Noninvasive Prognostic Biomarkers for Left-Sided Heart Failure as Predictors of Survival in Pulmonary Arterial Hypertension

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e-Appendix 1.

Supplemental Methods

Calculation of REVEAL risk scores and classification into risk categories

The REVEAL risk score is a weighted risk algorithm developed from the U.S.-based Registry to Evaluate Early and Long-term PAH Disease Management. The score incorporates patient-level data from a total of 12 possible evaluable clinical parameters. For all subjects in our cohort, REVEAL risk scores were calculated based on the available clinical parameters obtained at PAH Biobank enrollment using both originally published REVEAL parameters and cut points (so-called "REVEAL 1.0"),[1] as well as modified point values and cut points published in a recent update of the risk score ("REVEAL 2.0").[2] Two model parameters that were added and revised in the REVEAL 2.0 update, hospitalizations within the last six months and creatinine clearance, were not available for our cohort, and thus not included in our calculations. In both REVEAL 1.0 and 2.0 scoring systems, based on a subject's measured value of each evaluable clinical parameter, 1 or 2 points are either added to or subtracted from a baseline score of 6 to tabulate the final risk score. When REVEAL was established, it was expected that not every evaluable parameter would be available for a given subject at a given point in time, and REVEAL has been shown to maintain significant predictive power and calibration when at least seven evaluable parameters are available for inclusion into the score.[1,2] Therefore, missing parameters for subjects in our cohort were omitted from our scoring tabulations. REVEAL risk score was calculated for each subject as long as at least seven evaluable parameters were available.

Calculated REVEAL 1.0 scores ranged from 2 to 15, and REVEAL 2.0 scores ranged from 1 to 14. Based on risk score, each subject was classified into one of five previously defined risk categories[3,4] as follows: scores 1-7 were classified into category 1 (low risk); score 8 was classified into category 2 (average risk); score 9 was classified into category 3 (moderately high risk; scores 10-11 were classified into category 4 (high risk); and scores of 12 or higher were classified into category 5 (very high risk). The same classification scheme was utilized to create REVEAL 1.0 and REVEAL 2.0 risk categories based upon REVEAL 1.0 and REVEAL 2.0 risk scores, respectively.

Modification of risk scores to incorporate biomarkers that improve fit of base models

Assessments of likelihood ratio tests and Akaike Information Criteria showed that addition of the biomarker ST2 to base survival models improved model fit and predictive capacity. To incorporate information about ST2 into the risk scores for each subject, we evaluated ST2 thresholds for addition of risk points to initially calculated risk scores. REVEAL parameter point values and cut points were originally established by examining parameter data for measurement thresholds associated with worse than average survival in univariable analyses. For symmetry with the previously described five risk categories, untransformed ST2 measurements were divided into quintiles. Kaplan Meier analysis (shown in Supplemental Figure 1) and univariable Cox proportional hazard models demonstrated significantly worse survival among subjects in the fourth (HR 5.55, 95% CI 3.28-9.37) and fifth quintiles (HR 11.15, 95% CI 6.72-18.48) of ST2. Thus, one point was added to the previously calculated risk score for subjects with ST2 measurements in the fourth quintile, and 2 points were added to the previously calculated risk score for subjects with ST2 measurements in the fifth quintile. Once risk scores were modified to incorporate information about ST2, subjects were re-classified into the same 5 risk categories, as previously described.

e-Table 1. Age- and sex-adjusted linear regressions of biomarkers and continuous clinical variables, *overall cohort* (n= 2,017)

	(log) NT-proBNP	(log) Gal3	(log) ST2
RAP, mmHg	0.67 (0.51-0.83, <0.001)	0.62 (0.22-1.02, 0.002)	1.20 (0.87-1.54, <0.001)
mPAP, mmHg	1.15 (0.75-1.55, <0.001)	-0.27 (-1.26 – 0.72, 0.59)	1.32 (0.48-2.17, 0.002)
PAWP, mmHg	0.05 (-0.07-0.18, 0.40)	0.19 (-0.11 – 0.49, 0.21)	0.12 (-0.14 -- -0.38, 0.36)
PVR, Wood units	0.48 (0.30-0.65, <0.001)	-0.10 (-0.53 -- 0.33, 0.65)	0.55 (0.18-0.92, 0.003)
Cardiac output, L/min	-0.08 (-0.13- -0.02, 0.005)	0.06 (-0.07-0.19, 0.35)	-0.07 (-0.18 -- -0.04, 0.19)
Cardiac index, L/min/m2	-0.02 (-0.05- -0.02, 0.26)	0.001 (-0.08 – 0.09, 0.98)	-0.04 (-0.12-0.03, 0.24)
Stroke volume, L	-0.001 (-0.002- -0.00, 0.009)	0.00 (-0.003-0.002, 0.85)	-0.003 (-0.005-- -0.001, 0.001)
PA compliance, mL/mm Hg	-0.09 (-0.13- -0.05, <0.001)	0.07 (-0.02-0.16, 0.15)	-0.10 (-0.17 -- -0.02, 0.012)
RV stroke work, mmHg · L	-0.01 (-0.06- 0.03, 0.55)	-0.10 (-0.21- 0.01, 0.07)	-0.13 (-0.22- -0.04, 0.007)
RV stroke work index, g/m$_2$/beat	0.00 (0.00- 0.00, 0.87)	-0.001 (-0.002-0.00, 0.08)	-0.001 (-0.002-0.00, 0.013)
RV power, mmHg · L/min	1.73 (-2.61- 6.08, 0.43)	-0.97 (-11.45- 9.42, 0.86)	4.32 (-4.78- 13.42, 0.35)
HR, beats/min	0.23 (-0.33-0.80, 0.42)	1.67 (0.28-3.06, 0.019)	2.39 (1.22-3.57, <0.001)
6MWD, m	-19.44 (-24.88- -14.01, <0.001)	-20.42 (-33.97 -- -6.87, <0.001)	-28.16 (40 -- -16.28, <0.001)

All data presented as regression coefficient (95% CI, p value).
Definition of abbreviations: PA: pulmonary arterial; HR: heart rate. See Table 1 for all other abbreviations.

e-Table 2. Age- and sex-adjusted linear regressions of biomarkers and continuous clinical variables, *limited to subjects with serum obtained within 12 months of clinical tests* (n=454).

	(log) NT-proBNP	(log) Gal3	(log) ST2
RAP, mmHg	1.07 (0.71-1.42, <0.001)	0.95 (0.14-1.76, 0.02)	2.04 (1.29-2.78, <0.001)
mPAP, mmHg	1.66 (0.85-2.46, <0.001)	1.24 (-0.58-3.07, 0.18)	1.88 (0.19-3.57, 0.03)
PAWP, mmHg	-0.01 (-0.28-0.26, 0.95)	0.27 (-0.33-0.87, 0.38)	0.10 (-0.46-0.66, 0.73)
PVR, Wood units	0.80 (0.45-1.15, <0.001)	0.60 (-0.20-1.40, 0.14)	0.79 (0.05-1.53, 0.04)
Cardiac output, L/min	-0.11 (-0.22- -0.002, 0.04)	-0.18 (-0.42-0.06, 0.14)	-0.07 (-0.29-0.16, 0.54)
Cardiac index, L/min/m2	-0.07 (-0.13- -0.02, 0.013)	-0.10 (-0.23- 0.03, 0.12)	-0.09 (-0.21-0.04, 0.16)
Stroke volume, L	-0.002 (-0.004-0.00, 0.015)	-0.006 (-0.01- -0.002, 0.005)	-0.004 (-0.008- -0.001, 0.018)
PA compliance, mL/mm Hg	-0.17 (-0.26- -0.08, <0.001)	-0.10 (-0.25-0.05, 0.20)	-0.08 (-0.20-0.05, 0.23)
RV stroke work, mmHg · L	-0.03 (-0.10- 0.04, 0.42)	-0.20 (-0.37- -0.03, 0.018)	-0.18 (-0.32- 0.04, 0.013)
RV stroke work index, g/m2/beat	0.00 (-0.001- 0.00, 0.14)	-0.001 (-0.003- -0.00, 0.019)	-0.002 (-0.003- 0, 0.002)
RV power, mmHg · L/min	-3.82 (-13.16- 5.53, 0.42)	-4.03 (-25.51-15.44, 0.68)	-7.79 (-26.92-11.34, 0.42)
HR, beats/min	0.87 (-0.26-2.00, 0.13)	4.12 (1.54-6.69, 0.002)	2.45 (0.23-4.66, 0.03)
6MWD, m	-17.74 (-27.53- -7.94, <0.001)	-22.48 (-46.84-1.87, 0.07)	-45.38 (-66.86- -23.91, <0.001)

All data presented as regression coefficient (95% CI, p value).
Definition of abbreviations: PA: pulmonary arterial; HR: heart rate. See Table 1 for all other abbreviations.

e-Table 3. Age- and sex-adjusted linear regressions of biomarkers and continuous clinical variables, *limited to subjects with serum obtained within 6 months of clinical tests* (n=285).

	(log) NT-proBNP	(log) Gal3	(log) ST2
RAP, mmHg	1.32 (0.86-1.77, <0.001)	1.30 (0.28-2.31, 0.01)	2.47 (1.51-3.42, <0.001)
mPAP, mmHg	1.97 (0.99-2.95, <0.001)	1.16 (-1.00-3.31, 0.29)	2.34 (0.27-4.40, 0.027)
PAWP, mmHg	-0.07 (-0.42-0.28, 0.71)	0.37 (-0.38-1.11, 0.33)	0.02 (-0.69-0.74, 0.95)
PVR, Wood units	0.93 (0.51-1.34, <0.001)	0.51 (-0.41-1.43, 0.28)	0.96 (0.08-1.83, 0.032)
Cardiac output, L/min	-0.19 (-0.32- -0.06, 0.005)	-0.03 (-0.31-0.26, 0.86)	-0.15 (-0.43-0.12, 0.27)
Cardiac index, L/min/m2	-0.11 (-0.19- -0.04, 0.003)	-0.06 (-0.22- 0.10, 0.44)	-0.14 (-0.30-0.02, 0.08)
Stroke volume, L	-0.003 (-0.005- -0.001, 0.013)	-0.005 (-0.01- -0.001, 0.031)	-0.005 (-0.009- -0.001, 0.014)
PA compliance, mL/mm Hg	-0.17 (-0.26- -0.08, <0.001)	-0.09 (-0.27-0.08, 0.30)	-0.13 (-0.28-0.02, 0.08)
RV stroke work, mmHg · L	-0.06 (-0.14- 0.03, 0.18)	-0.20 (-0.39- -0.001, 0.05)	-0.16 (-0.33- 0.01, 0.057)
RV stroke work index, g/m2/beat	-0.001 (-0.001- 0.00, 0.037)	-0.002 (-0.003- -0.00, 0.06)	-0.002 (-0.003- 0.001, 0.005)
RV power, mmHg · L/min	-8.24 (-20.95- 4.46, 0.20)	10.11 (-15.74-35.96, 0.44)	-3.09 (-28.23-22.06, 0.81)
HR, beats/min	1.51 (0.14-2.89, 0.03)	5.47 (2.33-8.62, 0.001)	4.03 (1.31-6.75, 0.004)
6MWD, m	-24.88 (-39.30- -10.46, 0.001)	-45.92 (-81.31- -10.53, 0.11)	-73.55 (-104.30- -42.80, <0.001)

All data presented as regression coefficient (95% CI, p value).
Definition of abbreviations: PA: pulmonary arterial; HR: heart rate. See Table 1 for all other abbreviations.

e-Table 4. Sensitivity analyses of Cox multivariable hazard ratios for mortality, excluding covariates with significant missingness. Each model adjusted for age, sex, subtype of PAH, PAH-specific therapy, NYHA FC, RAP, mPAP, CI, PVR. The excluded covariate(s) is noted in the model label.

	(log) NT-proBNP	(log) Galectin 3	(log) ST2
Overall cohort	1.84, 1.62-2.10, <0.001	1.12, 0.85-1.47, 0.44	2.79, 2.21-3.53, <0.001
6MWD excluded	1.77, 1.62-1.94, <0.01	1.33, 1.08-1.63, 0.01	2.28, 1.98-2.63, <0.01
6MWD and NYHA FC excluded	1.77, 1.62-1.94, <0.01	1.37, 1.11-1.70, <0.01	2.29, 2.00-2.63, <0.01

All data presented as hazard ratio, 95% confidence interval, p value.

e-Table 5. Sensitivity analyses of Cox multivariable hazard ratios for mortality limited to a subgroup of patients enrolled into the cohort within 12 months of diagnostic RHC. Each model adjusted for age, sex, subtype of PAH, PAH-specific therapy, NYHA FC, RAP, mPAP, CI, PVR.

	(log) NT-proBNP	(log) Galectin 3	(log) ST2
Overall cohort	1.84, 1.62-2.10, <0.001	1.12, 0.85-1.47, 0.44	2.79, 2.21-3.53, <0.001
Enrolled within 12 mos of RHC	1.93, 1.43-2.61, <0.001	0.94, 0.56-1.57, 0.81	3.32, 1.96-5.63, <0.001
6MWD excluded	1.71, 1.40-2.09, <0.001	1.18, 0.81-1.71, 0.39	2.29, 1.59-3.28, <0.001
6MWD and NYHA FC excluded	1.64, 1.34-2.00, <0.001	1.20, 0.83-1.74, 0.33	2.20, 1.54-3.14, <0.001

All data presented as hazard ratio, 95% confidence interval, p value.

e-Table 6. Summary of candidate multivariable survival models.

Model	Terms	Model df	AIC
A	Age, sex, disease subtype, PAH therapy, NYHA FC, 6MWD, RAP, mPAP, CI, PVR, NT-proBNP	23	1812.56
B	Age, sex, disease subtype, PAH therapy, NYHA FC, 6MWD, RAP, mPAP, CI, PVR, NT-proBNP *plus ST2*	24	1791.18
C	Age, sex, disease subtype, PAH therapy, NYHA FC, 6MWD, RAP, mPAP, CI, PVR, NT-proBNP *plus ST2 and Gal 3*	25	1793.16
D	REVEAL terms	16	1109.81
E	REVEAL terms *plus ST2*	17	1105.03
F	REVEAL terms except 6MWD (excluded in sensitivity analysis)	15	2178.65
G	REVEAL terms except 6MWD (excluded in sensitivity analysis) *plus ST2*	16	2169.40
Definition of abbreviations. df: degrees of freedom; AIC: Akaike information criterion. See Table 1 for other abbreviations. REVEAL terms include disease subtype (presence of PAH associated with collagen vascular disease, portopulmonary hypertension, familial PAH etc), age, sex, functional class, heart rate, systolic blood pressure, right atrial pressure, pulmonary vascular resistance, 6 minute walk distance, and NT-proBNP.			

e-Table 7. Comparisons of null and extended models using likelihood ratio tests.

| Null Model	Extended Model	Δ df	Δ-2LL	p-value
A	B	1	23.38	<0.001
A	C	2	23.40	<0.001
D	E	1	6.77	0.009
F	G	1	11.25	<0.001
Definition of abbreviations: df: degrees of freedom; Δ-2LL: likelihood ratio test, expressed as the product of -2 times the change in log-likelihood. See Table 5 for model parameters.				

e-Table 8. Re-classification of subjects into risk categories based on modification of REVEAL 1.0 risk scores to incorporate ST2 measurements. Re-classification is shown for events (i.e. deaths) during follow up time versus non-events.

Risk Category	REVEAL 1.0 risk score at enrollment		Score modified to incorporate ST2	
	Nonevent	Event	Nonevent	Event
	(n=1660)	(n=324)	(n=1660)	(n=324)
	n (%)	n (%)	n (%)	n (%)
Category 1	1,013 (61)	107 (33)	858 (52)	64 (20)
Category 2	293 (18)	68 (21)	295 (18)	49 (15)
Category 3	187 (11)	77 (24)	199 (12)	62 (19)
Category 4	149 (9)	62 (19)	230 (14)	98 (30)
Category 5	18 (0.01)	10 (3)	78 (5)	51 (16)

e-Table 9. Re-classification of subjects into risk categories based on modification of REVEAL 2.0 risk scores to incorporate ST2 measurements. Re-classification is shown for events (i.e. deaths) during follow up time versus non-events.

Risk Category	REVEAL 2.0 risk score at enrollment		Score modified to incorporate ST2	
	Nonevent	Event	Nonevent	Event
	(n=1660)	(n=324)	(n=1660)	(n=324)
	n (%)	n (%)	n (%)	n (%)
Category 1	932 (56)	82 (25)	830 (50)	58 (18)
Category 2	297 (18)	64 (20)	248 (15)	37 (11)
Category 3	203 (12)	60 (18)	205 (12)	42 (13)
Category 4	188 (11)	93 (29)	272 (16)	104 (32)
Category 5	40 (2.4)	25 (8)	105 (6)	83 (26)

e-Figure 1. Kaplan-Meier analysis of quintiles of ST2. Log-rank p is <0.001.
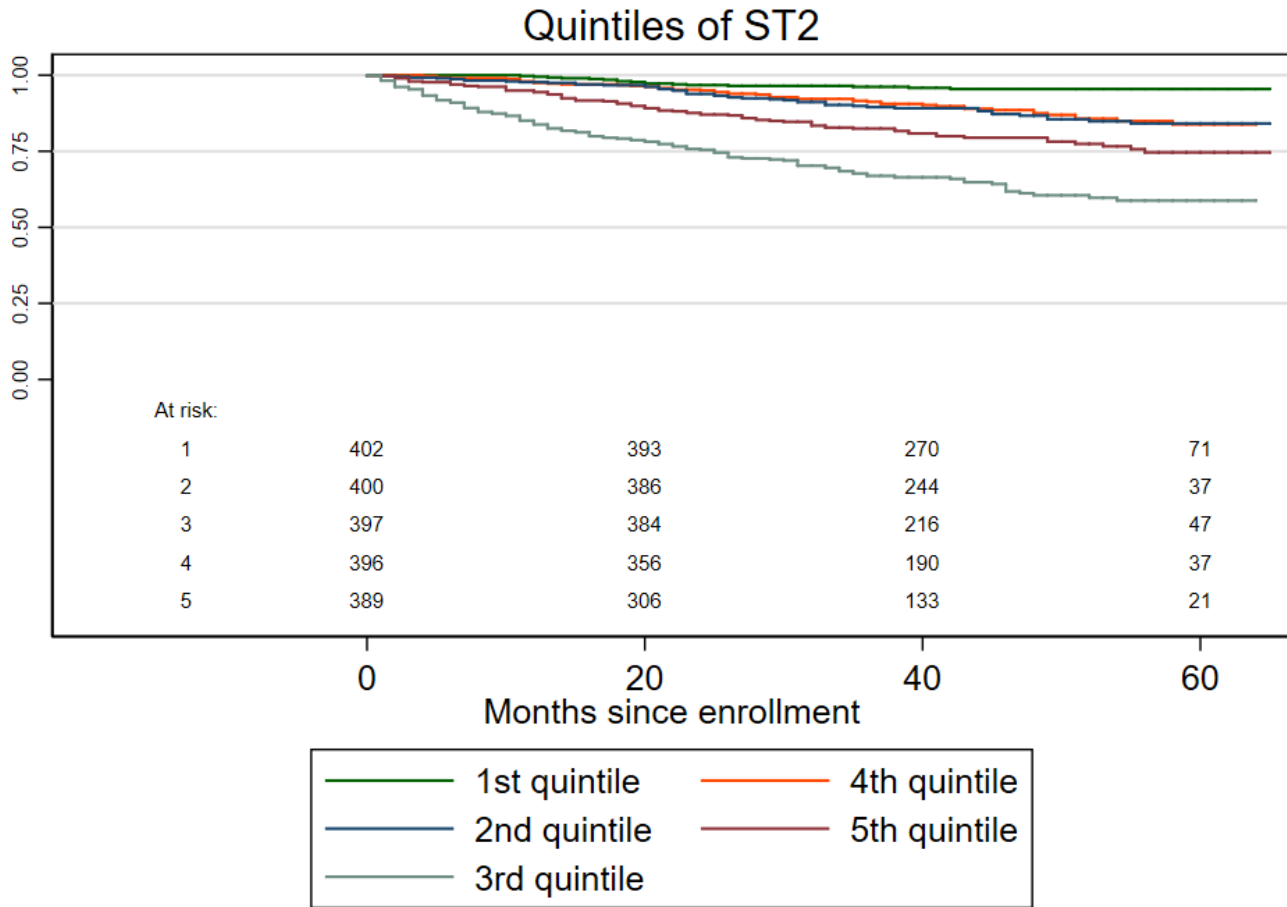
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