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## Prognostic value of multiple repeated biomarkers in pulmonary arterial hypertension associated with congenital heart disease

Pulmonary arterial hypertension (PAH) is a well-recognized complication of congenital heart disease (CHD), associated with alarmingly high morbidity and mortality.<sup>1</sup> PAH-CHD is considered a complex syndrome involving many pathophysiological mechanisms, the components of which can be represented by various biomarkers.

There has been a growing interest in biomarkers as prognostic markers in chronic heart disease, given the wide availability and reproducibility, non-invasive nature and low costs. Although a wide variety of biomarkers have been explored in PAH-CHD,<sup>2,3</sup> only natriuretic peptides have been incorporated by the European guidelines.<sup>4</sup> The use of multiple biomarkers in combination (the so-called 'multimarker' approach) may however be of greater prognostic value than a single biomarker approach. Moreover, little is known about the prognostic value of repeatedly measured biomarkers. Ideally, changes in biomarker levels over time should reflect disease progression more accurately. We therefore evaluated the prognostic value of repeated measurements of several biomarkers in a prospective cohort of patients with PAH-CHD. Pathways and corresponding candidate biomarkers studied were (i) myocardial stress [N-terminal pro brain natriuretic peptide (NT-proBNP)], (ii) myocyte injury [high-sensitive troponin T (hs-TnT)], (iii) cardio-renal dysfunction (cystatin-C) and (iv) extracellular matrix remodelling (galectin-3).

This observational dual-centre study included consecutive PAH-CHD adults who were prospectively followed at our institutions with first clinical assessment between January 2004 and January 2016. According to local protocol, patients underwent routine

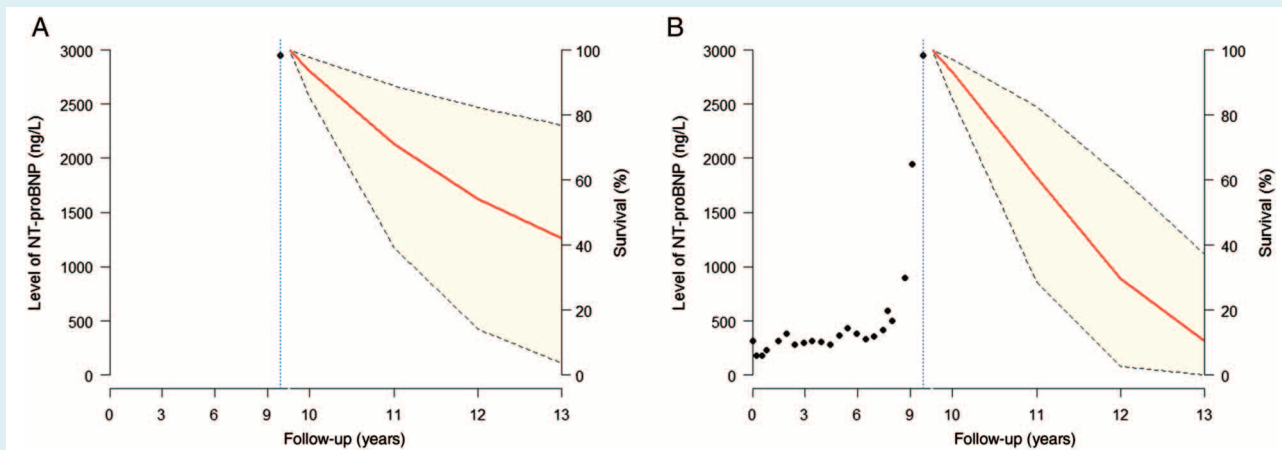
evaluation every 6–12 months at outpatient clinics, including regular assessment of serum biomarkers using commercially available immunoassays. The endpoint was all-cause mortality. Patient deaths and causes of death were site determined and verified by medical records documentation. Biomarker levels were log-transformed and expressed as one standard deviation (SD) increase for hazard ratios (HRs) and 95% confidence intervals (CIs). The association with mortality was assessed using fitted mixed-effect, Cox regression and joint models, adjusted for age, gender and Eisenmenger syndrome (ES).

The study cohort consisted of 98 patients (43 ± 16 years, 34% male, 37% Down syndrome), of whom 90% was treatment naive at baseline and started on advanced therapy within 1 month [interquartile range (IQR) 0.4–4.0]. The majority of patients had ES (69%), followed by closed defects (17%), systemic-to-pulmonary shunts (12%), and small defects (1%). Among ES patients, 47% had complex anatomy, 29% post-tricuspid shunts, and 24% pre-tricuspid shunts. During a median follow-up of 6.9 (IQR 4.1–10.7) years, 41 patients (42%) died. Half of ES patients (47%) died at 50 ± 12 years, whereas patients with systemic-to-pulmonary shunts (50%), closed defects (12%) or small defects (100%) died at older age (60 ± 16, 66 ± 16, and 77 years, respectively). Causes of death were validated in 38 (93%) cases, and primarily due to right heart failure (49%) and sudden cardiac death (12%). The average number of repeated measurements per patient during follow-up was nine for NT-proBNP, five for hs-TnT, and four for cystatin-C and galectin-3. Corresponding median levels of repeated measurements were: NT-proBNP 518 ng/L (IQR 223–1433), hs-TnT 11 ng/L (IQR 5–24), cystatin-C 0.97 mg/L (IQR 0.81–1.23), and galectin-3 15 µg/L (IQR 12–18). The correlations among the four biomarkers were weak to moderate (all  $r < 0.4$ ). All biomarker levels in patients who died progressively increased before time of death compared to those who remained alive during follow-up. Initiation of advanced therapy reduced biomarker levels in short-term, although levels deteriorated again after 1 year.<sup>5</sup>

After imputation and internal validation with 40 bootstrap samples, baseline

levels were associated with an increased risk of death, HRs as follows: NT-proBNP 1.90 (95% CI 1.30–2.78); hs-TnT 1.54 (95% CI 1.14–2.08); cystatin-C 1.69 (95% CI 1.21–2.37); galectin-3 1.58 (95% CI 1.12–2.19). During follow-up, one SD increase in biomarker level represented 250% increase of NT-proBNP, 150% of hs-TnT, and 50% of cystatin-C and galectin-3. Each SD increase in biomarker level was associated with a doubled risk of death at any particular time (all  $P < 0.001$ ), adjusted HRs as follows: NT-proBNP 2.17 (95% CI 1.64–2.89); hs-TnT 2.34 (95% CI 1.61–3.41); cystatin-C 1.81 (95% CI 1.35–2.43); galectin-3 1.79 (95% CI 1.27–2.74). Risk prediction with repeated measurements was more accurate than with single measurements. *Figure 1* illustrates the improved prognostic accuracy for an individual patient with NT-proBNP measurements; 95% CI is significantly broader using only the last single measurement compared with using repeated measurements. Of potential clinical value to physicians, we developed a free online risk stratification tool: <https://biomarkers-pah-chd.shinyapps.io/PAH-CHDbiomarkers/>. Of the four biomarkers, NT-proBNP, hs-TnT and cystatin-C achieved excellent predictive performance for 10-year mortality (c-index 0.81–0.92), whereas galectin-3 did not (c-index 0.60). In combination, the three strongest biomarkers, however, did not further improve discriminatory abilities (c-index 0.85).

We evaluated the prognostic value of a range of repeated biomarkers, individually and collectively, in patients with PAH-CHD. Our patient cohort had a high mortality rate, with 42% deceased by the end of follow-up. All repeated biomarkers individually were powerful predictors of mortality risk: patients with one SD increase had more than doubled the risk of death compared to those with no or less elevation. This is consistent with what has been found in previous studies in acquired heart failure<sup>6,7</sup> that have shown that serial measures provide superior prognostic power over a single biomarker measurement. Previous studies have suggested using absolute cut-off values.<sup>6</sup> From a clinical point of view, this approach may however neglect, at least to some extent, changes within a subject and not serve individual patients



**Figure 1** Dynamic survival predictions for an individual patient based on single (A) vs. repeated (B) N-terminal pro brain natriuretic peptide (NT-proBNP) measurements. (A) The probability that this patient will survive for the next 3 years after the last observed time (dotted vertical line) for which a longitudinal measurement (dot) was available is 45%, with wide 95% confidence interval (shading). (B) The survival probability of the same patient for the same next 3 years after the last observed time is only 18% based on the complete repeated biomarker history (dots), with narrower 95% confidence interval (shading). (JM package, R Project for Statistical Computing, Vienna, Austria). Online risk stratification tool: <https://biomarkers-pah-chd.shinyapps.io/PAH-CHDbiomarkers/>.

optimally. Based on our findings, we recommend to make use of relative changes (e.g. 100% increase) derived from our study rather than absolute (e.g. > 400 ng/L) change criterion, as this could help to initiate a more tailored approach. Altogether, it highlights the importance of serial measures in chronic diseases, in which multiple underlying pathophysiological mechanisms are ongoing dynamic processes that cannot be captured by a single biomarker measure at one point in time. Similar to data from acquired heart failure studies, NT-proBNP and hs-TnT were the most powerful prognostic biomarkers in our cohort. Contrary to studies in acquired heart failure,<sup>8</sup> we did not find a prognostic gain of using multiple repeated biomarkers compared to individual repeated biomarkers.

The following limitations of our study should be considered. It is a dual-centre study with relatively small sample size. There is potential for information bias given that the number of measurements was not equal for all biomarkers. The study focused on a panel of four biomarkers. Therefore, additional studies will be necessary to evaluate other markers of different pathways in PAH-CHD and validate our results in clinically distinct cohorts. Further research should certainly determine the optimal frequency of measurements required and investigate whether individual changes in biomarkers over time can also reflect response to therapy. This will enable further optimization of individual

follow-up strategies and timely initiation of therapeutic management.

In conclusion, repeated biomarker measurements were associated with an approximately two-fold higher mortality risk per SD increase and were more powerful predictors of mortality than single measurements. A multimarker approach provided no incremental prognostic value beyond the repeated measurements of individual biomarkers. Therefore, our findings support the concept of regular assessment of at least one biomarker, e.g. NT-proBNP, to help identify patients with PAH-CHD at greatest risk.

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