CHEST Online Supplement

ST2 Is a Biomarker of Pediatric Pulmonary Arterial Hypertension Severity and Clinical Worsening

Megan Griffiths, MD; Jun Yang, PhD; Catherine E. Simpson, MD, MHS; Dhananjay Vaidya, MBBS, PhD; Melanie Nies, MD; Stephanie Brandal, MS; Rachel Damico, MD, PhD; D. Dunbar Ivy, MD; Eric D. Austin, MD; Michael W. Pauciulo, MBA; Katie A. Lutz, BS; Erika B. Rosenzweig, MD; Russel Hirsch, MD; Delphine Yung, MD; William C. Nichols, PhD; and Allen D. Everett, MD

CHEST 2021; 160(1):297-306

 DOI: 10.1016/j.chest.2021.01.085

e-Appendix 1.

Supplemental Methods:

Cell Culture:

Primary human pulmonary artery endothelial cells (PAEC) and human pulmonary artery smooth muscle cells (PASMC) were available from PHBI Penn Cell Center (Philadelphia, PA). Cells primarily came from adult donors (both PAH and failed donor cells). Cells were maintained (passage 3-8) in a humidified 5% CO_2-supplemented incubator at 37°C (Napco 8000 DH, Thermo Scientific), and cultured in complete endothelial or smooth muscle cell medium from Lifeline Cell Technology (Vasculife VEGF-Mv Complete Medium (LL-0005) or Vasculife SMC Complete Medium (LL-0014), respectively). Cell conditioned media was harvested when cells reached 80% confluence, immediately stored at -80°C and subsequently assayed on the MSD multiplex ELISA platform.

We used TRIzol reagent (Cat# 15596026, ThermoFisher Scientific) to extract total RNA from PAECs and PASMCs in normal culture condition at 80-90% confluency, ArrayStar 6G RNAseq service (Rockville, MD) to perform RNAseq experiments as previously reported (1). Briefly, after RNAseq library preparation and quantification, the libraries were sequenced for 150 cycles for both ends on Illumina NovaSeq 6000 instrument. Transcript abundances for each sample was estimated with StringTie [4], and the FPKM [8] value for gene and transcript level were calculated with R package Ballgown [5–7]. ST2 gene expression levels (FPKM values) were extracted from the data analysis results.

Calculation of REVEAL risk scores, REVEAL risk categories and addition of ST2:

A REVEAL registry risk score was calculated based on the original REVEAL registry algorithm with updated cutoffs from the REVEAL 2.0 algorithm (REVEAL 2.0 score)(2). The REVEAL 2.0 score is a multi-variable weighted mortality risk prediction score that is well validated in adults; variables include age, sex, PAH type, RAP, PVR, heart rate, systolic blood pressure, functional class, 6MWD, diffusion capacity for carbon monoxide (DLCO), presence of a pericardial effusion, and BNP or NT-proBNP, with updated cutoffs used to calculated the REVEAL 2.0 score. The REVEAL 2.0 score includes additional variables, all cause hospitalization and renal function (eGFR) which were not available and thus not included in calculations. The REVEAL 2.0 score is not a pediatric specific risk score, including multiple variables that are not pediatric specific, but it has shown good performance in pediatric subjects(3). Calculation of REVEAL 2.0 scores includes 12 possible parameters, with points

added or subtracted based on clinical variables. While the REVEAL 2.0 score includes 12 possible parameters, not all are expected to be always available; the REVEAL score maintains validity as long as at least 7 parameters are available for calculation(4, 5). Thus, subjects' scores were calculated based on available parameters as long as at least 7 parameters were available. Notably, only 1 subject was excluded from the PAHB for an inadequate number of REVEAL score variables, making the total PAHB cohort 180 subjects available for analysis.

All eligible subjects in the PAHB (N=180) had REVEAL scores calculated using the REVEAL 2.0 algorithm with available variables. This is based on age, sex, PAH Type, heart rate, systolic blood pressure, NYHA functional class, 6-minute walk test, hemodynamic measures (right atrial pressure, pulmonary vascular resistance), and NT-proBNP at the time of enrollment. REVEAL scores ranged from 2-12 with a median score of 7 (Table 1). The original REVEAL score divides subjects into 5 risk categories. Thus, subjects REVEAL scores were divided into 5 REVEAL risk categories based on the original REVEAL registry algorithm(2). Classification of scores into risk categories was as follows: category 1, low risk, included scores 1-7; category 2, average risk, for a score of 8; category 3, moderately high risk, for a score of 9; category 4, high risk, for a score of 10-11; and category 5, very high risk, for a score greater than 12. Subjects were divided to generate REVEAL 2.0 risk categories based on REVEAL 2.0 scores.

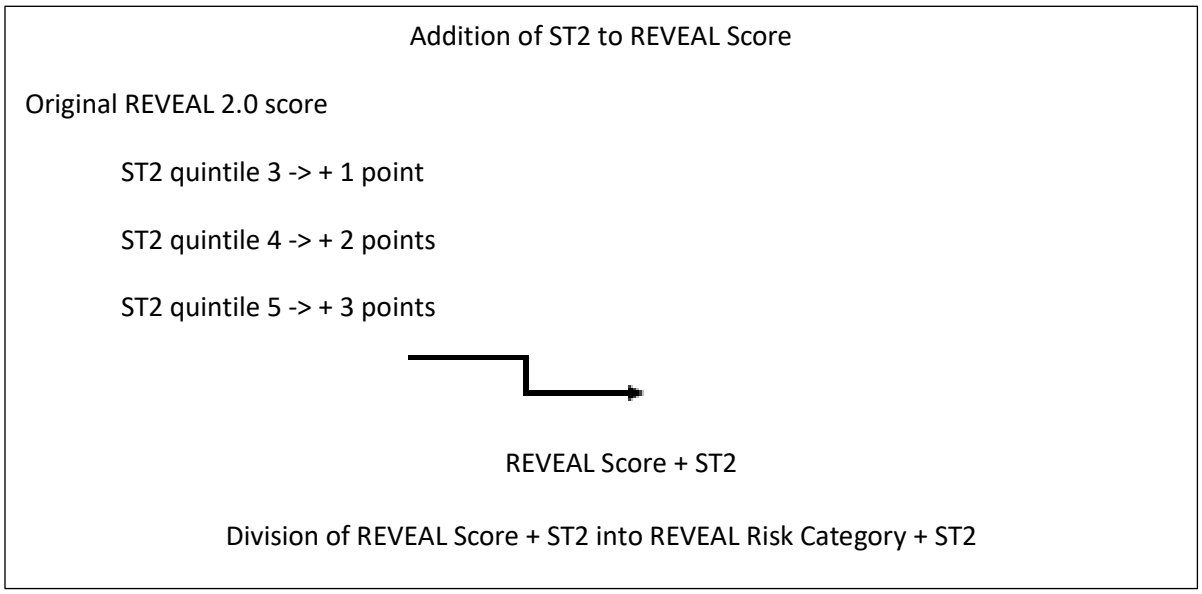
Division of score into REVEAL Risk Category

REVEAL score 1-7	Category 1, Low Risk
REVEAL score 8	Category 2, Avrage Risk
REVEAL score 9	Category 3, Moderately High Risk
REVEAL score 10-11	Category 4, High Risk
REVEAL score >12	Category 5, Very High Risk

In order to add ST2 into the REVEAL score, ST2 was divided into quintiles. Quintiles were pragmatically chosen in order to have multiple equal-sized ordered groups, allowing for the possibility of any threshold effects between ST2 and outcomes, and for symmetry with the validated REVEAL risk categories. Risk of event in each ST2 quintile was assessed by Cox proportional hazards ratio. The risk of event trended toward an increase in quintile 3, with the highest risk in quintile 5 (HR 4.2, 95% CI 0.9-20.2, p=0.07), although with 36

total subjects in each quintile, the model was underpowered. Points were then added to the REVEAL scores based on ST2 quintile with 1 point added for the third quintile, 2 points for the fourth quintile, and 3 points for the 5th quintile. After addition of ST2, the median REVEAL 2.0 score was 8 and ranged from 2-15. The REVEAL score + ST2 was then then re-categorized into the 5 REVEAL risk categories + ST2 based on the same breakdown of scores for each category.

The REVEAL risk category and REVEAL risk category +ST2 models were compared by C-statistic to discrimination of outcome of each model. Cox proportional hazard models were again developed for REVEAL scores and the REVEAL scores + ST2 as well as the respective REVEAL risk categories. Each model was compared to the model with ST2 added using Akaike Information Criterion (AIC)



1. Simpson CE, Chen JY, Damico RL, Hassoun PM, Martin LJ, Yang J, et al. Cellular sources of IL-6 and associations with clinical phenotypes and outcomes in PAH. Eur Respir J. 2020.

2. Benza RL, Gomberg-Maitland M, Miller DP, Frost A, Frantz RP, Foreman AJ, et al. The REVEAL Registry Risk Score Calculator in Patients Newly Diagnosed With Pulmonary Arterial Hypertension. Chest. 2012;141(2):354-62.

3. Barst RJ, McGoon MD, Elliott CG, Foreman AJ, Miller DP, Ivy DD. Survival in Childhood Pulmonary Arterial Hypertension: Insights From the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management. 2012;125(1):113-22.

4. Benza RL, Gomberg-Maitland M, Elliott CG, Farber HW, Foreman AJ, Frost AE, et al. Predicting Survival in Patients With Pulmonary Arterial Hypertension: The REVEAL Risk Score Calculator 2.0 and Comparison With ESC/ERS-Based Risk Assessment Strategies. Chest. 2019;156(2):323-37.

5. Benza RL, Miller DP, Gomberg-Maitland M, Frantz RP, Foreman AJ, Coffey CS, et al. Predicting Survival in Pulmonary Arterial Hypertension. Circulation. 2010;122(2):164-72.

e-Table 1. Demographics of subjects with any missing hemodynamic or functional variables

	PAH Biobank (N=16)	**Children's Hospital Colorado (CHC) (N=5)**
Demographics		
Age, years	13 (8-17.5)	5 (3-8)*
Sex, n female (%)	10 (62%)	37 (60)
Weight, kg	30.5 (10.5-54)	15.9 (11.7-26)*
Height, cm	137 (88-159)	104 (84-123)
BSA, m^2	1 (0.5-1.4)	0.69 (0.5-1)
Deaths, n (%)	0 (0)	1 (1.6)
Events, n (%)	0 (0)	11 (18)
Etiology, n (%)	-	-
APAH	9 (50)	28 (45)
IPAH	8 (44)	21 (34)
FPAH	1 (6)	5 (8)
PVOD/PCH	0	2 (3)
Other	0	5 (8)
Number of visits	-	2 (1-3)
Time between visits, months	-	16 (11-27)
Length of follow up, months	-	29 (8-87)
Time from enrollment to censor (months)	43 (32-56)	

Number missing is total number of subjects missing any hemodynamic variable. No subjects missing all hemodynamic variables.

e-Table 2. ST2 and NT-proBNP by PAH subtype

	PAH Biobank (n=182)		Children's Hospital of Colorado (n=61)	
	ST2 (pg/mL)	**NT-proBNP (ng/mL)**	**ST2 (pg/mL)**	**NT-ProBNP (ng/mL)**
IPAH	2979 (1979-4583, n=84)	135 (60-415, n=84)*	2730 (2090-7225, n=23)	222 (109-4853, n=23)
FPAH	3828 (2196-4458, n=11)	184 (61-439, n=11)	3522 (2231-4961, n=5)	100 (73-186), n=5)
APAH-CHD	3061 (2143-4736, n=69)	309 (174-588, n=69)*	2800 (1961-4513, n=29)	379 (213-734, n=29)
APAH-CTD	3973 (2978-4085, n=6)	154 (28-400, n=6)	-	-
APAH- Portopulmonary hypertension	6838 (1600-9671, n=3)	326 (216-422, n=3)	-	-
APAH-Drugs and Toxins	4965 (n=1)	96.1 (n=1)	-	-
APAH-Other	2934 (2874-3140, n=5)	289 (137-336, n=5)	-	-
PVOD/PCH	13522 (4232-22871, n=2)	4853 (191-8516, n=2)	1661 (n=1)	2201 (n=1)
Other Group 1 PAH	8192 (n=1)	14.4 (n=1)	35101(2075-68128, n=3)	4910 (43-9776, n=3)

All data presented as median (IQR, number of subjects).
** P<0.05*
See Table 1 for all other abbreviations.

e-Table 3. Subjects in each REVEAL Risk Category and in REVEAL Risk Category + ST2 in PAHB

REVEAL Category	Subjects in REVEAL 2.0 category total n/n with events (% with event)	Subjects in REVEAL 2.0 Category + ST2 total n/n with events (% with event)
1	101/6 (6%)	65/2 (3%)
2	38/4 (11%)	33/0 (0%)
3	20/2 (10%)	33/4 (12%)
4	18/3 (17%)	35/5 (14%)
5	4/3 (75%)	15/7 (47%)

Subjects divided into each REVEAL Risk Category (based on REVEAL score) in the PAHB at baseline. After addition of ST2 to REVEAL 2.0 score (REVEAL Score + ST2), subjects were reclassified into REVEAL Risk Categories. Total number of subjects in category, subject with adverse events shown, and percent with events shown.

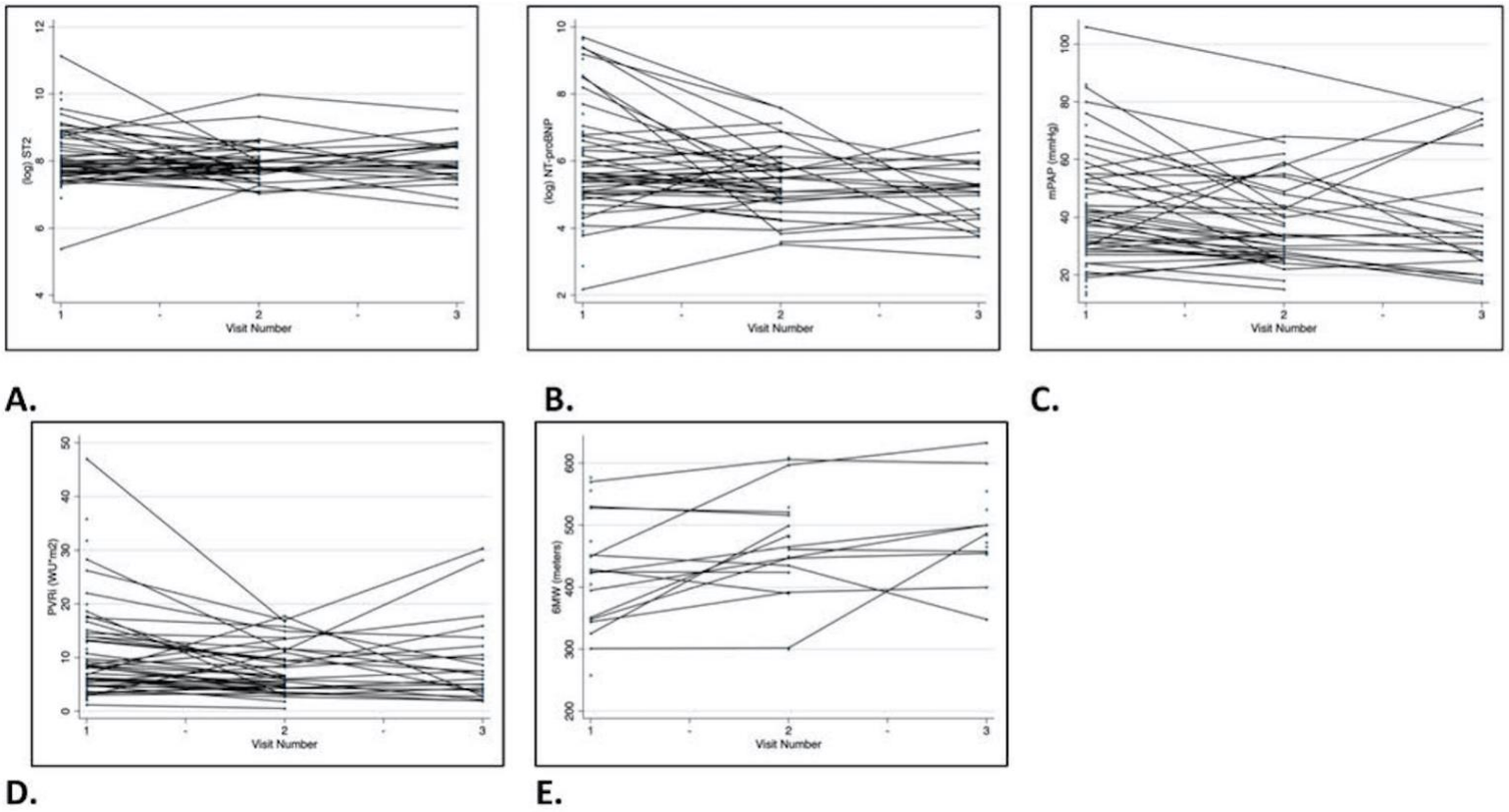
e-Table 4. Summary and comparison of candidate REVEAL score and REVEAL Score + ST2 risk models in PAHB using Akaike Information Criterion (AIC)

Model		AIC
A	**REVEAL 2.0**	101.4
C	**REVEAL 2.0 + ST2**	97.8
B	**REVEAL Risk Category**	103.9
D	**REVEAL Risk Category + ST2**	101.8

AIC comparison of model fit between REVEAL 2.0 and REVEAL 2.0+ ST2 model showing that Reveal 2.0 + ST2 model has better fit.
AIC comparison of model fit between REVEAL Risk Category and REVEAL Risk Category + ST2 showing that REVEAL Risk Category + ST2 has better model fit.

CHEST® Online Supplement

e-Figure 1: Biomarker level and clinical variables over clinic visits 1-3 in CHC cohort.



A. log ST2 in CHC subjects over visits 1-3. B. log NT-proBNP in CHC subjects over visits 1-3. C. Mean pulmonary artery pressure (mPAP) in CHC subjects over visits 1-3. D. Pulmonary Vascular Resistance index (PVRi) in CHC subjects over visits 1-3. E. Six-minute walk distance (meters) in CHC subjects over visits 1-3.

202691