A meta-analysis of soluble suppression of tumorigenicity 2 (sST2) and clinical outcomes in pulmonary hypertension

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Suppression of Tumorigenicity 2 (ST2) is a member of the interleukin (IL)-1 receptor family.[1] The ST2 receptor exists in two isoforms – ST2 ligand (ST2L) and soluble ST2 (sST2).[1] ST2L is a membrane receptor and sST2 is a truncated receptor which is soluble in the blood, allowing it to be detected in serum. IL-33 is a member of the IL-1 family of ligand and is the functional ligand of ST2L receptor.[2] It binds to the ST2L, thereby mediating its immune function.

Recently, it has been shown that IL-33 exerts an anti-hypertrophic action in the heart through binding to the ST2L receptors expressed in the cardiomyocytes.[3] sST2, however, is a decoy receptor for IL-33. Its binding to IL-33 reduces its beneficial effects on the heart.[3] In vitro studies showed that sST2 is elevated under conditions of mechanical stress to cardiomyocytes.[4] Levels of sST2 are elevated in various cardiovascular diseases, such as heart failure, coronary artery disease and after transcatheter aortic valve implantation.[5,6] There have been recent interests to examine its role as a potential prognostic biomarker for these cardiovascular diseases.

Pulmonary arterial hypertension (PAH) is a lung condition characterized by a progressive remodeling in the pulmonary arteries, resulting in elevations in arterial resistance and mean pulmonary artery pressure.[7] These changes increase the workload of the right heart, eventually right-sided heart failure. It is thought that the mechanical stress placed on the heart by PAH will increase the expression of sST2.[8,9] The levels of sST2 appear to relate to pulmonary vascular resistance, cardiac index, and clinical worsening.[9] Recently, it has also been shown in vitro that IL-33 knock-down increased the release of sST2 significantly from human endothelial cells in idiopathic PAH patients.[10] These results demonstrated the association between PAH and elevated level of sST2.

Although various studies have demonstrated an elevated level of sST2 in PAH patients,[9] its value in predicting the disease outcomes is not well-established. Therefore, we conducted a systematic review and meta-analysis on the value of sST2 in predicting disease outcomes in PAH patients.

The meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement. PubMed and Embase were searched for studies that investigated the prognostic value between soluble suppression of tumorigenicity-2 (sST2) in pulmonary
hypothesis using the following terms: [(Soluble suppression of tumorigenicity-2 OR sST2) and (pulmonary hypertension)]. The search period was from the beginning of the databases through to 8th October, 2017, with no language restrictions. The following inclusion criteria were applied: (1) the design was a case-control, prospective or retrospective cohort study in humans, (2) sST2 values were provided and related to clinical outcomes in pulmonary hypertension. The quality assessment of these studies included in our meta-analysis was performed using the Newcastle–Ottawa Quality Assessment Scale (NOS). The point score system evaluated the categories of study participant selection, comparability of the results, and quality of the outcomes. The following characteristics were assessed: (1) representativeness of the exposed cohort; (2) selection of the non-exposed cohort; (3) ascertainment of exposure; (4) demonstration that outcome of interest was not present at the start of study; (5) comparability of cohorts on the basis of the design or analysis; (6) assessment of outcomes; (7) follow-up period sufficiently long for outcomes to occur; and (8) adequacy of follow-up of cohorts. This scale varied from zero to nine stars, which indicated that studies were graded as poor quality if they met < 5 criteria, fair if they met 5 to 7 criteria, and good if they met > 8 criteria. The details of the NOS quality assessment are shown in Table 1.

Data from the different studies were entered in pre-specified spreadsheet in Microsoft Excel. All publications identified were assessed for compliance with the inclusion criteria. In this meta-analysis, the extracted data elements consisted of: (1) publication details: surname name of first author, publication year; (2) study design; (3) follow-up duration; (4) the quality score; and (5) the characteristics of the population including sample size, gender, age and cut-off points for sST2 levels. Two reviewers [Kingsum Luk and Christina Ip] independently reviewed each included study and disagreements were resolved by adjudication with input from a third reviewer (GT).

Mean differences in sST2 levels between survivors and non-survivors were extracted from each study and subsequently pooled in our meta-analysis. For the relationship between sST2 and clinical outcomes or mortality, multivariate adjusted hazard ratios (HR) with 95% CI were extracted and analyzed for each study. When values from multivariate analysis were not available, those from univariate analysis were used. When the latter were not provided, raw data were used to calculate unadjusted risk estimates where data were available.

Heterogeneity across studies was determined using Cochrane’s Q value, which is the weighted sum of squared differences between individual study effects and the pooled effect across studies, and the I2 statistic from the standard chi-square test, which describes the percentage of the variability in the effect estimates resulting from heterogeneity. I2 > 50% was considered to reflect significant statistical heterogeneity. The random-effects model using the inverse variance heterogeneity method was used with I2 > 50%. To locate the origin of the heterogeneity, sensitivity analysis excluding one study at a time, and subgroup analyses based on different disease conditions and different endpoints were performed. Funnel plots showing standard errors or precision against the logarithms of the odds ratio were constructed. Begg and Mazumdar rank correlation test and Egger’s test were used to assess for possible publication bias.

A flow diagram detailing the search strategy and study selection process is shown in Figure 1. A total of 12 and 25 entries were retrieved from PubMed and Embase. Of these studies, three met the inclusion criteria and were included in the final meta-analysis.[8,9,11] All three studies were prospective studies. Their baseline characteristics are shown in Table 2. The cut-off value, given as mean ± standard error, for sST2 was 37.0 ± 16.8 ng/mL.

The sST2 are significantly associated with mortality and poor clinical outcomes in pulmonary hypertension. A total of 166 patients (30% male, mean age 30.4 ± 7.6 years; mean follow-up duration of 17 months) were included. Two studies

| Studies       | Representative-ness of the exposed cohort | Selection of the non-exposed cohort | Ascertainment of exposure | Outcome of interest not present at start of study | Comparison of outcome | Adequacy of duration of follow-up | Adequacy of completeness of follow-up | Total score (0–9) |
|---------------|------------------------------------------|------------------------------------|---------------------------|-----------------------------------------------|----------------------|-------------------------------|-------------------------------|------------------|
| Placido 2017  | 1                                        | 0                                  | 1                         | 1                                             | 1 (age)              | 1                             | 1                             | 1                |
| Contractor 2016 | 1                                        | 0                                  | 1                         | 1                                             | 0                    | 1                             | 1                             | 0                |
| Chida 2014    | 1                                        | 0                                  | 1                         | 1                                             | 1 (age)              | 1                             | 1                             | 1                |
| Zheng 2014    | 1                                        | 0                                  | 1                         | 1                                             | 1 (age)              | 1                             | 1                             | 1                |

Table 1. NOS risk of bias scale for included cohort studies. NOS: Newcastle–Ottawa Quality Assessment Scale.
compared sST2 concentrations between survivors and non-survivors, both of which reported significantly higher values in non-survivors (Figure 2). Our meta-analysis showed a mean difference of 22.2 ng/mL (standard error: 11.3 ng/mL; \( P < 0.05 \)). \( I^2 \) took a value of 80%, indicating the presence of substantial heterogeneity. Sensitivity analysis excluding one study at a time did not significantly affect the pooled estimate. Three studies examined the relationship between high sST2 concentrations and mortality, all of which reported a significant association (Figure 3). Our meta-analysis showed that elevated sST2 concentrations were associated with an approximately seven-fold increase in all-cause mortality (HR: 7.18, 95% CI: 2.64 to 19.54, \( P < 0.0001 \)). \( I^2 \) took a value of 0%, indicating the absence of heterogeneity. Sensitivity analysis excluding one study at a time did not significantly affect the pooled estimate.

Finally, three studies examined the relationship between high sST2 concentrations and poor clinical outcomes. This was defined as death or hospitalization (\( n = 1 \)), poor prognosis (\( n = 1 \)) and any of five endpoints of death, lung trans-

Table 2. Characteristics of the studies included in this meta-analysis.

| Study | Population                  | sST2 cut-off, ng/mL | Sample size (\( n \)) | Age, yrs SD | No. of males | Follow-up, months | Variables in multivariate model                                                                 |
|-------|-----------------------------|---------------------|-----------------------|-------------|--------------|-------------------|------------------------------------------------------------------------------------------------------------------|
| Placido, 2017 | Group I, III, IV or V pulmonary hypertension | 68.6 | 43 | 59 | 15 | 12 | 34 | RA diameter (4-chamber), RA diastolic area, RA systolic area, RA fractional area, RV basal diameter, RV diastolic area, RV systolic area, RV fractional area, Lateral TASV, Estimated PASP, GFR (MDRD), renin, Log NT-proBNP, MR-proANP, copeptin, ET-1, MR-proADM |
| Chida, 2014 | Idiopathic or heritable pulmonary hypertension | 11.1 | 59 | 8.4 | 4.0 | 26 | 23 | NT-proBNP |
| Zheng, 2014 | Idiopathic pulmonary hypertension | 31.4 | 64 | 31.4 | 9.8 | 11 | 24 | Cardiac index, PVR |

ET-1: endothelin-1; GFR: glomerular filtration rate; MDRD: Modification of Diet in Renal Disease formula; MR-proADM: mid-regional pro-adrenomedullin; MR-proANP: mid-regional pro-atrial natriuretic peptide; NT-proBNP: N-terminal pro-brain natriuretic peptide; PASP: pulmonary artery systolic pressure; PVR: pulmonary vascular resistance; RA: right atrium; RV: right ventricle; sST2: soluble suppression of tumorigenicity 2; TASV: tricuspid annular systolic velocity.

Figure 2. Mean difference in sST2 levels between non-survivors and survivors in pulmonary hypertension. sST2: soluble suppression of tumorigenicity 2; CI: confidence interval.
plantation, hospitalization for pulmonary arterial hypertension, the initiation of a new therapy, or worsening WHO functional class (n = 1). All of these studies reported a significant relationship and our meta-analysis demonstrates a seven-fold increase in the likelihood of poor outcomes (HR: 7.17, 95% CI: 3.46 to 14.83, P < 0.0001). $I^2$ took a value of 0%, indicating the absence of heterogeneity. Sensitivity analysis excluding one study at a time did not significantly affect the pooled estimate. Begg and Mazumdar rank correlation suggested no significant publication bias (Kendall’s Tau value 1.0, P = 0.12). Egger’s test demonstrated significant asymmetry (intercept 1.7, t-value 0.8; P = 0.56).

The main findings of this systematic review and meta-analysis are that in the context of pulmonary hypertension, sST2 levels are higher in non-survivors than in survivors. High sST2 was significantly associated with a seven-fold increase in both mortality and likelihood of poor outcomes. N-terminal pro-brain natriuretic peptide (NT-proBNP) has been used as a biomarker for monitoring the severity of PAH. It is secreted mainly by ventricular cardiomyocytes as a result of higher stress from the elevations in pulmonary vascular resistance and pressure.[8] Other biomarkers such as sST2 are raised in patients with PAH. Increased serum sST2 levels has been associated with the degree of right ventricular dilatation and systolic dysfunction in the context of PAH.[9] Serum sST2 level has been correlated to right ventricle dimensions and function, which in turn related to the risk stratification of PAH patients. However, the precise role of sST2 as a biomarker in PAH and its prognostic value have not been well-defined.[12] The expression of sST2 can be induced in various cell types including endothelial cells, smooth muscle cells and cardiomyocytes by a wide range of signals, with highest expression in the lung, then kidney, heart and small intestine.[13] From in vitro, the secretion of sST2 by pulmonary alveolar cells and cardiomyocytes was found to be enhanced by inflammatory cytokines such as IL-1α, IL-1β, IL-6 and tumor necrosis factor-α.[14] In addition, sST2 level was also found to be elevated 24 h after patients underwent peripheral vascular surgery and arterial bypass. These findings suggested that sST2 may be related to arterial damage.[15]

Although the direct relationship between sST2 level and PAH has not yet been fully understood, it has been reported that there is an increase of sST2 in the serum and a decrease in IL-33 expression in lung tissue in idiopathic pulmonary arterial hypertension (IPAH) patients. In addition, endothelial dysfunction in lung arterial vessels has been reported to be associated with the loss of nuclear IL-33 in lung arterial endothelial cells. And the expression of IL-33 was also found to be reduced in the presence of inflammatory cytokines. Since the expression of sST2 was significantly increased with the loss of nuclear IL-33, it was suggested that IL-33 acts as a nuclear suppressor to reduce sST2 expression. In some cases, IL-33 level was almost undetectable in serum in IPAH patients. Thus, in addition to the role as a soluble decoy receptor of IL-33, sST2 has been suggested to act as a co-factor that contribute to the pulmonary vascular and right ventricular remodeling associated with IPAH.[16]
This suggestion is in parallel with the elevated remodeling of peripheral pulmonary arterial vasculature found in patients with IPAH.\(^9\) Furthermore, the increased circulating cytokines and chemokines in IPAH patients also point to a fact that there is an on-going inflammation during the course of PAH.\(^17\) These findings are supported by pre-clinical experiments involving prolonged IL-33 administration in mice, which led to increased serum soluble ST2, pulmonary arterial remodeling and right ventricular hypertrophy.\(^18\)

Serum sST2 has been suggested as a useful biomarker for vascular remodeling, and for the prediction of severity and outcome in patients with PAH.\(^9\) It is reported to be more sensitive and superior than NT-proBNP as a biomarker for PAH because the latter is secreted mainly from ventricular tissue.\(^8\) Our systematic evaluation demonstrated the prognostic role of sST2 as a biomarker, given its ability to independently predict mortality and outcomes in PAH.

There are many strengths of this study. Firstly, all of the included studies had the same design being prospective cohort studies, which would result in a lower likelihood of certain bias types such as recall bias. Secondly, multivariate analysis was used in all three studies, adjusting for confounding factors when assessing the risk of mortality and clinical outcomes. For meta-analysis, no heterogeneity was observed on pooling the hazard ratios, indicating the appropriateness to calculate an estimate from these studies. Nevertheless, some limitations should be noted. Only a few studies were identified in our systematic review. Larger prospective cohorts are needed to confirm the prognostic value of sST2 in this patient population. Currently, sST2 should be interpreted in the clinical context of the patients in conjunction with other laboratory test results for the purposes of risk stratification and to guide appropriate clinical management. With the growth of many biomarkers for risk stratification in pulmonary and non-pulmonary conditions,\(^19–21\) sST2 shows promising results in showing utility in predicting poor outcomes and mortality in pulmonary hypertension.

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References

1. Pascual-Figal DA, Januzzi JL. The biology of ST2: the International ST2 Consensus Panel. *Am J Cardiol* 2015; 115: 5B–7B.
2. Schmitz J, Ow Yang A, Oldham E, *et al.* IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005; 23: 479–490.
3. Sanada S, Hakuno D, Higgins LJ, *et al.* IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *J Clin Invest* 2007; 117: 1538–1549.
4. Weinberg EO, Shimpo M, De Keulenaer GW, *et al.* Expression and regulation of ST2, an interleukin-1 receptor family member, in cardiomyocytes and myocardial infarction. *Circulation* 2002; 106: 2961–2966.
5. Sinning C, Kempf T, Schwarz M, *et al.* Biomarkers for characterization of heart failure – Distinction of heart failure with preserved and reduced ejection fraction. *Int J Cardiol* 2017; 227: 272–277.
6. Dieplinger B, Egger M, Halmayer M, *et al.* Increased soluble ST2 predicts long-term mortality in patients with stable coronary artery disease: results from the Ludwigshafen risk and cardiovascular health study. *Clin Chem* 2014; 60: 530–540.
7. McLaughlin VV, Shah SJ, Souza R, *et al.* Management of pulmonary arterial hypertension. *Am Coll Cardiol* 2015; 65: 1976–1997.
8. Chida A, Sato H, Shintani M, *et al.* Soluble ST2 and N-terminal pro-brain natriuretic peptide combination. Useful biomarker for predicting outcome of childhood pulmonary arterial hypertension. *Circ J* 2014; 78: 436–442.
9. Zheng YG, Yang T, He JG, *et al.* Plasma soluble ST2 levels correlate with disease severity and predict clinical worsening in patients with pulmonary arterial hypertension. *Clin Cardiol* 2014; 37: 365–370.
10. Shao D, Perros F, Caramori G, *et al.* Nuclear IL-33 regulates soluble ST2 receptor and IL-6 expression in primary human arterial endothelial cells and is decreased in idiopathic pulmonary arterial hypertension. *Biochem Biophys Res Commun* 2014; 451: 8–14.
11. Placido R, Cortez-Dias N, Robalo Martins S, *et al.* Prognostic stratification in pulmonary hypertension: A multi-biomarker approach. *Rev Port Cardiol* 2017; 36: 111–125.
12. Oshikawa K, Yanagisawa K, Tominaga S, *et al.* ST2 protein induced by inflammatory stimuli can modulate acute lung inflammation. *Biochem Biophys Res Commun* 2002; 299: 18–24.
13. Mueller T, Dieplinger B. The Presage((R)) ST2 Assay: analytical considerations and clinical applications for a high-sensitivity assay for measurement of soluble ST2. *Expert Rev Mol Diagn* 2013; 13: 13–30.
14. Mildner M, Storka A, Lichtenauer M, *et al.* Primary sources and immunological prerequisites for ST2 secretion in humans. *Cardiovasc Res* 2010; 87: 769–777.
15. Willems S, Sels JW, Flier S, *et al.* Temporal changes of soluble ST2 after cardiovascular interventions. *Eur J Clin Invest* 2013; 43: 113–120.
16. Villacorta H, Maisel AS. Soluble ST2 testing: a promising biomarker in the management of heart failure. *Arq Bras Cardiol*.
Luk KS, et al. Meta-analysis of soluble ST2 in pulmonary hypertension

diol 2016; 106: 145–152.
17 Colvin KL, Dufva MJ, Delaney RP, et al. Biomarkers for pediatric pulmonary arterial hypertension – a call to collaborate. Front Pediatr 2014; 2: 7.
18 Ikutani M, Tsuneyama K, Kawaguchi M, et al. Prolonged activation of IL-5–producing ILC2 causes pulmonary arterial hypertrophy. JCI Insight 2017; 2: e90721.
19 Lee YT, Gong M, Chau A, et al. Pentraxin-3 as a marker of sepsis severity and predictor of mortality outcomes: a systematic review and meta-analysis. J Infect 2017; 76: 1–10.
20 Cheung A, Gong M, Bellanti R, et al. Cancer antigen-125 and risk of atrial fibrillation: a systematic review and meta-analysis. Heart Asia 2017.
21 Foris V, Kovacs G, Tscherner M, et al. Biomarkers in pulmonary hypertension: what do we know? Chest 2013; 144: 274–283.