Soluble ST2 and N-terminal pro-Brain Natriuretic Peptide Combination
– Useful Biomarker for Predicting Outcome of Childhood Pulmonary Arterial Hypertension –

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Background: Some potential biomarkers have been reported recently in patients with pulmonary arterial hypertension (PAH), but the most clinically useful among these potential biomarkers, especially in childhood PAH, has not been identified. Therefore, this study investigated which biomarker is useful in assessing severity of and patient prognosis in childhood idiopathic PAH (IPAH)/heritable PAH (HPAH).

Methods and Results: Fifty-nine patients who were younger than 16 years at onset of IPAH/HPAH were selected. The following 10 biomarker candidates were quantified: high-sensitivity troponin T, human heart fatty acid-binding protein, N-terminal pro-brain natriuretic peptide (NT-proBNP), pentraxin-3, soluble ST2 (sST2), angiopeitoin-2 (Ang-2), matrix metalloproteinase 2, tenascin C, endostatin (ES), and thymidine kinase. Functional characteristics and clinical outcomes were analyzed retrospectively. NT-proBNP, sST2, Ang-2, and ES correlated well with New York Heart Association class. On area under the receiver operating characteristic curve analysis, sST2 had a significantly good relationship with prognosis. On Kaplan-Meier curve and univariate Cox regression analyses, elevated sST2 had significantly worse prognosis among those with high NT-proBNP.

Conclusions: The sST2 and NT-proBNP combination is a useful biomarker to predict clinical condition and outcome in patients with childhood IPAH/HPAH. (Circ J 2014; 78: 436–442)

Key Words: Biomarker; Childhood pulmonary arterial hypertension; N-terminal pro-brain natriuretic peptide; Prognosis; Soluble ST2

Prediction of severity and outcome in pulmonary arterial hypertension (PAH) is important but often difficult, especially in childhood. Brain natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) are considered useful biomarkers of PAH in pediatric and adult patients. Lammers et al, however, reported that BNP had limited sensitivity (57%) for predicting death or the need for transplantation. Because BNP and NT-proBNP are secreted from the ventricular tissue as a consequence of right heart failure, they may not be considered as symbolic biomarkers of PAH. Furthermore, several potential biomarkers such as matrix metalloproteinase 2 (MMP-2), tenascin-C (TNC), pentraxin 3 (PTX3), cyclophilin A, angiopeitoin-2 (Ang-2), and soluble ST2 (sST2) have been recently reported to be present in patients with PAH, but have not been used in actual clinical practice because they have not yet been fully validated, and the optimal biomarker of PAH remains to be identified.

The aim of the present study was to investigate which biomarkers are useful for predicting severity and outcome of pediatric PAH. Emphasizing published data on functional...
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international consensus criteria, and mean pulmonary artery pressure >25 mmHg at rest or >30 mmHg during exercise. Patients with PAH associated with other diseases such as portal hypertension, congenital heart disease, or persistent pulmonary hypertension of the newborn were excluded from the study, as evaluated by trained cardiologists. The clinical characteristics of 59 patients are listed in Table 1.

Table 1. Baseline Clinical and Hemodynamic Data

| Variable | All patients (n=59) | Non-death group (n=48) | Death group (n=11) | P-value |
|----------|---------------------|------------------------|-------------------|---------|
| Age at onset (years) | 8.4±4.0 | 8.2±3.8 | 9.3±4.7 | 0.789 |
| Age at blood sampling (years) | 11.3±6.0 | 11.0±5.5 | 12.4±7.9 | 0.751 |
| Gender M/F | 26/33 | 29/19 | 4/7 | 0.147 |
| Family history of PAH | 12 (20) | 8 (17) | 4 (36) | 0.143 |
| Functional class at blood sampling, I/II/III-IV/unclear | 13/28/17/1 | 11/25/12/0 | 2/3/5/1 | 0.110 |

Hemodynamic parameters

| Variable | All patients (n=59) | Non-death group (n=48) | Death group (n=11) | P-value |
|----------|---------------------|------------------------|-------------------|---------|
| mPAP (mmHg) | 65.5±19.0 | 64.9±20.1 | 68.5±12.9 | 0.684 |
| RAP (mmHg) | 7.3±3.9 | 7.1±1.6 | 7.4±4.2 | 0.446 |
| CI (L · min⁻¹ · m⁻²) | 3.1±1.3 | 2.6±0.5 | 3.2±1.4 | 0.140 |
| TPR (wood · U · m⁻²) | 27.6±12.1 | 33.7±10.1 | 25.8±12.6 | 0.827 |
| PVR (wood · U · m⁻²) | 21.3±12.0 | 21.0±8.8 | 21.4±13.0 | 0.476 |
| PAWP (mmHg) | 9.4±2.6 | 10.6±2.8 | 9.2±2.6 | 0.903 |
| Current therapy | | | | |
| I.v. epoprostenol | 24 (41) | 21 (44) | 3 (27) | 0.316 |
| Cardiotonic drugs | 18 (31) | 15 (31) | 3 (27) | 0.796 |
| Sildenafil/tadalafil | 27 (46) | 24 (50) | 3 (27) | 0.172 |
| Bosentan/ambrisentan | 23 (39) | 21 (44) | 2 (18) | 0.117 |
| Other vasodilators per os | 23 (39) | 21 (44) | 2 (18) | 0.117 |
| Anticoagulant or antiplatelet agents | 26 (44) | 22 (46) | 4 (36) | 0.568 |
| Diuretics | 28 (47) | 24 (50) | 4 (36) | 0.414 |
| Oxygen | 31 (53) | 27 (56) | 4 (36) | 0.234 |

Data given as mean ± SD or n (%). Cardiac catheterization was performed at the nearest time to the collection of blood samples in 51 of 59 patients.

Methods

Study Design

We recruited 59 unrelated patients who were younger than 16 years when they received a diagnosis of idiopathic PAH (IPAH)/heritable PAH (HPAH). Some of the patients were also described in previous reports. In accordance with the Declaration of Helsinki, written informed consent was obtained from the parents or guardians of all the study subjects. This study was approved by the Institutional Review Committee of Tokyo Women’s Medical University.

Based on a previous review of the patients’ medical records, we retrospectively assessed New York Heart Association (NYHA) classification of functional capacity at blood sampling, data for cardiac catheterization performed at the nearest time to the collection of blood samples, and clinical outcome in each patient until March 2012. The median duration of follow-up was 23 months (range, 0–121 months). Patients whose blood samples were collected during active cardiopulmonary resuscitation were excluded from this study.

In all patients, diagnosis of IPAH/HPAH was made through clinical evaluation, chest radiography, electrocardiography, echocardiography, and cardiac catheterization based on current international consensus criteria, and mean pulmonary artery pressure >25 mmHg at rest or >30 mmHg during exercise. Patients with PAH associated with other diseases such as portal hypertension, congenital heart disease, or persistent pulmonary hypertension of the newborn were excluded from the study, as evaluated by trained cardiologists. The clinical characteristics of 59 patients are listed in Table 1.

Laboratory Analysis

Heparin plasma hsTnT, H-FABP, NT-proBNP, PTX3, sST2, Ang-2, MMP-2, TNC, endostatin (ES), and thymidine kinase (TK).

Statistical Analysis

Baseline characteristics are reported as mean±SD, median with range or counts and proportion, as appropriate. Univariate comparisons between the non-death group and death group were
done using t-test or the Wilcoxon test for continuous measures and the chi-squared test for categorical measures. The biomarkers were compared between the groups stratified according to NYHA functional class, using 1-way analysis of variance followed by the Tukey test.

To investigate the sensitivity and specificity of biomarker candidates, a logistic regression analysis for patient prognosis was performed. The optimal cut-off values of these biomarker candidates were determined according to the area under the receiver operating characteristic curves (AUC), respectively. Patients were divided into 2 groups according to the cut-off of each biomarker candidate. Overall survival in 2 groups, patients who had higher values, and those who had lower values was estimated using the Cox proportional hazard regression model, for each biomarker candidate. 

P<0.05 was considered statistically significant. All statistical analysis was done using SAS version 9.3 (SAS Institute, Cary, NC, USA).

Biomarker levels are listed in Table 2. Five (8 with t-test) of 10 biomarker candidate levels were significantly different between the non-death group and death group.

### Functional Class and Potential Biomarkers

As the first step, we compared NYHA functional class with the 10 biomarker candidates. NT-proBNP, sST2, Ang-2, and ES concentration were significantly different according to deterioration of NYHA functional class (Figure 1); the other 6 candidates, hsTnT, H-FABP, PTX3, MMP-2, TNC, and TK, were not.

#### sST2: Good Biomarker for Predicting Death in PAH

On AUC analysis, sST2, Ang-2 and ES had a significantly good relationship with prognosis (Table 3). In addition, an initial sST2 cut-off value of 11.1 ng/ml for death prediction was identified, with an AUC of 0.830, the highest among the AUC for all the candidates. Although NT-proBNP had a relatively high AUC, it was not statistically significant.

#### sST2 and NT-proBNP Elevation Associated With Poor Outcome

Proportion of overall survival according to Kaplan-Meier curve analysis for the respective cut-off levels of NT-proBNP and sST2 is shown in Figure 2A,B. Outcome was significantly better in the PAH patients with NT-proBNP concentration <537 pg/ml (P=0.004) and sST2 concentration <11.1 ng/ml (P<0.0001). Worse prognosis was seen for patients with higher levels of both sST2 and NT-proBNP, than in those with a lower level of 1 or both biomarkers (P<0.0001; Figure 2C).

As shown in Table 3, the Cox proportional hazards model for mortality indicated that elevated NT-proBNP and sST2 were independently and strongly associated with poor outcome (NT-proBNP: HR, 10.9; 95% confidence interval [CI]: 1.4–85.3; P=0.023; sST2: HR, 24.6; 95% CI: 3.1–192.9;
els of both biomarkers. Then, we used Cox proportional hazard regression models to identify the effect of sST2 in the PAH patients with high NT-proBNP level. PAH patients with elevated sST2 had a significantly worse prognosis among those with high NT-proBNP (HR, 8.9; 95% CI: 1.1–70.4; P=0.0387; Table 6).

Moreover, elevated NT-proBNP and sST2 were associated with poor prognosis (HR, 14.4; 95% CI: 3.1–66.7; P=0.002). In addition, we investigated whether sST2 would have an influence on clinical prognosis of PAH patients with high NT-proBNP. First, we stratified all the PAH patients into 4 groups according to NT-proBNP and sST2 cut-offs (Table 5). Of 17 patients, 9 (53%) died in the 2 groups with higher cut-off levels of both biomarkers. Then, we used Cox proportional hazard regression models to identify the effect of sST2 in the PAH patients with high NT-proBNP level. PAH patients with elevated sST2 had a significantly worse prognosis among those with high NT-proBNP (HR, 8.9; 95% CI: 1.1–70.4; P=0.0387; Table 6).

**Table 3. Candidate Biomarker Cut-Offs (Logistic Regression Analysis)**

| Biomarker | Cut-off | AUC    | AUC 95% CI      | P-value |
|-----------|---------|--------|-----------------|---------|
| hsTnT (ng/ml) | 0.03    | 0.752  | 0.593–0.911    | 0.061   |
| H-FABP (ng/ml) | 1.0     | 0.598  | 0.401–0.794    | 0.470   |
| NT-proBNP (pg/ml) | 537.0   | 0.796  | 0.643–0.950    | 0.084   |
| PTX3 (ng/ml) | 1.5     | 0.681  | 0.515–0.847    | 0.269   |
| sST2 (ng/ml) | 11.1    | 0.830  | 0.667–0.994    | 0.015   |
| Ang-2 (ng/ml) | 4.2     | 0.756  | 0.568–0.943    | 0.010   |
| MMP-2 (ng/ml) | 1,160.0 | 0.642  | 0.431–0.853    | 0.062   |
| TNC (ng/ml) | 122.7   | 0.646  | 0.454–0.838    | 0.138   |
| ES (ng/ml) | 87.2    | 0.753  | 0.598–0.908    | 0.024   |
| TK (U/L)   | 9.4     | 0.527  | 0.325–0.730    | 0.077   |

AUC, area under the receiver operating characteristic curve. Other abbreviations as in Table 2.

**Figure 1.** Relationship between plasma biomarker candidates and New York Heart Association (NYHA) classification. The NYHA functional classification grades were divided into 3 groups, that is, I, II, and III/IV, and compared using 1-way analysis of variance followed by the Tukey test. *P<0.05. Ang-2, angiopoietin-2; ES, endostatin; H-FABP, human heart fatty acid-binding protein; hsTnT, high-sensitivity troponin T; MMP-2, matrix metalloproteinase 2; NT-proBNP, N-terminal pro-brain natriuretic peptide; PTX3, pentraxin 3; sST2, soluble ST2; TK, thymidine kinase; TNC, tenascin C.
Figure 2. Kaplan-Meier survival curves: (A) low (<537 pg/dl, dotted line) and high (≥537 pg/dl, solid line) NT-proBNP level (log-rank test, P=0.0035); (B) low (<11.1 pg/dl, dotted line) and high (≥11.1 pg/dl, solid line) sST2 level (log-rank test, P<0.0001); (C) high levels of both biomarkers (NT-proBNP ≥537 pg/dl and sST2 ≥11.1 pg/dl, solid line) and lower levels of both/either of the biomarkers (dotted line; log-rank test, P<0.0001). NT-proBNP, N-terminal pro-brain natriuretic peptide; sST2, soluble ST2.

Table 4. Indicators of Poor Outcome† in All PAH Patients (n=59)

|                       | HR   | 95% CI | P-value |
|------------------------|------|--------|---------|
| High NT-proBNP (≥537 pg/dl) | 10.9 | 1.4    | 85.3    | 0.023* |
| High sST2 (≥11.1 ng/ml)   | 24.6 | 3.1    | 192.9   | 0.002* |
| Both NT-proBNP and sST2 high | 14.4 | 3.1    | 66.7    | 0.001* |

*P<0.05. †Death. Patients were divided into 2 groups according to biomarker cut-offs. HR, hazard ratio. Other abbreviations as in Tables 1,2.

Table 5. Deaths in NT-proBNP and sST2 Subgroups

| sST2     | NT-proBNP Lower | NT-proBNP Higher |
|----------|-----------------|------------------|
| Lower    | 0/26            | 1/11             |
| Higher   | 1/5             | 9/17             |

n, deaths/all patients. Abbreviations as in Tables 1,2.

Table 6. Effect of sST2 in 28 PAH Patients With High NT-proBNP

| sST2 (≥11.1 ng/ml) | HR     | 95% CI Lower | 95% CI Upper | P-value |
|--------------------|--------|--------------|--------------|---------|
| High sST2          | 8.9    | 1.1          | 70.4         | 0.0387* |

*P<0.05. Patients with high NT-proBNP were divided into 2 groups according to sST2 cut-off (≥11.1 ng/ml). Abbreviations as in Tables 1,2,4.
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Discussion

We simultaneously compared 10 biomarker candidates and this is the first study to identify the usefulness of the combination of sST2 and NT-proBNP in childhood IPAH/HPAH. Recent studies have suggested several potential biomarkers in patients with PAH, but the optimal biomarker was not clearly indicated and hence could not be used in clinical practice. Furthermore, some of the previous studies did not give sufficient consideration to the relationship between the potential biomarker and clinical outcome. In the present study, we narrowed down the targets to sST2 and NT-proBNP as the 2 most useful biomarkers. Using these markers, current condition of PAH patients was able to be estimated, as with NYHA functional class. In addition, we found that elevated sST2 and NT-proBNP level predicted poor outcome in patients with childhood IPAH/HPAH. Given the specificity of the present results, they may be applied in the clinical setting.

sST2 in Cardiovascular Disease

ST2 is a member of the interleukin-1 (IL-1) receptor host defense and inflammation family, and encodes the soluble (sST2) and transmembrane types. According to GeneCards website (www.genecards.org), ST2 is produced in various cells and tissues including endothelial cells, smooth muscle cells and cardiomyocytes. Circulating sST2 is a biomarker of cardiac stress. Weinberg et al showed that myocyte stretch induced markedly upregulated myocardial ST2 gene expression. Furthermore, the production of IL-33, the ligand of sST2, is known to be induced by myocyte stretch via its anti-hypertrophic and anti-fibrotic activities. Seki et al found that IL-33 reduced ventricular dilation and improved contraction function in wild-type mice but not in ST2−/− mice with myocardial infarction. SST2 may prevent the binding of IL-33 to a membrane-bound receptor version of ST2.

After Weinberg et al reported in 2003 that sST2 was a novel biomarker of heart failure in their patients, several studies suggested that sST2 is one of the most useful prognostic biomarkers of acute heart failure, chronic heart failure, and ST-elevation myocardial infarction. Furthermore, Shah et al showed that sST2 concentration was associated with right ventricular systolic pressure. Sato et al showed that sST2 level was elevated in adult Kawasaki disease and correlated with impaired myocardial relaxation. The median age of their patients with Kawasaki disease was 2.8 years. Sato et al noted that sST2 might also be considered useful in the assessment of myocardial damage in children.

sST2 and NT-proBNP in PAH

Carlonagino et al were the first to report increased serum sST2 level in patients with PAH, suggesting that the increase was strictly related to the degree of right ventricular dilatation and systolic dysfunction. Their study, however, did not describe the relationship between sST2 level and clinical prognosis, and the superiority of sST2 over other potential biomarkers. Hence, we conducted the present study to determine whether sST2 is the optimal biomarker. On AUC analysis sST2 had a significantly good relationship with prognosis. Furthermore, we found that elevated sST2 level was associated with poor prognosis. In addition, elevated levels of both sST2 and NT-proBNP predicted poor outcome in patients with childhood IPAH/HPAH.

The role of sST2 in PAH is speculative but may be related to its role in cardiac fibrosis in response to increasing right ventricular pressure induced by PAH. Although the relationship between sST2 level and the pulmonary artery has not yet been described, Willems et al found that sST2 level was significantly increased 24 h after patients underwent peripheral vascular surgery, arterial bypass, and femoral endarterectomy. Their findings suggest that sST2 may be related to arterial damage. Moreover, IL-33 mRNA expression in human pulmonary arterial smooth muscle cells (hPASMCs) was reported to be high. Further studies are required, however, to investigate the relationship between sST2 level and the pulmonary artery, and to explain why sST2 may be considered the most useful among the other potential biomarkers and the more sensitive biomarker than NT-proBNP, which is secreted predominantly from ventricular tissues.

Study Limitations

We identified several limitations of the present study. The number of subjects was relatively low. Because we measured the concentration of all the biomarker candidates just once per patient, we could not obtain the longitudinal change in these indicators. To confirm the validity of the sST2 and NT-proBNP combination, further prospective studies may be needed before clinical application.

Although we did not obtain significant results relating to current condition and clinical outcome, in almost all the PAH patients, TK was higher than the reference range (data not shown). Hence, the marker may be associated with the pathogenesis of PAH itself.

It may be worth investigating hPASMCs. In addition, further investigations using human pulmonary artery and animal PAH models are required to elucidate the biological role of sST2.

Conclusions

The sST2 and NT-proBNP combination is a useful biomarker for accurately predicting current condition and outcome of patients with childhood IPAH/HPAH.

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Disclosures

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