One-year follow-up

Supplementary Material

Innsbruck PAH registry

2021-10-07

Contents

Supplementary Methods

Data transformation, visualization, descriptive statistic

Data transformation, analysis and result visualization was accomplished by R version 4.0.5 with *tidyverse* environment. Figures were generated with *cowplot* package (1), Supplementary Material file was built with *rmarkdown* environment (packages *knitr*, *rmarkdown*, *kableExtra* and *bookdown*) (2).

For univariable survival modeling and construction of candidate risk signatures, a set of categorical 23 demographic, biochemical, right-heart catheter, laboratory, ultrasound and lung function parameters recorded at PH diagnosis was used. For the list of modeling variables and their stratification scheme, see: **Supplementary Table S1**.

Hypothesis testing, multiple comparisons

As the majority of the analyzed numeric variables were non-normally distributed as checked by Shapiro-Wilk test, differences in median values between analysis groups were investigated by Mann-Whitney and Kruskal-Wallis test, as appropriate. Differences in survival between groups were compared by Kaplan-Meier (KM) analysis, Mentel-Henszel and log-rank test (3), as appropriate. KM analysis and visualization of its results were done with tools provided by *survival* and *survminer* packages (4) and home-developed wrappers (https://github.com/PiotrTymoszuk/KM-Toolbox).

For each analysis and cohort, p values were corrected for multiple comparisons with Benjamini-Hochberg method (5).

Univariable Cox survival modeling

Association of candidate categorical variables (**Supplementary Table S1**) with overall survival time was assessed by series of univariable Cox proportional hazard models constructed for the Innsbruck and Linz/Vienna cohort using *survival* package (6) and home-developed wrappers (https://github.com/PiotrTymoszuk/KM-Toolbox). Significance of the hazard ratio estimates was determined by Wald Z test. P values were corrected for multiple comparisons with Benjamini-Hochberg method (5). For the full modeling results, see: **Supplementary Table S2**.

Development of custom risk signatures

Candidate risk signatures (n = 10879) were constructed as all possible combinations of 2 - 4 modeling variables (**Supplementary Table S1**). The development and selection of significant risk signatures involved a 3-step procedure (**Figure 3A**):

(1) Identification of the significant signatures in the training Innsbruck cohort. Correlation of the candidate signatures with overall survival time was investigated in the training Innsbruck cohort by multivariable Cox proportional hazard modeling (*survival* package_) (6). Out of 10879 tested signatures a total of 30 variable combinations displayed (**A**) significant hazard ratios for all model components (Wald Z test), (**B**) significant Harrell's concordance index (C-index, 2.5% confidence interval limit > 0.5, *concordance()* function, *survival* package) (6,7) and (**C**) Benjamini-Hochberg-adjusted significance in likelihood ratio test versus the respective NULL model. For complete multivariable Cox modeling results in the test cohort, see: **Supplementary Table S3**.

(2) Cross-validation (CV) in the training Innsbruck cohort. Ability of the signatures identified in Step 1 to reproducibly predict overall survival in the training Innsbruck cohort was assessed with 20-fold cross-validation essentially as described in (8). Briefly, for each training - test split of the Innsbruck cohort and the given variable combination, a multi-parameter Cox model was constructed in the training subset,

and its linear predictor values calculated in the test subset. Association of the linear predictor score in the test subset with survival was measured by C-index statistic. Out of the 30 signatures subjected to CV, 13 showed significant C-index values (2.5% confidence interval limit > 0.5).

(3) External validation in the test Linz/Vienna cohort. The external validation procedure was based on procedure described in (9). For the signatures passing the CV selection in Step 2 linear predictor scores based on the training cohort Cox model estimates (see: **Supplementary Table S5** for the formulas) were calculated in the test Linz/Vienna cohort. Correlation of the linear predictor scores with overall survival in the testing cohort was assessed by Cox proportional hazard modeling and C-index statistic. All 13 CV-passing risk signatures demonstrated significant association with overall survival in the test cohort

Values of mean squared errors and C-indexes in the training cohort (Step 1), cross-validation (Step 2) and test (Step 3) cohort are presented in **Figure 2** and **Supplementary Figure S1**.

Modeling of 5-year mortality

Linear predictor scores for the developed 13 risk signatures were calculated in the test Innsbruck and training Linz/Vienna cohort. Association of the score values with 5-year mortality was assessed with logistic regression (generalized linear modeling, log-link function, binomial family) and receiver-operator characteristic (ROC). Optimal score cutoffs were determined by maximizing the Youden J statistic. Score cutoff, sensitivity, specificity and area-under the curve value determination were done with *optimal.cutpoints* package (10). ROC curve plotting was accomplished by *plotROC* package tools (11).

Clustering

Clustering of the study participants in respect to numeric values of six-minute walking distance, WHO functional classification, sex (0: male, 1: female), age at diagnosis and 5-year mortality (0: survivor, 1: deceased) was performed with DBSCAN algorithm ($minPts = 5$, Euclidean distance, package *dbscan* and https://github.com/PiotrTymoszuk/cluster_tools) (12,13) separately in the Innsbruck and Linz/Vienna cohorts. The optimal *eps* value was determined by inspection of the plot of 5-nearest neighbor distance plot as described (14). By this means 3 participant subsets in each cohort were identified. To visualize the clustering assignment, the clustering variables were subjected to two-dimensional principal component analysis (PCA) using *pcaPP* package (function *PCAgrid()*) (15) and PCA scores for each observation displayed in a point plot.

Data and code availability

The study data set is available at serious request to the corresponding author. The analysis R code was deposited on GitHub (https://github.com/PiotrTymoszuk/PAH-biomarker).

Supplementary Tables

Table S1: **Study variables.** Variable: variable name used in R code, Variable label: variable label used in plots and tables, Variable type: indicator whether the variable was used as a modeling response, independent variable (signature development) or comparator risk tool. Stratification: variable stratification scheme.
The table is available online.

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Table S2: **Results of univariable Cox proportional hazard modeling.** Variable: variable name and strata, HR: hazard ratio with 95% confidence interval, N: number of complete observations, pFDR: Benjamini-Hochberg-corrected p value for HR significance.
The table is available online.

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Table S3: **Results of multivariable Cox proportional hazard modeling for the candidate risk signatures in the training Innsbruck cohort.** Significant HRs: indicator if all model betas were significant, pLRT FDR: Benjamini-Hochberg-corrected p value for the likelihood ratio test, pWald FDR: Benjamini-Hochberg-corrected p value for the Wald test, C: concordance index with 95% confidence intervals, N: number of complete observations.
The table is available online.

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Table S4: **Results of multivariable Cox proportional hazard modeling for the established PH risk assessment tools in the training Innsbruck and test Linz/Vienna cohort.** pLRT FDR: Benjamini-Hochberg-corrected p value for the likelihood ratio test, pWald FDR: Benjamini-Hochberg-corrected p value for the Wald test, C: concordance index with 95% confidence intervals, N: number of complete observations.

Cohort	Risk scale	pLRT FDR	pWald FDR	C	N
IBK	mRASP	8.0e-07	1.9e-06	0.72 [0.64 - 0.8]	101
	COMPERA	7.6e-06	1.8e-05	0.7 [0.63 - 0.77]	101
	SPAHR	3.0e-07	4.0e-07	0.72 [0.66 - 0.79]	101
	FRENCH3p	8.1e-06	2.1e-04	0.66 [0.58 - 0.74]	101
	FRENCH4p	1.2e-05	1.8e-04	0.69 [0.61 - 0.76]	101
	Reveal Lite	3.2e-06	1.1e-04	0.69 [0.61 - 0.77]	101
	Reveal 2.0	7.6e-06	2.1e-04	0.68 [0.61 - 0.76]	101
LZ/W	mRASP	4.1e-05	1.4e-04	0.67 [0.6 - 0.75]	113
	COMPERA	1.5e-03	1.5e-03	0.64 [0.57 - 0.72]	113
	SPAHR	1.7e-04	2.8e-04	0.66 [0.59 - 0.73]	113
	FRENCH3p	5.4e-06	2.2e-04	0.71 [0.63 - 0.79]	113
	FRENCH4p	6.7e-03	9.1e-03	0.66 [0.57 - 0.75]	113

Table S5: **Formulas of linear predictor scores for the developed risk signatures.**

Signature	Score formula
Signature 3	4.8 × Age: >65 + 0.36 × CI: 2 - 2.5 + 0.2 × CI: ≥2.5
Signature 14	6.6 × Age: >65 + 2.5 × Percard. Eff: yes
Signature 16	4.9 × Age: >65 + 3.3 × RAA: >20.7
Signature 17	6.1 × Age: >65 + 2.4 × RDW: >14.5
Signature 18	6.7 × Age: >65 + 0.45 × Sex: female
Signature 127	2 × GFR: <60 + 3.9 × RAA: >20.7
Signature 181	2.2 × mPAP: ≥49 + 3.5 × RAA: >20.7
Signature 182	2.7 × mPAP: ≥49 + 2.1 × RDW: >14.5
Signature 301	5.4 × Age: >65 + 0.43 × CI: 2 - 2.5 + 0.27 × CI: ≥2.5 + 2.5 × mPAP: =49
Signature 309	5.1 × Age: >65 + 0.33 × CI: 2 - 2.5 + 0.21 × CI: ≥2.5 + 0.44 × Sex: female
Signature 410	7.1 × Age: >65 + 3.9 × mPAP: ≥49 + 3.2 × Percard. Eff: yes
Signature 464	5 × Age: >65 + 2.8 × RAA: >20.7 + 2.1 × RDW: >14.5
Signature 2525	5.9 × Age: >65 + 0.4 × CI: 2 - 2.5 + 0.29 × CI: ≥2.5 + 2.2 × mPAP: =49 + 0.49 × Sex: female

Table S6: **Prediction of 5-year mortality by the developed signatures and established PH risk assessment tools investigated by receiver-operator characteristic.** J: Youden J statistic, AUC: area under the ROC curve with 95% confidence interval.

Cohort	Signature	Cutoff	Sensitivity	Specificity	J	AUC
IBK	Signature 3	0.000	0.90	0.57	0.48	0.79 [0.699 - 0.881]
	Signature 14	0.900	0.90	0.42	0.33	0.692 [0.587 - 0.796]
	Signature 16	2.800	0.71	0.66	0.38	0.729 [0.634 - 0.825]
	Signature 17	2.700	0.52	0.82	0.35	0.724 [0.606 - 0.843]
	Signature 18	1.900	0.57	0.90	0.47	0.754 [0.625 - 0.883]
	Signature 127	1.300	0.90	0.48	0.38	0.738 [0.636 - 0.84]
	Signature 181	2.000	0.62	0.86	0.48	0.788 [0.681 - 0.895]
	Signature 182	1.000	0.62	0.81	0.43	0.77 [0.656 - 0.884]
	Signature 301	0.900	0.71	0.79	0.50	0.832 [0.736 - 0.927]
	Signature 309	0.000	0.81	0.76	0.57	0.823 [0.725 - 0.921]
	Signature 410	2.500	0.62	0.85	0.47	0.798 [0.688 - 0.907]
	Signature 464	2.400	0.81	0.61	0.42	0.771 [0.672 - 0.87]
	Signature 2525	1.700	0.62	0.95	0.57	0.84 [0.74 - 0.94]
	mRASP	2.000	0.43	0.91	0.34	0.734 [0.623 - 0.846]
	COMPERA	2.000	1.00	0.28	0.28	0.708 [0.618 - 0.799]
	SPAHR	2.000	1.00	0.30	0.30	0.735 [0.65 - 0.821]
	FRENCH3p	2.000	0.95	0.31	0.26	0.635 [0.528 - 0.742]
	FRENCH4p	3.000	0.86	0.45	0.31	0.684 [0.575 - 0.792]
	Reveal Lite	2.000	0.90	0.41	0.32	0.679 [0.578 - 0.779]
	Reveal 2.0	3.000	0.81	0.52	0.33	0.678 [0.579 - 0.777]
	Signature 3	0.530	0.53	0.81	0.34	0.638 [0.462 - 0.814]
	Signature 14	0.900	0.76	0.49	0.25	0.607 [0.484 - 0.73]
	Signature 16	2.800	0.35	0.84	0.20	0.628 [0.483 - 0.772]
	Signature 17	1.800	0.59	0.56	0.15	0.584 [0.44 - 0.728]
	Signature 18	0.000	0.82	0.44	0.26	0.653 [0.515 - 0.791]
	Signature 127	1.300	0.60	0.62	0.22	0.58 [0.435 - 0.725]
	Signature 181	0.780	0.76	0.51	0.28	0.635 [0.504 - 0.766]
	Signature 182	0.750	0.94	0.27	0.21	0.616 [0.496 - 0.737]
	Signature 301	0.850	0.59	0.72	0.31	0.64 [0.47 - 0.81]
	Signature 309	0.075	0.53	0.84	0.37	0.685 [0.525 - 0.845]
	Signature 410	2.500	0.41	0.83	0.25	0.651 [0.504 - 0.798]
	Signature 464	2.700	0.35	0.84	0.20	0.624 [0.483 - 0.766]
	Signature 2525	0.095	0.65	0.69	0.33	0.683 [0.525 - 0.841]
	mRASP	1.000	0.94	0.36	0.31	0.66 [0.56 - 0.76]
	COMPERA	2.000	0.88	0.38	0.26	0.642 [0.535 - 0.75]

	SPAHR	2.000	0.94	0.34	0.28	0.671 [0.579 - 0.763]
	FRENCH3p	3.000	0.65	0.65	0.29	0.674 [0.556 - 0.792]
	FRENCH4p	2.000	0.94	0.36	0.31	0.645 [0.532 - 0.759]

Supplementary Figures

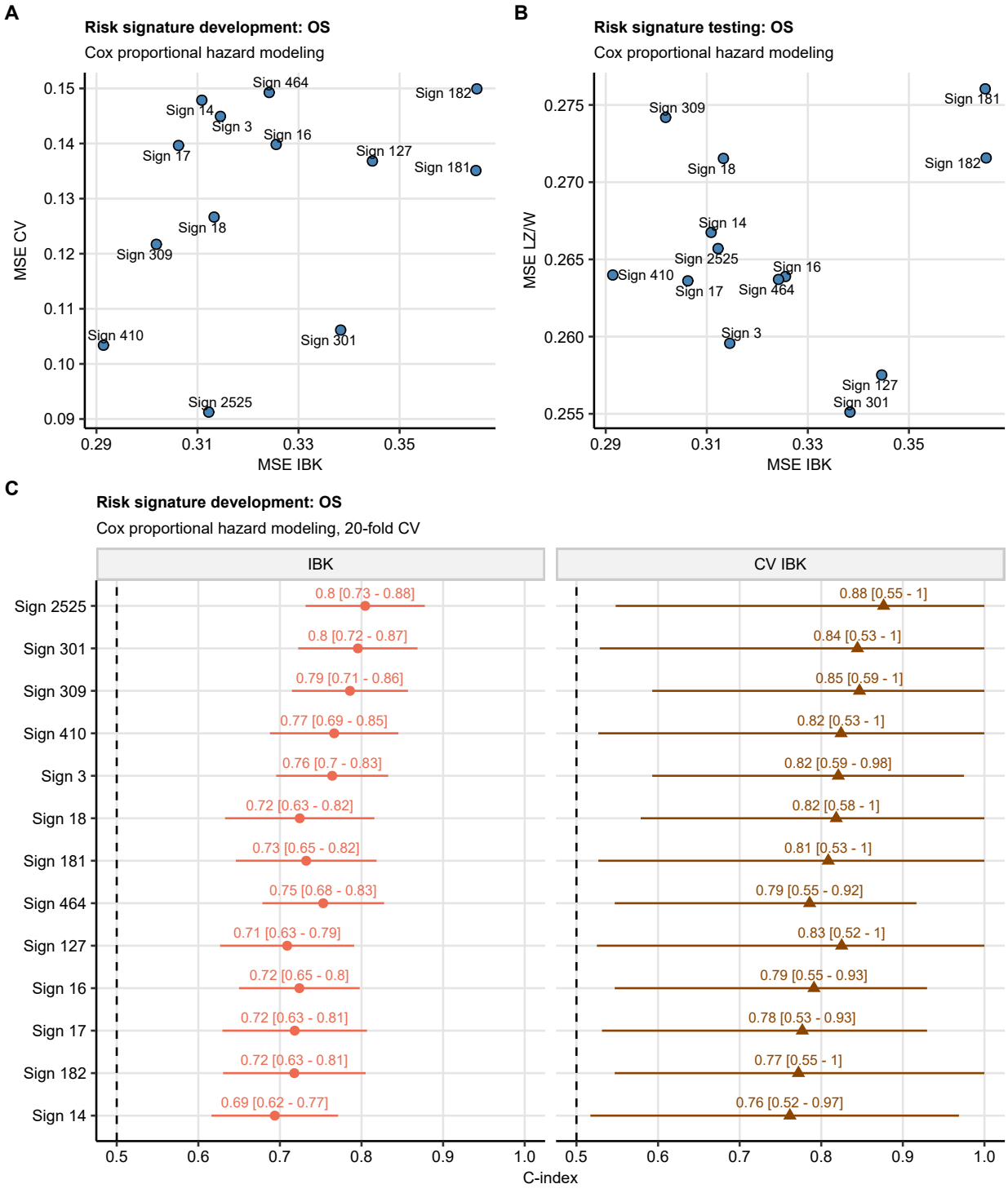

Figure S1: Fit errors and overall survival prediction performance for the significant risk signatures.

Supplementary Figure S1. Fit errors and overall survival prediction performance for the significant risk signatures.

Correlation of the candidate 2 - 4 parameter risk signatures (all possible combinations of 23 variables, **Supplementary Table S1**) with overall survival (OS) in the Innsbruck training cohort (IBK) was investigated by Cox proportional hazard modeling and verified by 20-fold cross-validation (CV) (**Figure 3**). Model fit parameters (MSE: mean squared error) and prediction accuracy measures (concordance index: C-index) for the 13 developed significant signatures are presented.

(A, B) MSE in the training cohort, cross-validation and test cohort.

(C) C-index values with 95% confidence intervals in the training and cross-validation.

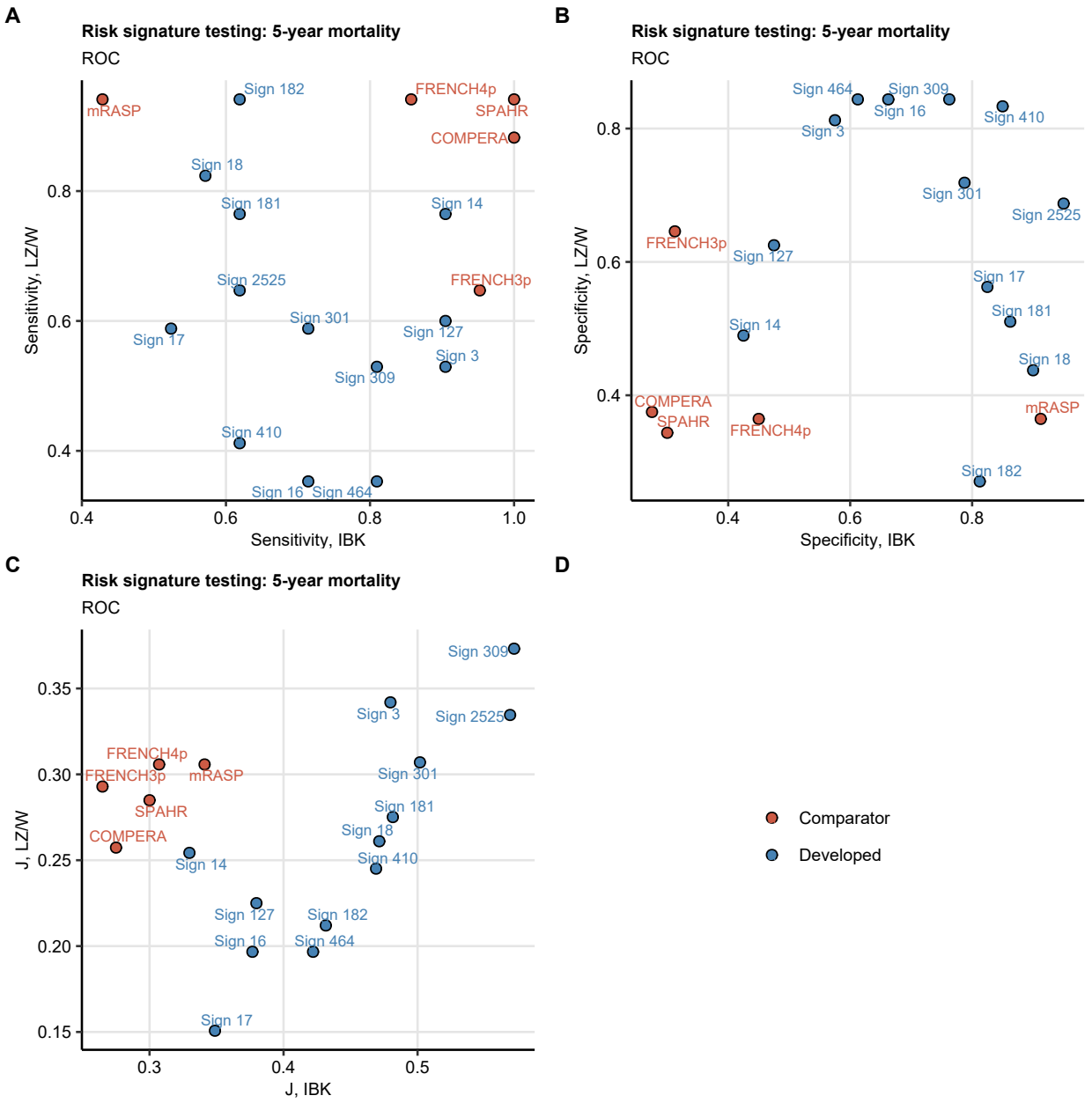

Figure S2: Sensitivity and specificity at predicting 5-year mortality.

Supplementary Figure S2. Sensitivity and specificity at predicting 5-year mortality for the developed risk signature and established PH risk assessment tools.

Association of the developed risk signatures (**Figure 3**) and established PH risk assessment tools with 5-year mortality was assessed with receiver-operator characteristic (ROC, **Supplementary Table S6**). Optimal risk score value was determined by the maximum of Youden J statistic. Sensitivity (**A**), specificity (**B**) and J values at the score cutoff in the training Innsbruck (IBK) and the test Linz/Vienna (LZ/W) cohorts are shown.

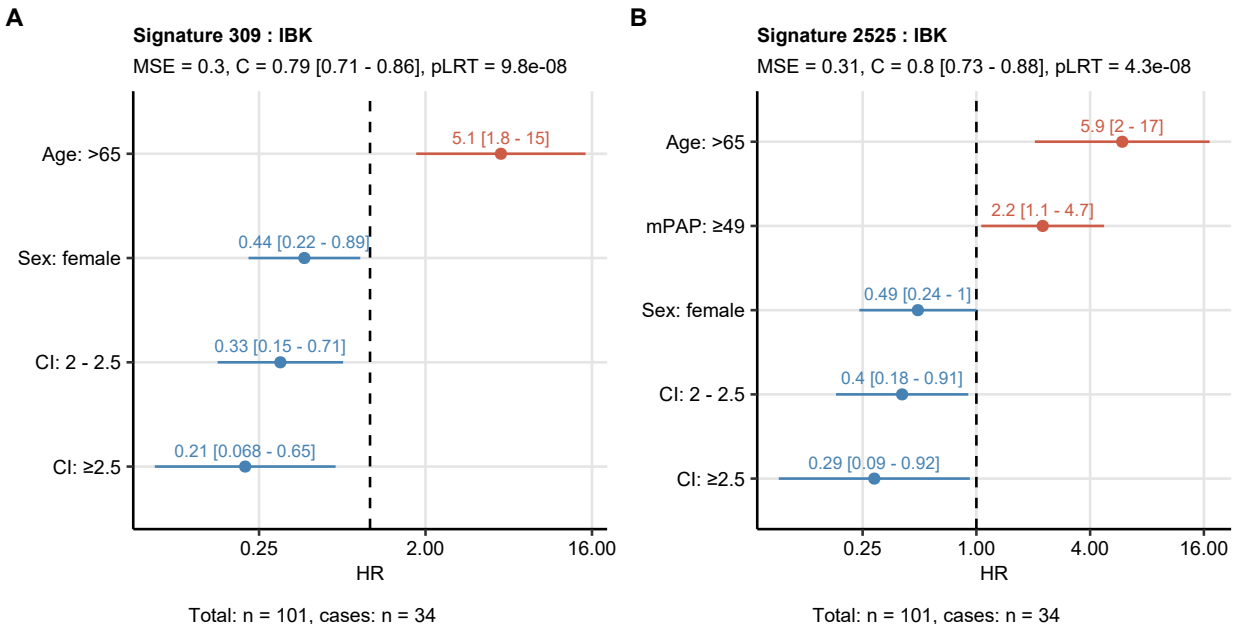

Figure S3: Hazard ratio estimates of the Cox model components for the developed risk signatures 309 and 2525.

Supplementary Figure S3. Hazard ratio estimates of the Cox model components for the developed risk signatures 309 and 2525.

The risk signatures predicting overall survival in PH were developed as presented in **Figures 3**. Values of hazard ratio estimates of the Cox proportional hazard models for the Signatures 309 and 2525 in the training Innsbruck (IBK) cohort are shown with 95% confidence intervals. Mean squared errors (MSE), concordance indexes (C) with 95% confidence intervals and significance in likelihood ratio test (pLRT) are displayed in the plot captions.

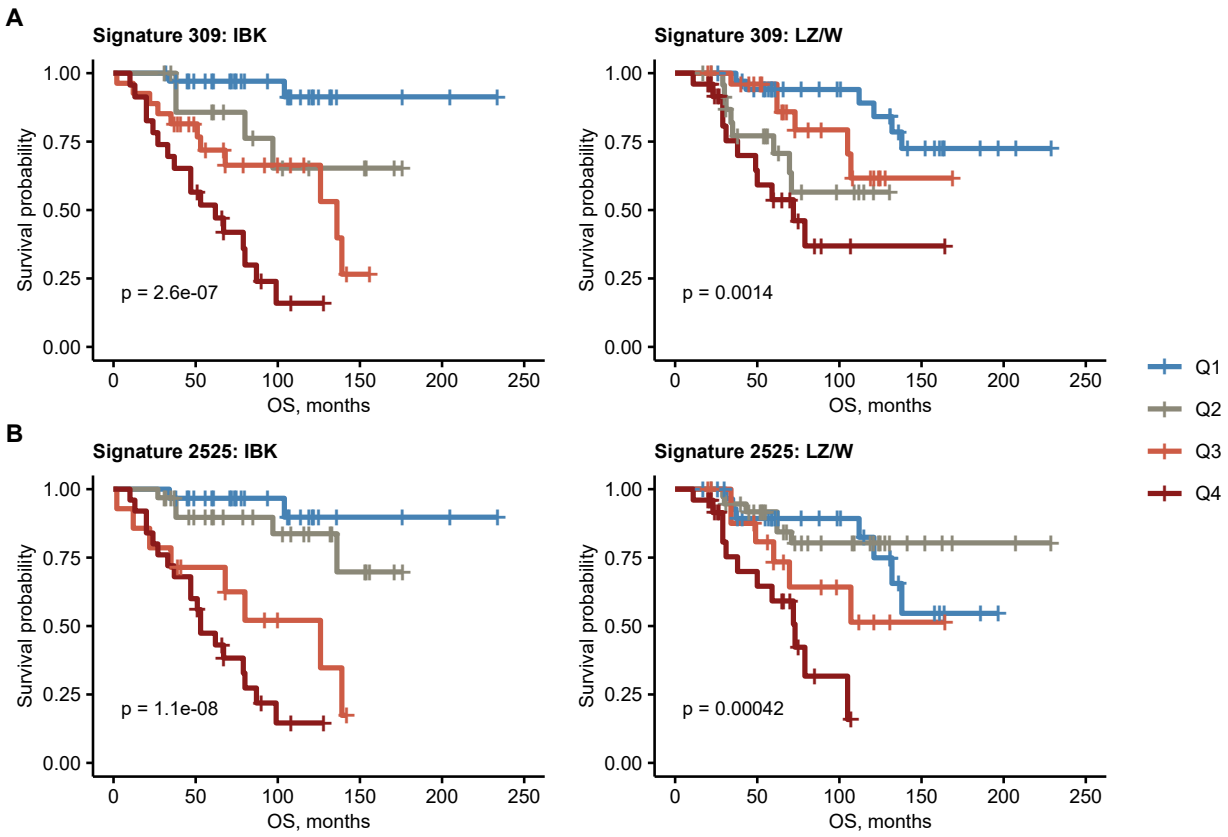

Figure S4: Values of the risk signatures 309 and 2525 scores and overall survival.

Supplementary Figure S4. Values of the risk signatures 309 and 2525 scores and overall survival.

The risk signatures predicting overall survival in PH were developed as presented in **Figures 3**. The signature linear scores (**Supplementary Table S5**) in the training Innsbruck (IBK) and test Linz/Vienna cohort (LZ/W) were stratified by quartiles (Q1: 1^{st}, Q2: 2^{nd}, Q3: 3^{rd} and Q4: 4^{th}) and the survival differences between the score strata were compares by Kaplan-Meier analysis and log-rank test. P values corrected for multiple comparisons with Benjamini-Hochberg method are shown in the plots.

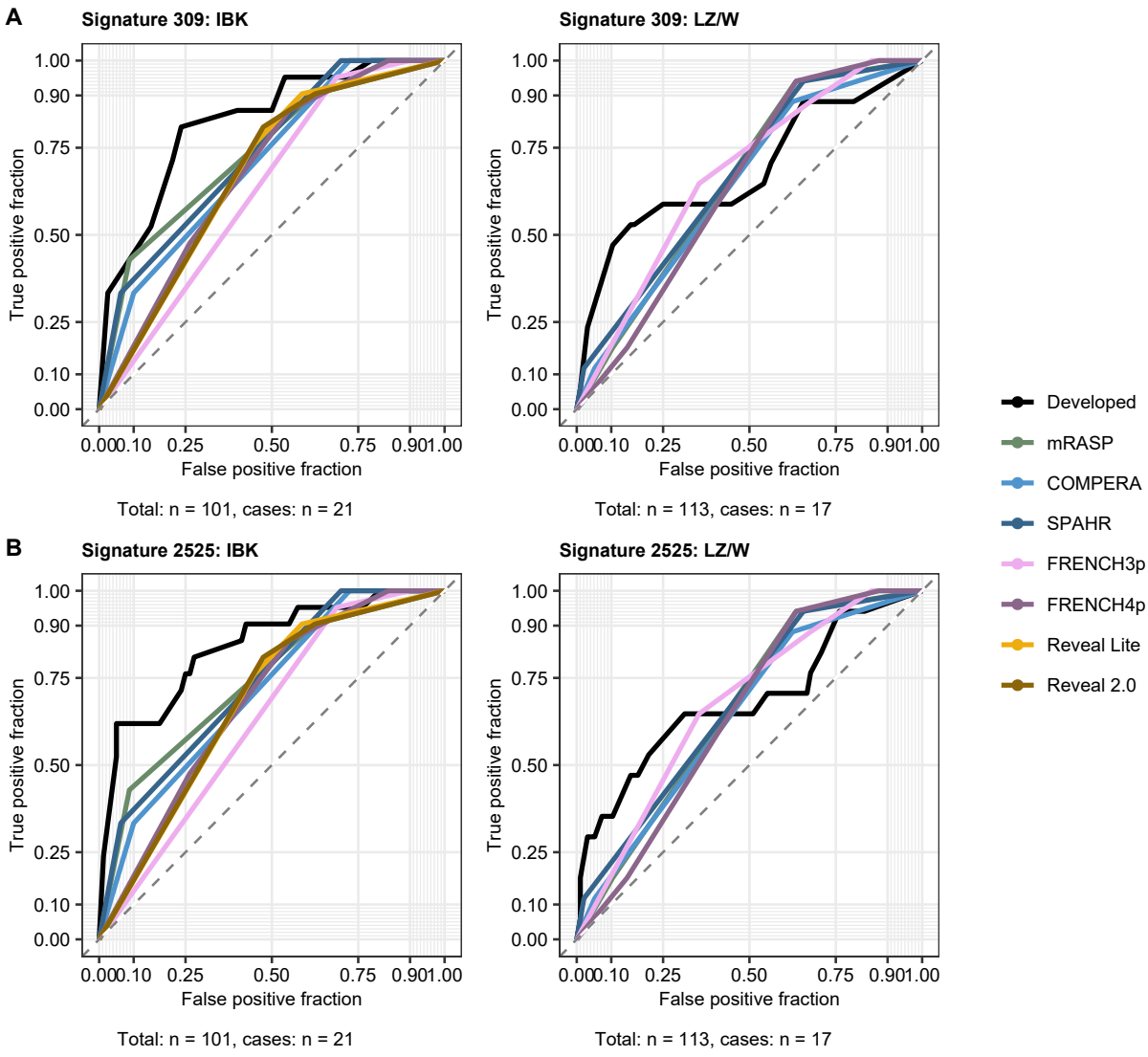

Figure S5: Values of the risk signatures 309 and 2525 scores and 5-year survival analyzed by receiver-operator characteristic.

Supplementary Figure S5. Values of the risk signatures 309 and 2525 scores and 5-year survival analyzed by receiver-operator characteristic.

The risk signatures predicting overall survival in PH were developed as presented in **Figures 3**. The ability of the signature linear scores (**Supplementary Table S5**) to predict five-year survival in the training Innsbruck (IBK) and test Linz/Vienna cohort (LZ/W) was assessed with receiver-operator characteristic (ROC, **Supplementary Table S6**). ROC curves for the signatures 309 and 2525 and the established PH risk assessement tools are shown.

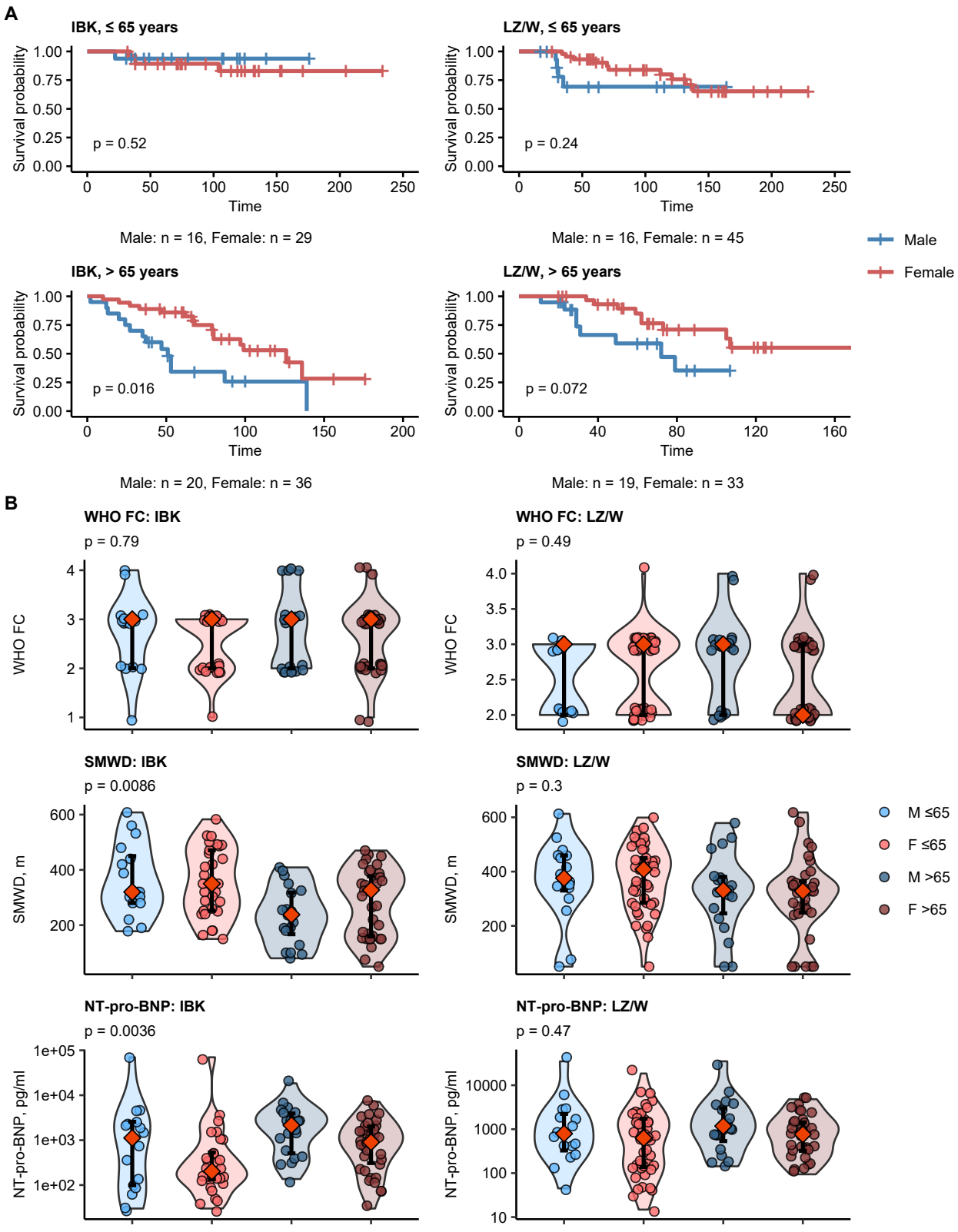

Figure S6: Interplay of gender and age affects PH survival independently of functional classification, motility and NT-pro-BNP levels.

Supplementary Figure S6. Interplay of gender and age affects PH survival independently of functional classification, motility and NT-pro-BNP levels.

(A) Differences in PH survival between the participants stratified by age class and sex (IBK: Innsbruck, LZ/W: Linz/Vienna cohort) were assessed by Kaplan-Meier analysis and Mentel-Henszel test. P values corrected for multiple comparisons with Benjamini-Hochberg method are shown in the plots. Numbers of males and females in the age strata are indicated below the plots.

(B) Differences in WHO functional classification (WHO FC), six-minute walking distance (SMWD) and circulating NT-pro-BNP levels at PH diagnosis in the participants stratified by age class and sex were assessed by Kruskal-Wallis test. P values corrected for multiple comparisons with Benjamini-Hochberg method are shown in the plot captions.

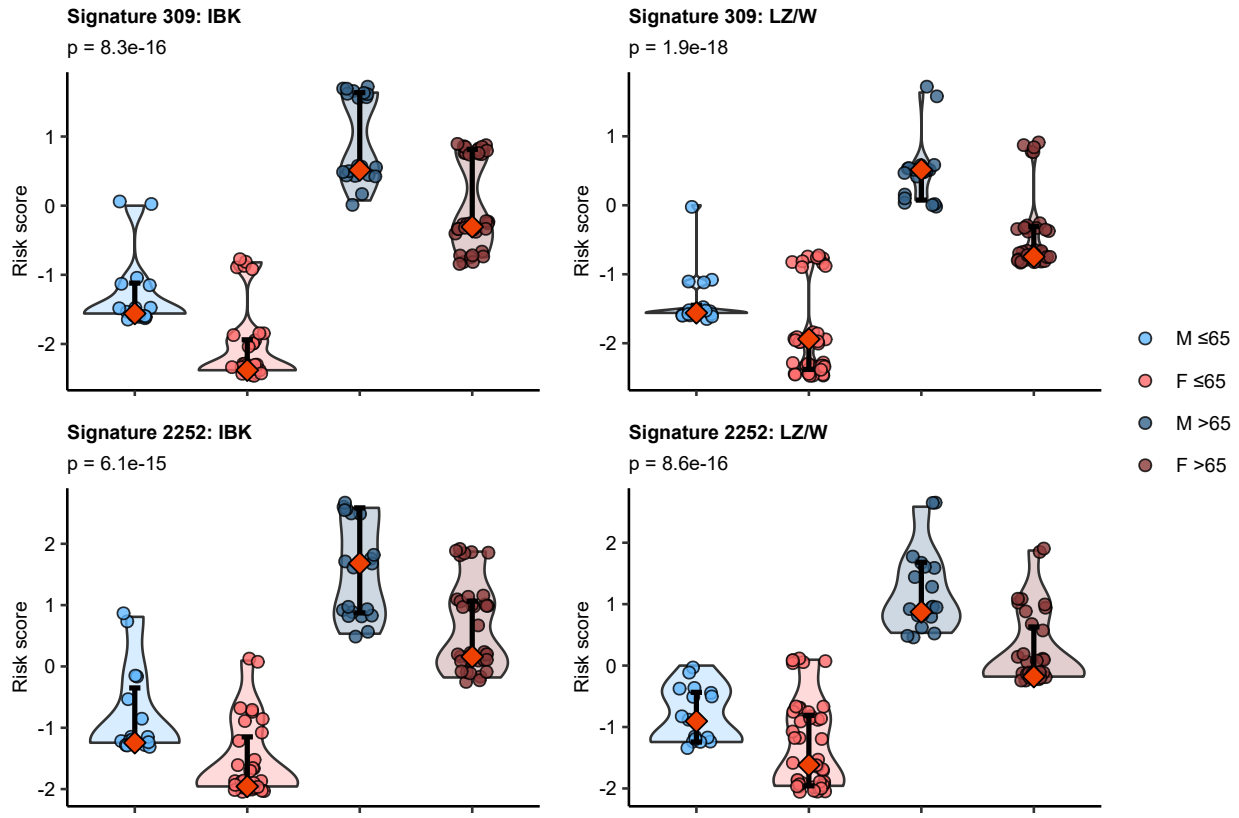

Figure S7: Values of the risk signatures 309 and 2525 scores in gender and age strata.

Supplementary Figure S7. Values of the risk signatures 309 and 2525 scores in gender and age strata.

The risk signatures predicting overall survival in PH were developed as presented in **Figures 3**. The differences in the signature linear scores (**Supplementary Table S5**) between the participants stratified by age class and sex (IBK: Innsbruck, LZ/W: Linz/Vienna cohort) were assessed by Kruskal-Wallis test. P values corrected for multiple comparisons with Benjamini-Hochberg method are shown in the plot captions. IBK $\leq$ 65: male n = 16, female n = 29, IBK >65: male n = 20, female n = 36, LZ/W $\leq$ 65: male n = 16, female n = 45, LZ/W >65: male n = 19, female n = 33.

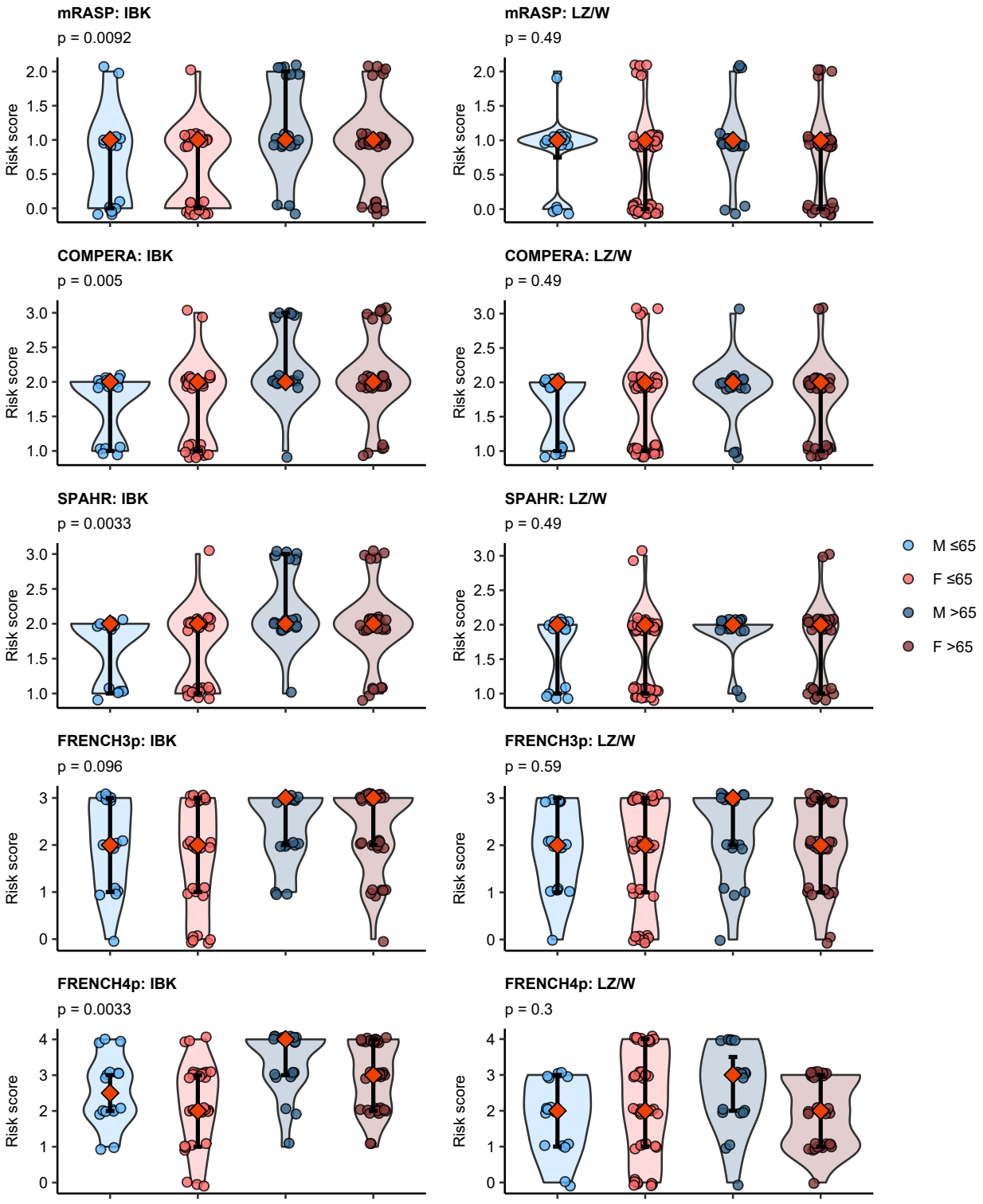

Figure S8: Scoring by the established PH risk assessment tools in gender and age strata.

Supplementary Figure S8. Scoring by the established PH risk assessment tools in gender and age strata.

The differences in scoring by the established PH risk assessment tools between the participants stratified by age class and sex (IBK: Innsbruck, LZ/W: Linz/Vienna cohort) were assessed by Kruskal-Wallis test. P values corrected for multiple comparisons with Benjamini-Hochberg method are shown in the plot captions. IBK $\leq$ 65: male n = 16, female n = 29, IBK >65: male n = 20, female n = 36, LZ/W $\leq$ 65: male n = 16, female n = 45, LZ/W >65: male n = 19, female n = 33.

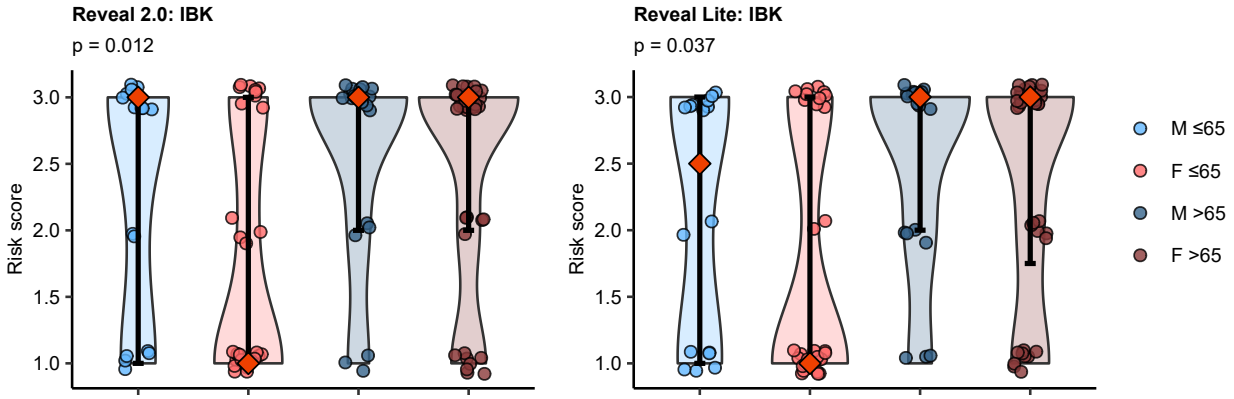

Figure S9: Scoring by the REVEAL risk assessment tools in gender and age strata.

Supplementary Figure S9. Scoring by the REVEAL risk assessment tools in gender and age strata.

The differences in REVEAL 2.0 and REVEAL Lite scoring between the participants stratified by age class and sex in the Innsbruck (IBK) cohort were assessed by Kruskal-Wallis test. P values corrected for multiple comparisons with Benjamini-Hochberg method are shown in the plot captions. IBK $\leq$ 65: male n = 16, female n = 29, IBK >65: male n = 20, female n = 36.

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