker values cut at the 50th and 90th percentile of the values in the present study, was analysed by Cox-proportion hazard model. Proportionality was validated by log–log plots.

Parametric data are expressed as mean ± standard error of the mean (SEM), non-parametric as medians with inter quartile ranges, HR and AUCs are expressed with standard errors (SE). Odds ratios (OR), sensitivity, specificity, PPV and NPV are expressed with 95% CIs. Analyses of troponin-T were made on all patients (n = 208) and separately in patients examined after introduction of the high-sensitivity method (n = 164). Results based on the analysis of patients after the introduction of the new method are shown.

A p-value below 0.05 was regarded as statistically significant.

Results

Patients

Characteristics of the 212 participants in the study are described in details elsewhere. Briefly, 23% had idiopathic pulmonary fibrosis (IPF), 15% had non-specific interstitial pneumonitis, 6% had desquamative interstitial pneumonitis, 7% had hypersensitivity pneumonitis, 19% had ILD associated with a rheumatological disorder, 10% had sarcoidosis stage II–IV and 20% had other types of ILD. The mean total lung capacity, diffusion capacity, forced expiratory volume in one second and forced vital capacity were 69 ± 1, 45 ± 1, 67 ± 1 and 71 ± 2% of expected, respectively. Mean 6-min walk test was 424 ± 8 m. A total of 16% of patients had a history of cardiac disease defined as heart failure, ischemic heart disease, previous myocardial infarction, or atrial fibrillation.

Time of inclusion was defined as the date for analysis of plasma biomarkers. One hundred and ninety-five patients had echocardiography performed at inclusion. Seventeen were examined prior to inclusion (median 1.5 (1–2.5)
Table 1 Distribution of diagnoses in patients without and with PH

<table>
<thead>
<tr>
<th>ILD diagnosis</th>
<th>No PH, n = 179</th>
<th>PH, n = 29</th>
<th>P PH vs no PH</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPF</td>
<td>21</td>
<td>41</td>
<td>0.01*</td>
</tr>
<tr>
<td>NSIP</td>
<td>16</td>
<td>3</td>
<td>0.07</td>
</tr>
<tr>
<td>DIP</td>
<td>7</td>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td>Hypersens. pneumonitis</td>
<td>8</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>Rheumatologic disease</td>
<td>19</td>
<td>21</td>
<td>0.8</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>9</td>
<td>10</td>
<td>0.9</td>
</tr>
<tr>
<td>Endstage</td>
<td>3</td>
<td>10</td>
<td>0.05*</td>
</tr>
<tr>
<td>Other</td>
<td>17</td>
<td>10</td>
<td>0.3</td>
</tr>
</tbody>
</table>

n indicates number of patients in each group. Data were available in all patients. *p < 0.05 vs no PH analysed by proportion test. Results are expressed as proportions. Other comprises: eosinophilic pneumonia, Respiratory bronchiolitis associated-ILD, cryptogenic organizing pneumonia, lymphoid interstitial pneumonia, alveolar proteinosis, Histiocytosis X, medically induced ILD, inorganic dust exposure, acute interstitial pneumonitis, granulomatous interstitial lung disease, unclassified. Hypersens. Pneumonitis: hypersensitivity pneumonitis. Proportion significantly higher than in patients with no PH.

months). Thirty patients were screened positive for PH by echocardiography. Nine patients with PH had dilatation of the right ventricle. In four, PH status was undeterminable. RHC was performed in 18 of these and in only one patient, the diagnosis was discarded. Hence, 29 were defined as PH. All diagnostic groups except for hypersensitivity pneumonitis were represented in the PH-group, and compared to non-PH patients a larger fraction of patients with PH had IPF and endstage ILD (Table 1). Patients in whom PH was confirmed by RHC had significantly higher NT-proBNP and the presence of PH (area under ROC curve (AUC) = 0.86) (Fig. 1A). With a cut-off value of 95 ng/l, the sensitivity, specificity, PPV and NPV was 96 (82–100), 52 (44–60), 25 (17–33) and 99 (94–100)%, respectively. Forty-five percent of patients had NT-proBNP values below 95 ng/l (Fig. 1B).

NT-proBNP values above the 50th percentile and 90th percentile were associated with a higher mortality compared to patients with values less than 106 ng/l, respectively) (Fig. 1C & Table 3).

Troponin-T

AUC for troponin-T to detect PH was 0.62 ± 0.007

Levels of troponin-T were higher in PH patients when compared to non-PH patients (Fig. 2A). Furthermore, PH patients with dilatation of the right ventricle had higher Troponin-T levels than PH patients without right ventricular dilatation (Median: 25 (16–84) vs 9 (9–15) ng/l, p = 0.02).

Troponin-T values above the 90th percentile were associated with a higher mortality (Fig. 3A & Table 3). Only with regard to mortality, differences between the results based on the analysis of the whole population and the sub population of patients with the high-sensitivity Troponin-T analysis were present (see Table 3).

Uric acid

AUC for detection of PH was 0.53 ± 0.07. There were no significant differences between uric acid values in patients with and without PH (Fig. 2B). However, values above the normal reference level (0.4 mmol/l), raised the odds for PH (OR = 3.1 (95 % CI: 1.1–8.8)).

Values above the 50th and 90th percentile were associated with a higher mortality (Fig. 3B & Table 3).

Fibrin D-dimer

AUC for the detection of PH was 0.44 ± 0.07. There were no differences in values between PH and non-PH patients (Fig. 2C). A total of 34% of measured fibrin D-dimer values were above reference value (0.5 mg/l). OR for PH in those with abnormally elevated values were not increased (OR = 1.4 (0.6–3.2) p = 0.4).

NT-proBNP

There was a significant relationship between higher values of NT-proBNP and the presence of PH (area under ROC curve (AUC) = 0.86) (Fig. 1A). With a cut-off value of 95 ng/l, the sensitivity, specificity, PPV and NPV was 96 (82–100), 52 (44–60), 25 (17–33) and 99 (94–100)%, respectively. Forty-five percent of patients had NT-proBNP values below 95 ng/l (Fig. 1B).

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Table 2 Patients with pulmonary hypertension established by right heart catheterisation or echocardiography.

<table>
<thead>
<tr>
<th>Dlco (% of expected)</th>
<th>RHC confirmed PH</th>
<th>PH based on echo</th>
<th>p (PH vs No PH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC</td>
<td>65 ± 4</td>
<td>17</td>
<td>37 ± 6</td>
</tr>
<tr>
<td>FVC</td>
<td>59 ± 6</td>
<td>17</td>
<td>61 ± 6</td>
</tr>
<tr>
<td>FEV1 (% of expected)</td>
<td>54 ± 6</td>
<td>16</td>
<td>57 ± 6</td>
</tr>
<tr>
<td>6 MWT distance (m)</td>
<td>275 ± 25</td>
<td>16</td>
<td>358 ± 29</td>
</tr>
<tr>
<td>TR (mmHg)</td>
<td>65 ± 4.3</td>
<td>16</td>
<td>53 ± 4</td>
</tr>
<tr>
<td>TAPSE (cm)</td>
<td>1.8 ± 0.13</td>
<td>14</td>
<td>2.01 ± 0.14</td>
</tr>
</tbody>
</table>

Comparison of patients with PH verified by right heart catheterisation and patients with a diagnosis of PH based on echocardiography. RHC, right heart catheterisation; echo, echocardiography; Dlco, diffusion capacity; TLC, total lung capacity; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; 6MWT, 6-min walk test; TR, tricuspid regurgitation gradient; TAPSE, tricuspid annular plane systolic excursion. n indicates number of patients from whom data were available. Results are means ± SEM.
However, mortality was increased in patients with fibrin D-dimer levels in the upper 90th percentile (Fig. 3C and Table 3). Exhaled NO

AUC for the detection of PH was 0.44 ± 0.07. A total of 14% of patients could not cooperate to exhaled NO-measurements, primarily due to breathlessness. Patients from whom values were not obtained had significantly lower lung function parameters (data not shown).

There were no relations between values of exhaled NO and PH (Fig. 2D) or mortality (Fig. 3D, Table 3).

**Discussion**

The main findings of this study were that a value of NT-proBNP below 95 ng/l effectively ruled out PH in ILD patients. Abnormally high uric acid levels was a risk factor for PH. High values of NT-proBNP, troponin-T, uric acid and fibrin d-dimer were associated with a worse prognosis. Exhaled NO had no relation to the presence of PH or mortality.

**NT-proBNP**

To our best knowledge, the present study shows for the first time that NT-proBNP may be used as a rule-out test for PH in ILD patients. Studies with a limited number of ILD patients have shown that BNP were able to detect severe PH in ILD,7,8 while another study, primarily including COPD patients, concluded that NT-proBNP was not able to detect mild PH, because NT-proBNP levels were generally beneath the reference value.9 In accordance with that, we found that a very low cut-off value of NT-proBNP should be used if NT-proBNP should be applied as a rule-out test. By using a cut-off value of 95 ng/l, the presence of PH could be excluded in 45% of patients, with a low risk of missing cases of PH. NT-proBNP may therefore be used as a first-line screening tool.

The results from the present study are supported by our recent COPD study10 and findings in a study predominated by asthma and COPD patients, where NT-proBNP values below 93 pg/ml in men had a high NPV for the exclusion of heart diseases,11 and also corresponds well with results from Bonderman et al.12 who showed that a value of NT-proBNP below 80 pg/ml in combination with parameters on the electrocardiogram could be used to preclude pre-capillary PH in various types of patients with suspected PH.

The finding that higher levels of NT-proBNP are associated with a poor prognosis in ILD patients also concurs with previous findings of BNP as a prognostic marker in ILD patients.13

**Troponin-T, uric acid, fibrin D-dimer and exhaled NO**

Troponin-T may reflect myocardial injury caused by right ventricular failure and was found to be related to a worse prognosis in patients with precapillary PH.14,15 In accordance, in the present study, an association between higher levels of troponin-T and increased mortality was observed in ILD patients. Furthermore, patients with PH had higher troponin-T levels than non-PH patients, a difference that may be ascribed to right ventricular dilatation.

In a study in patients with idiopathic pulmonary arterial hypertension (iPAH), a notably larger proportion of iPAH patients had abnormal uric acid values16 than observed in the present study. This incongruence is probably explained...
by higher MPAPs and more severe affection of the nucleic acid metabolisation in iPAH patients. However, it appears from the present study that abnormal levels of uric acid in ILD patients are associated with both the presence of PH and a higher mortality.

In iPAH, fibrin D-dimer is elevated. Few data exist on fibrin D-dimer levels in ILD patients. In a study in IPF patients, levels of fibrin D-dimer were 1.9–2.1 µg/ml (reference value 0.5 µg/ml as in the present study). In accordance, we found that a high percentage of ILD patients had elevated levels. The absence of a relationship to PH in the present study may be explained by the role of fibrin D-dimer as an inflammatory marker. Inflammation and fibrin deposition in the airways in ILD patients may override the increase related to pulmonary vascular fibrin turnover. The relation to a poorer prognosis may, accordingly, be related to a more pronounced parenchymal affliction rather than to pulmonary vascular disease.

To our knowledge this is the first study addressing the use of exhaled NO as a diagnostic tool for PH and a prognostic marker in ILD. Inflammation in the lungs that increases NO production, may mask decreased endothelial-derived NO in PH, and may explain why

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**Table 3**  
Hazard ratio for death based on levels of biomarkers.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>High sensitivity analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT-proBNP</td>
<td>164</td>
<td>206</td>
</tr>
<tr>
<td>Troponin-T</td>
<td>9 ng/l</td>
<td>0.37</td>
</tr>
<tr>
<td>Uric acid</td>
<td>165</td>
<td>206</td>
</tr>
<tr>
<td>Fibrin D-dimer</td>
<td>50 ng/l</td>
<td>0.37</td>
</tr>
<tr>
<td>Exhaled NO</td>
<td>0.15 ppb</td>
<td>0.15 ppb</td>
</tr>
</tbody>
</table>

* indicates the number of patients from whom data were available. Data are expressed as hazard ratios (HR) ± standard error. 50 pct, 50th percentile of the values in the population examined. 90 pct, 90th percentile of the values in the population examined. *p < 0.05 vs 50th percentile; **p < 0.05 vs 50–90th percentile.

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**Figure 2**  
(A) Scatterplots of troponin-T in non-PH (n = 141) and PH-patients (n = 21). Data are shown as medians with inter quartile ranges. (B) Uric acid values in non-PH (n = 175) and PH-patients (n = 28). Data are shown as mean with SEM. (C) Fibrin D-dimer values in non-PH (n = 176) and PH-patients (n = 27). Error bars show median with inter quartile ranges. (D) Exhaled NO values in patients without (n = 146) and with PH (17). Data are shown as mean with SEM. *p < 0.05 vs no PH tested by Student’s t-test (A and C) or rank sum test (B and D).
exhaled NO was not a marker of PH. Recording of exhaled NO was difficult to obtain in patients with more severe lung function impairment, and we cannot exclude this may also have contributed to the lack of predictability of exhaled NO for PH in ILD patients.

Study limitations

It is a limitation that not all patients in the present study were evaluated for PH by RHC. In a previous study, the sensitivity for PH of an echo-estimated systolic pulmonary blood pressure above 45 mmHg was high (85 %) in patients with lung diseases.20 In the present study, we used an overall evaluation of both the tricuspid pressure gradient and right ventricular abnormalities. We rely on these criteria in our clinical practice to exclude the presence of PH, but the risk of false negative echocardiograms for PH, and a following overestimation of the NPV of NT-proBNP cannot be eliminated. The differences between PH-patients with and without RHC, indicates that those 12 patients in whom RHC was not performed had milder PH, but it cannot be excluded that a subset of them may not

Figure 3  (A) Survival curves for patients with troponin-T values within the 0–50 (% n = 114), 50–90 (% n = 34) and 90–100th percentile (% n = 20). (B) Survival curves for patients with uric acid values within the 0–50 (% n = 113), 50–90 (% n = 74) and 90–100th percentile (% n = 20). (D) Survival curves for patients with exhaled NO values within the 0–50 (% n = 86), 50–90 (% n = 63) and 90–100th percentile (% n = 16). *p < 0.05 vs 0–50th percentile. †p < 0.05 vs 50–90th percentile.
fulfil the diagnostic criteria. These potential false positives would, though, not reduce the NPV of NT-proBNP.

The relatively small number of PH patients and the wide spectrum of ILD diagnoses may also be a limitation; the diagnostic value of the biomarkers could be confounded by the distribution of ILD in PH- and non-PH patients, rather than the presence of PH per se. However, this does not appear to be the case as nearly all diagnostic categories appeared in the PH-group, although IPF was over-represented. The main source of circulating NT-proBNP has been shown to be the heart. Thus, the NT-proBNP results may not be largely affected by the specific ILD type, while this concern may be greater for lung-derived biomarkers such as exhaled NO. Conversely, the heterogeneity of the population in the present study may also be an advantage, because it reflects reality at centres for evaluation of ILD. Consequently, this design may improve applicability in practice.

Finally, the use of routine blood sample analyses with the resulting intra assay variation can also be regarded as a limitation. However, since routine tests are used in practice, results obtained by this approach may also be more readily applicable in the clinic.

Conclusions

A value of NT-proBNP below 95 ng/l may be used as a rule-out test for PH in ILD, and abnormally high levels of uric acid should increase the suspicion of PH in ILD patients. Furthermore, NT-proBNP, troponin-T, uric acid and fibrin D-dimer have prognostic value in ILD patients, while exhaled levels of NO does not predict PH or mortality.

Conflict of interest

None.

References


