Severe Pulmonary Arterial Hypertension Is Characterized by Increased Neutrophil Elastase and Relative Elafin Deficiency

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**e-Table 1. NE and elafin dose series for pulmonary artery endothelial cell culture studies.**

| Dose series (mass based) | NE/elafin (ng/mL) | NE:elafin ratio | NE/elafin (µM) | NE:elafin molar ratio |
|--------------------------|-------------------|-----------------|-----------------|---------------------|
|                          | 0/0               | -               | 0/0             | -                   |
|                          | 400/0             | 8:1             | 13.2/0          | 1.60                |
|                          | 400/50            | 4:1             | 13.2/8.33       | 0.80                |
|                          | 400/100           | 2:1             | 13.2/16.67      | 0.40                |
|                          | 400/200           | 1:1             | 13.2/33.33      | 0.20                |
|                          | 400/400           | 1:2             | 13.2/66.67      | 0.10                |
|                          | 400/800           |                 | 13.2/133.3      |                     |
**e-Table 2. NE and elafin in relation to PAH clinical severity metrics. Upper panel:** NE and elafin univariate relationships with various clinical metrics. **Lower panel:** Median regression analysis of the same relationships adjusted for age, sex, and BMI (see footnotes for additional details).  

### Unadjusted correlation analysis.\(^a\)

| Clinical variable | NE Spearman’s rho (P-value) | Elafin Spearman’s rho (P-value) |
|-------------------|-----------------------------|---------------------------------|
| 6MWD              | -0.300 (P=0.006) **         | 0.036 (P=0.580)                  |
| NT-proBNP         | 0.250 (P=0.011) *           | -0.058 (P=0.362)                 |
| DLCO              | -0.047 (P=0.478)            | 0.037 (P=0.582)                  |
| Echo TAPSE        | -0.231 (P=0.019) *          | 0.016 (P=0.807)                  |
| mPAP              | 0.205 (P=0.039) *           | -0.018 (P=0.784)                 |
| RAP               | 0.092 (P=0.153)             | -0.008 (P=0.902)                 |
| PVR               | 0.077 (P=0.227)             | -0.043 (P=0.500)                 |

### Median regression analysis adjusted for age, sex, and BMI.\(^b\)

| Clinical variable | NE (regression coefficient p-value) | Elafin (regression coefficient p-value) |
|-------------------|-------------------------------------|-----------------------------------------|
| 6MWD              | ↓ 34.2 meters (P=0.004) **          | ↑ 3.0 meters (P=0.510)                  |
| NT-proBNP         | relative ↑ 20.2% (P=0.032) *        | relative ↓ 16.5% (P=0.087)              |
| DLCO              | ↓ 5.5% of predicted (P=0.221)       | ↑ 0.6% of predicted (P=0.734)           |
| Echo TAPSE        | ↓ 0.18 cm (P=0.041) *               | ↑ 0.03 cm (P=0.427)                    |
| mPAP              | ↑ 4.1 mmHg (P=0.037) *              | ↑ 0.3 mmHg (P=0.769)                   |
| RAP               | ↑ 0.1 mmHg (P=0.884)                | ↓ 0.01 mmHg (P=0.986)                  |
| PVR               | ↑ 15.8 dynes-sec/cm\(^5\) (P=0.686) | ↓ 41.8 dynes-sec/cm\(^5\) (P=0.357)     |

\(^a\) Correlation analysis: Spearman’s rho statistic estimated a rank-based measure of association, and p-values shown are based on a two-sided null hypothesis (no direct or inverse relationship).

\(^b\) Median regression analysis adjusted for age, sex, and BMI: Molecular biomarkers (NE, elafin) were independently assessed as predictors of each PAH clinical metric with age, sex, and BMI included as covariates in all models. NE, elafin, and NT-proBNP were log-transformed for regression as distributions were right-skewed. The regression coefficient p-values are shown for each molecular predictor. Regression coefficients were used to determine the magnitude each clinical marker differs with two-fold higher NE and elafin. Because NT-proBNP was log-transformed for analysis, \(\Delta\) NT-proBNP per two-fold higher NE and elafin is expressed in relative percentages (rather than absolute units).
### Risk of death associated with NE >168.5 ng/ml threshold (adjusted multivariable analysis)

| Stepwise addition of adjustment covariates (added=italic) | HR (95% CI) | P-value |
|----------------------------------------------------------|-------------|---------|
| Age (≥60 years)                                          | 3.12 (1.75 – 5.56) | 0.0001 *** |
| Age + sex (male)                                         | 3.12 (1.75 – 5.58) | 0.0001 *** |
| Age + sex + etiology (CTD-APAH or PoPH)                  | 3.22 (1.80 – 5.76) | 0.000007 *** |
| Age + sex + etiology + incident PAH status               | 3.24 (1.81 – 5.80) | 0.000007 *** |
| Age + sex + etiology + incident PAH + prostacyclin       | 3.13 (1.75 – 5.60) | 0.0001 *** |
| Age + sex + etiology + incident PAH + prostacyclin + NYHA functional class (III or IV) | 2.60 (1.44 – 4.69) | 0.0014 ** |
| Age + sex + etiology + incident PAH + prostacyclin + NYHA FC + NTproBNP (>1400 pg/mL) | 2.45 (1.34 – 4.48) | 0.0036 ** |
| Age + sex + etiology + incident PAH + prostacyclin + NYHA FC + NTproBNP + RAP (≥14 mmHg) (full model) | 2.52 (1.36 – 4.65) | 0.0032 ** |

### Risk of death for each covariate in full multivariable model

| Covariate:                                   | HR (95% CI) | P-value |
|----------------------------------------------|-------------|---------|
| NE >168.5 ng/mL                              | 2.52 (1.36 – 4.65) | 0.0032 ** |
| Age ≥60 years                                 | 1.60 (0.91 – 2.79) | 0.101   |
| Male sex                                     | 1.81 (0.99 – 3.30) | 0.053   |
| CTD-APAH or PoPH etiology                    | 2.00 (1.19 – 3.36) | 0.009   |
| Incident PAH status (vs prevalent)            | 2.25 (1.12 – 4.51) | 0.022 *  |
| Prostacyclin treatment                       | 1.69 (0.93 – 3.05) | 0.084   |
| NYHA functional class III or IV              | 2.94 (1.53 – 5.63) | 0.0012 ** |
| NT-proBNP >1400 pg/mL                       | 2.51 (1.47 – 4.28) | 0.0008 *** |
| Right atrial pressure ≥14 mmHg               | 1.15 (0.65 – 2.02) | 0.634   |

*P<0.05, **P<0.01, ***P<0.001
e-Table 4. NE adds incremental value to established PAH risk scores for outcome prediction. Tabulated statistics are displayed from pairs of nested Cox regression models, which evaluated the incremental prognostic value added by NE to three different validated PAH risk scores: the Registry to Evaluate Early and Long-Term PAH Disease Management (REVEAL) risk calculator version 2.0, the French Pulmonary Hypertension Registry (FPHR) strategy, and the Comparative Prospective Registry of Newly Initiated Therapies for Pulmonary Hypertension (COMPERA) algorithm, which each stratified low, intermediate, and high-risk groups (as described in the footnotes and e-Appendix 6). Cox models fit with NE and each risk score are compared to nested risk score-only models. Corresponding goodness-of-fit statistics (log likelihood and chi-square \([\chi^2]\) values) are shown for each model. \(P\)-values reflect the likelihood ratio test, which evaluated whether NE-inclusive models added significant value in the prediction of death or transplant at 5 years.

| Model | Predictor | HR (95% CI) | \(P\)-value for predictor | \(\chi^2\) statistic for model | Log likelihood for model | \(P\)-value model comparison |
|-------|-----------|-------------|----------------------------|-------------------------------|--------------------------|----------------------------|
| Model 1a | REVEAL score (per risk group) | 2.52 (1.87-3.40) | <0.0001 | 40.21 | -314.26 | 0.0003 |
| Model 1b | REVEAL score NE >168.5 ng/mL | 2.27 (1.69-3.04) | <0.0001 | 49.06 | -320.70 |
| Model 2a | FPHR score (per risk group) | 2.15 (1.49-3.10) | <0.0001 | 17.09 | -325.82 | 0.0016 |
| Model 2b | FPHR score NE >168.5 ng/mL | 1.88 (1.31-2.71) | <0.0001 | 28.93 | -330.77 |
| Model 3a | COMPERA score (per risk group) | 2.37 (1.65-3.42) | <0.0001 | 21.43 | -323.65 | 0.0026 |
| Model 3b | COMPERA score NE >168.5 ng/mL | 2.16 (1.50-3.11) | <0.0001 | 34.12 | -328.17 |

\(a\) REVEAL 2.0 risk score: composite risk score from a variety of clinical variables including PAH subtype, presence of renal insufficiency, age, sex, NYHA functional class, systolic blood pressure, heart rate, six-minute walk distance, NT-proBNP, echocardiographic presence of pericardial effusion, diffusion capacity of the lung, mean right atrial pressure, pulmonary vascular resistance, and all-cause hospitalization in preceding 6 months. Patients were stratified into 3 risk groups according to score: low (0-6), intermediate (7-8), and high (>9).

\(b\) FPHR risk score: risk stratification algorithm based on the number of low-risk PAH clinical variables (including functional class I/II, six-minute walk distance <440 meters, right atrial pressure <8 mmHg, and cardiac index >= 2.5 L min\(^{-1}\) m\(^{-2}\)). Patients were divided into 3 risk groups: low (3 or 4 low-risk criteria), intermediate (1 or 2 low-risk criteria), and high risk (no low-risk criteria).

\(c\) COMPERA risk score: risk tool based on six variables (functional class, six-minute walk distance, NT-proBNP, right atrial pressure, cardiac index, and mixed venous oxygen saturation) which were each split by thresholds defining low risk (1 point), intermediate risk (2 points), and high risk (3 points). For each patient, the total number of points was summed across all available variables, then divided by number of available variables, and rounded to nearest integer to yield overall risk stratification into 3 groups (1=low risk, 2=intermediate risk, 3=high risk).

Additional details about risk score calculations are provided in e-appendix 6c.
**Table 5. Baseline characteristics of United Kingdom validation cohorts A and B.**
Descriptive statistics are shown for various clinical features at the time of baseline NE measurement. Data from the Stanford PAH primary analysis cohort is also provided for reference.

|                      | UK IPAH validation cohort A (N=75) | UK IPAH validation cohort B (N=357) | Stanford PAH primary analysis cohort (N=249) |
|----------------------|-----------------------------------|-----------------------------------|--------------------------------------------|
| **Age, years, median (IQR)** | 55 (39-70)                        | 39 (30-48)                        | 49 (38-59)                                |
| **Gender, n (%)**     |                                   |                                   |                                           |
| Female               | 45 (60.0%)                        | 263 (73.7%)                       | 191 (76.7%)                               |
| Male                 | 30 (40.0%)                        | 94 (26.3%)                        | 58 (23.3%)                                |
| **Race/ethnicity, n (%)** |                                  |                                   |                                           |
| White                | 59 (78.7%)                        |                                  | 139 (55.8%)                               |
| Black                | 3 (4.0%)                          |                                  | 14 (5.6%)                                 |
| Hispanic             | 0 (0.0%)                          |                                  | 40 (16.1%)                                |
| Asian                | 11 (14.7%)                        |                                  | 43 (17.3%)                                |
| Other                | 2 (2.7%)                          |                                  | 13 (5.2%)                                 |
| **PAH subtype, n (%)** |                                  |                                   |                                           |
| IPAH + HPAH          | 75 (100.0%)                       | 357 (100.0%)                      | 69 (27.7%)                                |
| D&T-APAH             | -                                 | -                                 | 47 (18.9%)                                |
| CTD-APAH             | -                                 | -                                 | 79 (31.7%)                                |
| PoPH                 | -                                 | -                                 | 16 (6.4%)                                 |
| CHD-APAH             | -                                 | -                                 | 38 (15.3%)                                |
| **NYHA FC, n (%)**   |                                   |                                   |                                           |
| Class I              | 1 (1.3%)                          | 8 (2.3%)                          | 14 (5.6%)                                 |
| Class II             | 14 (18.7%)                        | 68 (19.9%)                        | 93 (37.3%)                                |
| Class III            | 41 (54.7%)                        | 215 (63.0%)                       | 114 (45.8%)                               |
| Class IV             | 19 (25.3%)                        | 50 (14.7%)                        | 28 (11.2%)                                |
| **6MWD, meters, median (IQR)** | 258 (120-369)                    | 352 (270-431)                     | 423 (341-513)                             |
| **Therapy extent, n (%)** |                                   |                                   |                                           |
| Naïve                | 19 (25.3%)                        | -                                 | 87 (35.0%)                                |
| Monotherapy          | 34 (47.9%)                        | -                                 | 70 (28.1%)                                |
| Dual therapy         | 20 (28.2%)                        | -                                 | 69 (27.7%)                                |
| Triple therapy       | 2 (2.7%)                          | -                                 | 23 (9.2%)                                 |
| **Hemodynamics, median (IQR)** |                                   |                                   |                                           |
| Right atrial pressure, mmHg | 12 (8-18)                      | 8 (6-12)                          | 7 (5-11)                                  |
| mPAP, mmHg           | 51 (46-62)                        | 55 (47-63)                        | 50 (40-60)                                |
| Cardiac index, L/min/m² | 2.20 (1.71-2.59)              | 2.04 (1.66-2.56)                 | 2.09 (1.76-2.43)                          |
| PVR, dynes-sec/cm⁵   | 817 (600-1048)                    | 935 (637-1324)                   | 821 (506-1197)                            |

6MWD = 6-minute walk distance, APAH = associated PAH, CHD = congenital heart disease, CTD = connective tissue disease, D&T = drugs and toxins, HPAH = hereditary PAH, IPAH = idiopathic PAH, IQR = interquartile range (25-75%), mPAP = mean pulmonary arterial pressure, NYHA FC = New York Heart Association functional class, PoPH = portopulmonary hypertension, PVR = pulmonary vascular resistance
**Table 6. Overview of clinical characteristics at baseline (T0) and follow-up (T1) blood sampling in patients with repeated measurements of NE and elafin (n=70).**

[A] Demographic features, PAH etiology, and background PAH therapies. [B] Clinical disease metrics. Clinical data was omitted from analysis when not available with one month of blood sampling (available n shown when data missing for some subjects). The paired Wilcoxon signed rank test or McNemar’s test was applied to assess for significant changes in therapies and clinical metrics from T0 to T1. [e-Table 6 continued on next page]

**[A]**

| demographic features, PAH etiology, and background PAH therapies. Clinical data was omitted from analysis when not available with one month of blood sampling (available n shown when data missing for some subjects). The paired Wilcoxon signed rank test or McNemar’s test was applied to assess for significant changes in therapies and clinical metrics from T0 to T1. [e-Table 6 continued on next page] |
|---|---|---|
| Interval from T0, years, median (IQR) | - | 0.9 (0.5, 1.4) |
| Age, years, mean ±SD | 48.5 ±13.7 | 49.5 ±13.9 |
| Female sex, n (%) | 53 (75.7%) | - |
| PAH etiology, n (%) | | |
| IPAH & FPAH | 20 (28.6%) | - |
| D&T-APAH | 17 (24.3%) | - |
| CTD-APAH | 18 (25.7%) | - |
| PoPH | 7 (10.0%) | - |
| CHD-APAH | 8 (11.4%) | - |
| PAH therapy extent, n (%) | | |
| Treatment naive | 19 (27.1%) | 0 (0.0%) |
| Monotherapy | 21 (30.0%) | 28 (40.0%) |
| Dual therapy | 24 (34.3%) | 32 (45.7%) |
| Triple therapy | 6 (8.6%) | 10 (14.3%) |
| Any therapy escalation, n (%) | - | 32 (45.7%) |
| PAH therapy class, n (%) | | |
| PDE-5 inhibitor | 36 (51.4%) | 55 (78.6%) |
| ERA | 22 (31.4%) | 30 (42.9%) |
| Prostacyclin | 29 (41.4%) | 43 (61.4%) |
| Therapy intervention T0 to T1, n (%) | | |
| PDE-5 inhibitor addition | - | 19 (27.1%) |
| ERA addition | - | 8 (11.4%) |
| Prostacyclin addition | - | 14 (20.0%) |

\*P<0.05, \**P<0.01, \***P<0.001

\[b\] Wilcoxon signed rank test (paired test version) used to assess whether overall PAH therapy extent changed significantly from T0 to T1, and McNemar’s test applied to examine changes for each therapy class during this interval.
**Table 6 (continued). Overview of clinical characteristics at baseline (T0) and follow-up (T1) blood sampling in patients with repeated measurements of NE and elafin (n=70).**

**A** Demographic features, PAH etiology, and background PAH therapies. **B** Clinical disease metrics. Clinical data was omitted from analysis when not available with one month of blood sampling (available n shown when data missing for some subjects). The paired Wilcoxon signed rank test or McNemar’s test was applied to assess for significant changes in therapies and clinical metrics from T0 to T1.

|                       | Baseline (T0) | Follow-up (T1) | P-value a |
|-----------------------|---------------|----------------|-----------|
| **NYHA FC, n (%)**    |               |                |           |
| I                     | 5 (7.1%)      | 8 (11.4%)      |           |
| II                    | 33 (47.1%)    | 34 (48.6%)     |           |
| III                   | 24 (34.3%)    | 23 (32.9%)     |           |
| IV                    | 8 (11.4%)     | 5 (7.1%)       |           |
| **NYHA functional class trend, n (%)** |               |                | 0.183     |
| Improved              | -             | 24 (34.3%)     |           |
| Stable                | -             | 31 (44.3%)     |           |
| Worsened              | -             | 15 (21.4%)     |           |
| **6MWD, m, median (IQR)** | 433 (367, 512) | 482 (389, 569) | 0.049 *   |
| △ 6MWD T0 to T1, m, median (IQR) | -            | +26 (-25, +79) |           |
| **NT-proBNP, pg/mL, median (IQR)** | 216 (75, 934) | 157 (58, 771) | 0.430     |
| △ NT-proBNP, pg/mL, median (IQR) | -            | +10 (-55, +180) |           |
| **Hemodynamics, median (IQR)** |               |                |           |
| Right atrial pressure, mmHg | 7.0 (4.0, 12.0) | 48.0 (38.0, 55.3) | 0.665     |
| mPAP, mmHg            | 52.0 (42.3, 59.8) | 52.0 (38.0, 55.3) | 0.025 *   |
| Cardiac index, L/min/m² | 2.07 (1.70, 2.36) | 2.31 (1.97, 2.50) | 0.005 **  |
| PVR, dynes-sec/cm²    | 841 (596, 1177) | 666 (404, 971) | 0.001 **  |
| △ Right atrial pressure, mmHg | -            | -3.0 (-1.0, +2.0) |           |
| △ mPAP, mmHg          | -             | -0.0 (-2.8, +3.0) |           |
| △ Cardiac index, L/min/m² | -          | +0.2 (-0.1, +0.5) |           |
| △ PVR, dynes-sec/cm²  | -             | -0.105 (-329, +0) |           |

*P<0.05, **P<0.01, ***P<0.001

a Wilcoxon signed rank test (paired test version) used to assess whether each clinical variable significantly changed from T0 to T1.
e-Table 7. Linear mixed effects models to associate changes in NE and elafin with clinical features. [A] NE, [B] elafin. Methods are detailed in the table footnotes. [e-Table 7 continued on next page]

[A]

| Predictors: | Model intercept (SE) | Predictor coefficient (SE) | P-value b |
|-------------|----------------------|---------------------------|-----------|
| Time (T1 ref to T0) | 5.257 (0.121) | -0.390 (0.169) | 0.023 * |
| Age - time interaction | 5.594 (0.444) | 0.016 (0.012) | 0.185 |
| Female sex (ref to male) - time interaction | 5.254 (0.138) | -0.425 (0.391) | 0.278 |
| PAH etiology (ref to others) - time interaction | | | |
| IPAH or FPAH | 5.383 (0.142) | 0.366 (0.372) | 0.327 |
| CTD-APAH | 5.194 (0.139) | -0.553 (0.381) | 0.149 |
| D&T-APAH | 5.296 (0.139) | 0.424 (0.391) | 0.281 |
| CHD-APAH | 5.191 (0.127) | -0.812 (0.522) | 0.123 |
| PoPH | 5.240 (0.127) | 0.393 (0.561) | 0.485 |
| Any therapy escalation - time interaction | 5.318 (0.158) | 0.143 (0.343) | 0.676 |
| PDE-5 inhibitor addition - time interaction | 5.359 (0.141) | 0.315 (0.378) | 0.407 |
| ERA addition - time interaction | 5.240 (0.128) | 0.122 (0.531) | 0.818 |
| Prostacyclin addition - time interaction | 5.179 (0.135) | -0.680 (0.379) | 0.035 * |
| NYHA class change - time interaction | | | |
| Improved | 5.125 (0.146) | -0.887 (0.340) | 0.011 * |
| Stable | 5.207 (0.162) | -0.034 (0.340) | 0.907 |
| Worsened | 5.403 (0.132) | 1.245 (0.384) | 0.002 ** |

*p<0.05, **p<0.01, ***p<0.001

a Various linear mixed effects models were fit to log-transformed NE (dependent variable). A random effect was introduced in all models to account for baseline differences across subjects. In the first model (shaded row), which sought to determine if the cohort demonstrated a significant directional NE change from baseline (T0) to follow-up (T1), time was included as a fixed effect (T1 referenced to T0) along with the random effect. In all subsequent models (unshaded rows), which evaluated the relationship between NE changes and clinical variables over time, the clinical variable-time interaction term served as the fixed effect.

b To assess if the cohort had a significant directional NE change (first p-value, shaded row), analysis of variance (ANOVA) was performed to compare two linear mixed models: a null model with random effect only (subject) vs. a model including time as a fixed effect. To determine if each clinical variable was associated with changes in NE over time (all subsequent p-values, unshaded rows), ANOVA was again applied to compare two models: a model including time and the clinical variable as separate fixed effects vs. a model where the time-clinical variable interaction was the only fixed effect. P-values reflect the significance of this interaction term.
e-Table 7 (continued). Linear mixed effects models to associate changes in NE and elafin with clinical features. [A] NE, [B] elafin. Methods are detailed in the table footnotes.

[B]

| Log elafin (dependent variable): in relation to time and the clinical variable-time interaction (predictors) | Model intercept (SE) | Predictor coefficient (SE) | P-value b |
|---|---|---|---|
| Time (T1 ref to T0) | 3.880 (0.067) | -0.172 (0.052) | 0.001 ** |
| Age - time interaction | 3.524 (0.242) | 0.001 (0.004) | 0.784 |
| Female sex (ref to male) - time interaction | 3.888 (0.077) | 0.018 (0.120) | 0.878 |
| PAH etiology (ref to others) - time interaction | | | |
| IPAH or FPAH | 3.878 (0.079) | -0.037 (0.114) | 0.748 |
| CTD-APAH | 3.857 (0.078) | -0.044 (0.118) | 0.711 |
| D&T-APAH | 3.918 (0.076) | 0.008 (0.120) | 0.944 |
| CHD-APAH | 3.900 (0.071) | 0.144 (0.161) | 0.374 |
| PoPH | 3.851 (0.070) | -0.003 (0.172) | 0.985 |
| Any therapy escalation - time interaction | 3.739 (0.085) | -0.188 (0.102) | 0.268 |
| PDE-5 inhibitor addition - time interaction | 3.833 (0.077) | 0.044 (0.116) | 0.707 |
| ERA addition - time interaction | 3.846 (0.070) | -0.193 (0.160) | 0.231 |
| Prostacyclin addition - time interaction | 3.840 (0.074) | -0.341 (0.122) | 0.006 ** |
| NYHA class change - time interaction | | | |
| Improved | 3.877 (0.082) | -0.142 (0.107) | 0.187 |
| Stable | 3.857 (0.090) | -0.062 (0.103) | 0.584 |
| Worsened | 3.900 (0.075) | 0.192 (0.121) | 0.122 |

p<0.05, **p<0.01, ***p<0.001

a Various linear mixed effects models were fit to log-transformed elafin (dependent variable). A random effect was introduced in all models to account for baseline differences across subjects. In the first model (shaded row), which sought to determine if the cohort demonstrated a significant directional elafin change from baseline (T0) to follow-up (T1), time was included as a fixed effect (T1 referenced to T0) along with the random effect. In all subsequent models (unshaded rows), which evaluated the relationship between elafin changes and clinical variables over time, the clinical variable-time interaction term served as the fixed effect.

b To assess if the cohort had a significant directional elafin change (first p-value, shaded row), analysis of variance (ANOVA) was performed to compare two linear mixed models: a null model with random effect only (subject) vs. a model including time as a fixed effect. To determine if each clinical variable was associated with changes in elafin over time (all subsequent p-values, unshaded rows), ANOVA was again applied to compare two models: a model including time and the clinical variable as separate fixed effects vs. a model where the time-clinical variable interaction was the only fixed effect. P-values reflect the significance of this interaction term.
e-Table 8. Circulating immune cell subset counts (absolute cells/mm$^3$) in relation to NE and elafin.

|                      | Spearman’s rho (P-value) |               |
|----------------------|--------------------------|---------------|
|                      | NE                      | Elafin        |
| White blood cell     | 0.216 (p=0.018) *        | -0.025 (p=0.790) |
| count                |
| Neutrophil count     | 0.375 (p=0.0007) ***     | 0.027 (p=0.767) |
| Lymphocyte count     | -0.070 (p=0.444)         | -0.045 (p=0.625) |
| Neutrophil/lymphocyte ratio | 0.325 (p=0.004) **  | 0.085 (p=0.356) |
| Monocyte count       | -0.098 (p=0.288)         | 0.107 (p=0.269) |
| Eosinophil count     | -0.111 (p=0.227)         | 0.015 (p=0.871) |
| Basophil count       | -0.005 (p=0.960)         | 0.089 (p=0.335) |

*P<0.05, **P<0.01, ***P<0.001

a Spearman’s rho statistic estimates a rank-based measure of association, and p-values shown are based on a two-sided alternative hypothesis (no direct or inverse relationship, rho=0).
**Table 9. Analysis of NE, cytokines, and chemokines as predictors of 5-year mortality risk among patients with available same-day NE and multiplex immunoassay measurements (n=228).** Univariate Cox regression models were fitted with a cubic spline function to evaluate the relationship between each cytokine/chemokine and mortality risk (all models demonstrated a non-linear relationship). Bootstrapped model estimates were obtained across the measured biomarker range and used to identify an optimal prognostic threshold (cytokine/chemokine level beyond which mortality risk remained significantly increased), as was done for NE in Figure 3B. These identified prognostic biomarker thresholds were then entered into univariate and multivariable Cox models to obtain unadjusted and adjusted mortality risk (see footnote for adjustment covariates). The inflammatory markers found to be independent predictors of risk (NE, IL-1α, IL-6, IL-10, and TNF-α) were carried forward to the analysis shown in the following e-Table 10.

| **Univariate analysis** | **Multivariable analysis** |
|------------------------|---------------------------|
| **NE (> 168.5 ng/mL)** | **Unadjusted HR (95% CI)** | **Adjusted HR (95% CI)** |
| 3.21 (1.77-5.83)       | < 0.0001***               | 2.67 (1.45-4.94)          |
| IL-1α                  | No prognostic threshold   |
| IL-1α (z-score > 0.40) | 2.09 (1.27-3.44)          | 1.70 (1.01-2.86)          |
| IL-2 (z-score > 0.79)  | 2.37 (1.39-4.04)          | 1.57 (0.88-2.79)          |
| IL-6 (z-score > 0.48)  | 2.01 (1.22-3.31)          | 1.73 (1.04-2.92)          |
| IL-8                   | No prognostic threshold   |
| IL-10 (z-score > 0.74) | 2.86 (1.70-4.79)          | < 0.0001***               |
| IL-12 (z-score > 0.55) | 1.90 (1.15-3.14)          | 1.45 (0.85-2.47)          |
| IL-13                  | No prognostic threshold   |
| TNF-α (z-score > 0.56) | 2.09 (1.26-3.46)          | 1.75 (1.03-3.00)          |
| HGF (z-score > 0.37)   | 2.44 (1.42-4.19)          | 1.57 (0.87-2.82)          |
| CXCL10                 | No prognostic threshold   |
| CXCL12                 | No prognostic threshold   |
| CCL2                   | No prognostic threshold   |
| VEGF (z-score > 0.69)  | 2.12 (1.27-3.56)          | 1.42 (0.81-2.48)          |

*P<0.05, **P<0.01, ***P<0.001

A Biomarkers for which no association was found with mortality risk in cubic spline models (IL-1α, IL-8, IL-13, CXCL10, CXCL12, CCL2).

B Biomarkers associated with mortality risk in univariate analysis were each evaluated in independent multivariable Cox proportional hazards models with the following adjustment covariates: age, sex, PAH etiology (CTD or PoPH vs other), incident vs prevalent PAH status, NYHA functional class (III/IV vs other), right atrial pressure, and prostacyclin treatment.
e-Table 10. NE predicts mortality risk independent of other inflammatory markers.
A multivariable Cox proportional hazards model was fitted with NE, the cytokines found to be significant risk predictors in e-Table 9 (IL-1β, IL-6, IL-10, and TNF-α), age, sex, PAH etiology, and incident vs. prevalent PAH status.

| Model covariates                  | Adjusted HR (95% CI) | P-value |
|-----------------------------------|----------------------|---------|
| NE (> 168.5 ng/mL)                | 2.63 (1.42-4.87)     | 0.001** |
| IL-1β (z-score > -0.40)           | 1.05 (0.42-2.59)     | 0.881   |
| IL-6 (z-score > -0.48)            | 1.55 (0.61-4.06)     | 0.368   |
| IL-10 (z-score > -0.74)           | 1.27 (0.52-3.12)     | 0.604   |
| TNF-α (z-score > -0.56)           | 1.45 (0.69-3.10)     | 0.337   |
| Age, per 10 years                 | 1.11 (0.92-1.36)     | 0.295   |
| Sex, male (vs. female)            | 1.58 (0.85-2.97)     | 0.150   |
| Etiology, CTD-APAH or PoPH (vs. others) | 2.32 (1.29-3.98) | 0.009** |
| Incident PAH status (vs. prevalent) | 2.17 (1.08-4.48) | 0.031*  |

*P<0.05, **P<0.01, ***P<0.001

e-Table 11. PAEC culture study: cohort characteristics. Clinical features are described at the time of lung transplantation for [A] PAH patients (explanted lungs) (n=3) and [B] donor controls (unused lungs) (n=3). The tissues were procured under informed consent at the following transplant procurement centers of the Pulmonary Hypertension Breakthrough Initiative: Allegheny General Hospital (Pittsburgh, PA), Baylor College of Medicine (Houston, TX), Cleveland Clinic (Cleveland, OH), Stanford University (Stanford, CA), University of Alabama at Birmingham (Birmingham, AL), and Vanderbilt University (Nashville, TN). De-identified patient data were obtained from the Data Coordinating Center at the University of Michigan (Ann Arbor, MI).

[A]

| Age | Sex | Race | Etiology | PAP s/d (m) | PVR (dynes/s/cm²) | 6MWD (meters) | Therapy |
|-----|-----|------|----------|-------------|-------------------|---------------|---------|
| 27  | F   | White| IPAH     | 110/49 (69) | 969               | 421            | Sildenafil Bosentan Treprostinil IV |
| 32  | F   | White| IPAH     | 68/38 (49)  | 1227              | 238            | Bosentan Epoprostenol               |
| 33  | F   | Black| FPAH     | 75/33 (48)  | 1247              | 326            | Sildenafil Bosentan Epoprostenol IV |

[B]

| Age | Sex | Race | Cause of death          |
|-----|-----|------|-------------------------|
| 57  | F   | White| Acute myocardial infarction |
| 1   | M   | White| Drowning,Anoxia         |
| 46  | F   | White| Intracerebral hemorrhage |
e-Table 12. Changes in clinical metrics, NE, and elafin from T0 to T1 according to interval therapeutic interventions. Unless indicated, data are represented as median and 25-75% interquartile range. For NE, elafin, and the ratio, fold change = T1 biomarker concentration/T0 biomarker concentration (where fold $\Delta = 1$ corresponds to no change, fold $\Delta >1$ indicates an increase, and fold $\Delta <1$ denotes a reduction).

| Therapeutic intervention during interval between T0 and T1 | Stable regimen (N=38) | PDE5i added (N=19) | ERA added (N=8) | PGI2 added (N=14) |
|----------------------------------------------------------|-----------------------|--------------------|-----------------|-------------------|
| NYHA FC, n (%) | Improved 11 (28.9%) 8 (42.1%) 1 (12.5%) 8 (57.1%) | Stable 17 (44.7%) 7 (36.8%) 7 (87.5%) 5 (35.7%) | Worsened 10 (26.3%) 4 (21.1%) 0 (0.0%) 1 (7.1%) |
| 6MWD, meters | Absolute $\Delta$ +18 (-33, +43) +23 (-1, +67) +2 (-57, +44) +47 (+18, +98) |
| NE, ng/mL | Absolute $\Delta$ -25.5 (-107.7, +49.6) -43.8 (-160.1, +35.4) -39.7 (-175.3, +44.1) -114.5 (-250.3, -82.4) | Fold $\Delta$ 0.81 (0.63, 1.39) 0.82 (0.50, 1.39) 0.96 (0.50, 1.33) 0.52 (0.36, 0.67) |
| Elafin, ng/mL | Absolute $\Delta$ -3.9 (-9.6, +3.8) 0.0 (-17.0, +4.2) -18.2 (-25.8, 0.0) -21.1 (-37.9, -8.6) | Fold $\Delta$ 0.88 (0.71, 1.08) 1.00 (0.75, 1.13) 0.67 (0.59, 1.0) 0.67 (0.50, 0.77) |

PDE5i= phosphodiesterase-5 inhibitor, ERA= endothelin receptor antagonist, PGI2= prostacyclin
e-Figure 1. Stanford PAH cohort identification. The Vera Moulton Wall Center for Pulmonary Vascular Disease biorepository was screened to identify Group 1 PAH patients evaluated at Stanford University (Stanford, CA) with at least one plasma sample collected between May 1, 2008 and December 15, 2013 (n=291). Subjects with recent acute illness, chronic infection, ILD, obstructive lung disease, aortic or mitral valve disease, or left ventricular systolic dysfunction were then excluded, as specified (n=42). In the remaining Stanford PAH primary analysis cohort (n=249), follow-up plasma samples were available for a subset (n=70). BMPR2= bone morphogenetic protein receptor-2, EF= ejection fraction, FEV1= forced expiratory volume in 1 second, FVC= forced vital capacity, ILD= interstitial lung disease, PBMCs= peripheral blood mononuclear cells. PH= pulmonary hypertension, VMWC= Vera Moulton Wall Center.
e-Figure 2. NE and elafin in PAH and health. [A] Receiver operating characteristic (ROC) curves display the PAH discriminatory power of NE, elafin, and the NE/elafin ratio. Calculated c-statistics (AUC) and ideal discrimination cut-offs (derived from Youden’s index) are indicated. [B] A scatter plot shows that no significant correlation is observed between NE and elafin at baseline sampling in PAH or health. P-values reflect the Spearman’s rho statistic (two-sided null hypothesis of no direct or inverse relationship).
e-Figure 3. NE and elafin in relation to various demographic features. [A] Log-transformed NE and elafin related to age for PAH patients (green) and health controls (gray). P-values reflect Spearman’s rho statistic (two-sided null hypothesis). [B] Log-transformed NE and elafin by sex (females= pink, males= blue) for PAH patients and healthy controls. Boxes represent the median and 25-75% interquartile range (IQR), and whiskers denote data within 1.5*IQR. For each biomarker, the Wilcoxon rank sum test was applied to compare females vs. males among PAH patients and controls (p-values above solid black lines), female PAH vs female controls (p-values above pink dashed lines), and male PAH vs male controls (p-values above blue dashed lines). [C] Log-transformed NE and elafin by race in PAH (White= light purple, Black= green, Hispanic= yellow, Asian= red). Each biomarker was compared across races via the Kruskal-Wallis test, and p-values are shown. [D] Log-transformed NE and elafin related to body mass index in PAH. P-values reflect Spearman’s rho statistic (two-sided null hypothesis).
e-Figure 4. NE and elafin are independent of illness duration (time from PAH diagnosis to baseline blood sampling). [A] Histogram of illness distribution in the PAH cohort with median indicated (dashed vertical line). [B] Log-transformed biomarker levels for PAH patients in various illness duration subgroups (0–3 months, 3 months–2 years, 2–5 years, and >5 years, colored with varying shades of red) and healthy controls (gray). Levels are compared across illness duration groups by Kruskal-Wallis test (P-value on dashed red line), and each illness duration group is compared to controls by Dunn’s test with Benjamini-Hochberg adjustment for multiple comparisons (P-values at top).
e-Figure 5. Baseline NE and elafin levels are independent of treatment status and extent of background therapy. NE and elafin (log-transformed) stratified by the extent of background PAH-directed therapy (naïve, monotherapy, dual therapy, or triple therapy). Within boxplots, P-values reflect comparison of each PAH group to controls (top of plots, Dunn’s test with Benjamini-Hochberg adjustment) and an assessment for directional trend across ordered groups (bottom of plots, Cuzick test).
e-Figure 6. Baseline NE and elafin levels do not associate with any class of background PAH therapy. Biomarkers were analyzed with respect to each major class of background PAH therapy, including [A] prostacyclins (PGI2), [B] phosphodiesterase-5 inhibitors (PDE5i), and [C] endothelin receptor antagonists (ERA). For each class of therapy, corresponding boxplots show log-transformed biomarker levels for 4 subgroups of PAH patients who were either (i) treated with the class as monotherapy (dark purple), (ii) treated with the class in combination with other classes (light purple), (iii) treated with alternative classes only (blue), or (iv) therapy naïve (white). Biomarkers are compared across these PAH subgroups by Kruskal-Wallis test (P-value on purple dashed line). Healthy controls biomarker levels are also shown (gray) and compared to each PAH therapy subgroup, via Dunn’s test with Benjamini-Hochberg adjustment (P-values at top).
e-Figure 7. Neither NE nor elafin levels differ among patients on immune modulating therapy (IMT). For [A] NE and [B] elafin, boxplots show log-transformed biomarker levels for 4 subgroups of PAH patients who were either treated with (i) IMT alone without PAH-specific therapy (dark gold, IMT+/PAHtx−), (ii) IMT in combination with PAH therapy (light gold, IMT+/PAHtx+), (iii) PAH therapy alone without IMT (blue, IMT−/PAHtx+), (iv) or neither (white, IMT−/PAHtx−). Biomarkers are compared across these PAH subgroups by Kruskal-Wallis test ($P$-value on gold dashed line). Healthy controls biomarker levels are also shown (gray) and compared to each IMT subgroup via Dunn’s test, with post-hoc Benjamini-Hochberg adjustment ($P$-values at top). Background IMT agents included prednisone, mycophenolate mofetil, leflunomide, azathioprine, tacrolimus, anakinra, rituximab, and cyclophosphamide.

[A] NE

[B] Elafin
e-Figure 8. Elafin levels do not prognosticate. [A] Kaplan-Meier estimates of five-year transplant-free survival according to elafin quartiles (Q1 <15.3, Q2 15.3–32.0, Q3 32.0–59.1, Q4 >59.1 ng/mL), with across-quartile comparison by log-rank test. [B] Plot relating elafin to mortality risk (hazard ratio= solid line, 95% confidence interval= shaded gray area), which reflects bootstrapped estimates from a Cox regression model fitted with a cubic spline function. Model estimates are shown across the measured range of elafin, with patient-level measurements indicated along the x-axis rug plot. No significant relationship between elafin and mortality risk is observed.
e-Figure 9. Patient-level changes in NE and elafin over time during treatment of PAH. Among the subgroup with available follow-up measurements (N=70), absolute log-scale biomarker changes are displayed for each patient as single arrows (baseline= arrow tail, follow-up= arrow head). Arrows are colored across a spectrum of biomarker fold-changes on the raw scale (darker red= relative increase, and darker blue= relative decrease). For reference, y-axis intercept lines show the healthy control cohort median (solid black), ideal PAH discrimination cut-off (dashed black), and optimal prognostic threshold (dashed maroon, note that no elafin threshold predicted outcomes).
e-Figure 10. NE levels over time in relation to PAH therapy interventions. [A] NE changes for stable therapy vs. any therapy added. [B] NE changes according to class of PAH therapy added: phosphodiesterase-5 inhibitor (PDE5i), endothelin receptor antagonist (ERA), or prostanoid (PGI2). Boxplots show log-transformed NE at baseline (T0) and follow-up (T1). Overlying spaghetti plots display the corresponding patient-level NE changes, where line color represents fold-change on the raw scale (red= relative increase, blue=relative decrease) and the bold dashed line denotes mean change. To evaluate the significance of relationships between each therapy intervention and NE change, a mixed effects model was fit to NE as a function of time (T1 referenced to T0), the therapy intervention, and their interaction (detailed in e-Table 7A). P-values are shown for the interaction term, indicating whether the temporal relationship between therapy and NE is significant.

[A] Stable therapy (N=38) vs. Therapy escalation (any agent) (N=32)

[B] PDE-5 inhibitor added (N=19) vs. ERA added (N=8) vs. Prostanoid added (N=14)
e-Figure 11. Elafin levels over time in relation to PAH therapy interventions. [A] Elafin changes for stable therapy vs. any therapy escalation (regardless of therapy class added). [B] Elafin changes according to class of PAH therapy added: phosphodiesterase-5 inhibitor (PDE5i), endothelin receptor antagonist (ERA), or prostanoid (PGI2). Boxplots show log-transformed elafin at baseline (T0) and follow-up (T1). Overlying spaghetti plots display the corresponding patient-level elafin changes, where line color represents fold-change on the raw scale (red= relative increase, blue= relative decrease) and the bold dashed line denotes mean change. To evaluate the significance of relationships between each therapy intervention and elafin change, a mixed effects model was fit to elafin as a function of time (T1 referenced to T0), the therapy intervention, and their interaction (detailed in e-Table 7B). P-values are shown for the interaction term, indicating whether the temporal relationship between therapy and elafin is significant.
e-Appendix 1. Study population: supplemental methods

1a. Stanford PAH (primary cohort) - Vera Moulton Wall Center (VMWC) biobank: The VMWC biobank (Stanford, CA) is a comprehensive repository that includes plasma, serum, blood mononuclear cells, exhaled breath condensate, and urine samples from patients with all forms of pulmonary hypertension (Stanford University IRB #14083). Initiated in 2007, the VMWC biobank has captured samples from over 700 well-characterized subjects who were recruited at the time of first evaluation in Stanford University adult pulmonary hypertension clinic. Each sample is linked to comprehensive clinical data captured in the Stanford Pulmonary Hypertension database.

The VMWC biobank was screened to identify all consecutive Group 1 PAH patients (N=249) evaluated at Stanford University who had at least one plasma sample collected for the biobank between 2008 and 2013. No a priori sample size calculations were performed, and ultimate cohort size was dictated by biological sample availability. PAH was diagnosed according to guidelines and required mean pulmonary arterial pressure (mPAP) ≥25 mmHg, pulmonary vascular resistance (PVR) >240 dynes·sec/cm, and wedge pressure ≤15 mmHg.1 We excluded patients with known interstitial lung disease (radiologic chronic ground glass infiltrates, reticulation, or fibrosis), COPD (FEV1/FVC ratio < 0.70), left ventricular systolic dysfunction (ejection fraction <55%), non-tricuspid valve disease (echocardiographic severity > mild), any chronic infection, or any acute illness in the preceding month.

1b. Healthy Controls - Stanford Healthy Aging Population cohort: Control plasma samples (N=106) were acquired from the Stanford University Cardiovascular Institute Biomarker and Phenotype Core Laboratory biorepository (Stanford University IRB #40869). Samples were collected from healthy volunteers between 2009 and 2013, as part of the Stanford Healthy Aging Population study (Stanford University IRB #20942). Subjects ≥18 years-old with cardiovascular and immune health were enrolled. Health was rigorously established by comprehensive questionnaire, clinical assessment, laboratory studies, echocardiography, and multi-site vascular ultrasound (abdominal aorta, carotid, lower extremity), which were used to screen for the following exclusion criteria:

- History of essential hypertension, or blood pressure >140/90 during screening clinical assessment
- History of symptomatic heart failure, or NT-proBNP 300 pg/mL during laboratory screening, or subclinical left ventricular systolic dysfunction demonstrated by screening echocardiography (ejection fraction < 50%)
- History of known heart valvular disease, or any valve abnormality > mild demonstrated by screening echocardiography
- Symptomatic atherosclerotic disease, or asymptomatic with >30% carotid or femoral stenosis by screening ultrasound
- Abdominal aortic aneurysm (> 5 cm) demonstrated by screening abdominal aortic ultrasound
- History of coronary artery disease
- History of known arrhythmia, or electrocardiogram demonstrating arrhythmia during screening clinical assessment
- History of blood clot within the last 10 years
- History of known pulmonary hypertension, or any right ventricular enlargement/dysfunction or estimated right ventricular systolic pressure >30 mmHg demonstrated by screening echocardiography
- History of any known chronic pulmonary disease (parenchymal or airways) requiring prior consultation or treatment
- History of known dyslipidemia, or dyslipidemia demonstrated during laboratory screening
- History of known diabetes mellitus, or hemoglobin A1C >7.0% during laboratory screening
- Body mass index >35 kg/m2
- History of atopy
- History of known chronic autoimmune or systemic inflammatory disease
- Recent viral illness or cold/flu-like symptoms, or antibiotic/antiviral/antifungal within the last month
- Long-term treatment (> 2 weeks) with any antibiotic, antiviral, or antifungal within the last year
- Active anti-inflammatory of immune modulating therapy
- Any vaccine within the last month
- History of HIV/AIDS or chronic hepatitis
- History of malignancy within the last 10 years
- Abnormal liver function tests or renal dysfunction (estimated GFR < 60 ml/min/1.73 kg m2) during laboratory screening
- History of Alzheimer’s Disease
- History of major depression with psychotic or melancholic features
- Active tobacco use (self-reported) or substance abuse within the last year (self-reported)
e-Appendix 2. Sample and data collection: supplemental methods

2a. Stanford PAH blood sampling: After informed consent was obtained, internal jugular venous samples were collected from PAH patients during right heart catheterization in the fasting state. **Plasma sample processing:** Blood was drawn from the patient into EDTA vacutainers under standard sterile precautions. Collection tubes were immediately placed upright into a rack at room temperature. Within 30 minutes, the sample was inverted several times to mix components, and subsequently centrifuged at 1300 rpm for 10 minutes. The plasma layer was then carefully removed by pipette without disturbing the buffy coat, and transferred to Eppendorf tubes in 200 µL aliquots. Aliquoted samples were secured and stored upright at -80°C in the VMWC biobank. **Blood mononuclear cell processing:** Blood was drawn from the patient into a CPT™ vacutainer (BD Biosciences, Franklin Lakes, NJ). Collection tubes were immediately placed upright into a rack at room temperature. Within 30 minutes, the sample was inverted several times to mix components, and subsequently centrifuged at 1300 rpm for 10 minutes. The plasma layer was then carefully removed by pipette without disturbing the buffy coat, then the buffy coat was pipetted into a centrifuge tube with PBS to bring volume to 15 mL. After centrifugation for 10 minutes at 300 rpm, supernatant was removed without disturbing cell pellet, and then the cell pellet was re-suspended in 15 mL PBS by vortexing. The same centrifugation, supernatant removal, pellet re-suspension process was thereafter repeated, though re-suspension occurred in 1000 uL of 1:1 DMSO/human serum type AB. Blood mononuclear cell samples were subsequently stored in the VMWC biobank in liquid nitrogen.

2b. Stanford PAH clinical data collection: Demographic, clinical, hemodynamic, and outcomes data were obtained from the Stanford Pulmonary Hypertension Database (SPHD). Established in 2000, the SPHD has enrolled consecutive patients evaluated at Stanford University with hemodynamically confirmed PAH (Stanford University IRB#12338). This observational relational database captures nearly 400 unique variables in a longitudinal manner. Clinical data were omitted if not available within one month of plasma sampling for NE and elafin. The included demographic variables were age, sex, and self-reported race/ethnicity. Subjects were categorized into a Group 1 PAH subtype based on the World Health Organization classification scheme, which defines subtypes according to predisposing factors that underlie PAH \(^1\). We also collected the dates of PAH diagnosis (including diagnoses that preceded referral to Stanford) and symptom onset (patient-reported). Incident PAH cases included patients who were (a) diagnosed with PAH on the day of blood sampling or within preceding 3 months and (b) treatment naïve. Extracted PAH clinical metrics included right heart catheterization hemodynamic measures, non-invasive disease markers (NYHA functional class, six-minute walk distance, NT-proBNP, diffusion capacity of the lung for carbon monoxide, echocardiographic variables), and peripheral blood white blood cell count data with differential subsets. In addition, we captured background PAH-specific therapies (phosphodiesterase-5 inhibitors, endothelin receptor antagonists, and prostanoids) and immune modulating agents (prednisone, mycophenolate mofetil, hydroxychloroquine, leflunomide, azathioprine, tacrolimus, bortezomib, anakinra, rituximab, or cyclophosphamide). The SPHD and electronic medical records were also used to identify patients who died from any cause or underwent lung/heart-lung transplantation prior to database lock (November 2018).

2c. Healthy control blood sampling: Venous blood samples were drawn in the fasting state from the antecubital fossa, and subsequently processed and stored utilizing the same protocol that was applied for PAH samples (see above).
3. NE and elastin measurements - enzyme linked immunosorbent assay (ELISA) protocol:

Overview: Diluted plasma samples (1:40) were added in duplicate to microtiter plates (100 µL/well) coated with analyte-specific antibody. Standards were prepared and included on each plate. Biotinylated tracer antibody was introduced to sandwich captured analytes, followed by streptavidin-peroxidase conjugate to bind these complexes, and tetramethylbenzidine to incite detectable enzymatic reaction. Absorbance was measured by spectrophotometer (450 nm) and duplicate-averaged for each sample set. Analyte concentrations were determined from the standard curve.

Detailed protocol:

1) Thaw plasma samples on ice, then centrifuge (3,000G) for 10 min at 4°C.
2) Passively bring reagents and sample to room temperature.
3) Dilute 10X dilution Buffer to 1X: add 10 mL of 10X dilution Buffer + 90 mL dH2O (100 mL total of 1X dilution Buffer, sufficient for 2 x 96 tests)
4) Reconstitute standard by adding 0.5 ml dH2O (must be used within 1 hour, cannot be stored for re-use)
5) Serial dilution of standard: vortex to mix each of the following standards in series

| Tube | Vol. dilution buffer | Vol. standard | Conc. (ng/mL) |
|------|----------------------|---------------|---------------|
| 1    | 1.104 mL             | 300 µL vial 3 | 50            |
| 2    | 1.104 mL             | 150 µL vial 3 | 25            |
| 3    | 225 µL               | 225 µL tube 2 | 12.5          |
| 4    | 225 µL               | 225 µL tube 3 | 6.3           |
| 5    | 225 µL               | 225 µL tube 4 | 3.1           |
| 6    | 225 µL               | 225 µL tube 5 | 1.6           |
| 7    | 225 µL               | 225 µL tube 6 | 0.8           |
| 8    | 225 µL               | 225 µL tube 7 | 0.4           |
| 9    | 225 µL               | --            | 0             |

6) Dilute each experimental sample using 1X dilution Buffer for duplicate runs:

| Sample Dilution | Vol. dilution buffer | Vol. Plasma (µL) |
|-----------------|----------------------|------------------|
| 1:40            | 312 µL               | 8                |

7) Take out microwell strips and label.
8) Transfer 100µL in duplicate of standard, samples, and controls into appropriate wells.
9) Apply an adhesive cover to the tray. Gently tap the tray to eliminate any air bubbles.
10) Incubate the plate for 1 hour at room temperature.
11) Dilute 40X wash buffer to 1X: add 15 mL of 40x wash buffer + 585 mL of dH2O (600 mL total of 1X wash buffer, sufficient for 2 x 96 tests)
12) Prepare tracer solution (1:12): First, reconstitute tracer solution by adding 1 mL deionized water. Next, prepare diluted tracer by adding 22 mL of 1X Dilution Buffer to 2 mL reconstituted of tracer (24 mL total, sufficient for 2 x 96 tests)
13) Prepare streptavidin-peroxidase solution: Reconstitute stock vial by adding 1 mL dH2O, then combine 23 mL 1X dilution buffer + 1 mL reconstituted streptavidin-peroxidase (24mL total, sufficient for 2 x 96 tests)

After 1 hour of plate incubation (standards, samples, and controls):

14) Wash plates 4 times with wash buffer using multi-channel pipette as follows: Carefully remove adhesive cover. Empty plate by inverting plate, shaking contents out over waste bin, and tapping dry on a thick layer of tissues. Add 200 µL of wash buffer to each well, wait 20 seconds, empty plate as described earlier. Repeat washing procedure three more times for total of 4 washes. Empty plate after final wash, but do not let wells dry
15) Add 100 µL of diluted tracer to each well. Do not touch the sides or bottom of the wells.
16) Cover tray with adhesive cover and incubate for 1 hour at room temperature.
17) After 1 hour, repeat wash procedure 4 times as described earlier.
18) Add 100 µL of diluted streptavidin-peroxidase to each well. Do not touch the sides or bottom of wells.
19) Cover tray with adhesive cover and incubate 1 hour at room temperature.
20) Repeat wash procedure 4 times as described earlier.
21) Add 100 µL of TMB substrate to each well. Do not touch side or bottom of wells.
22) Cover tray with new adhesive cover and incubate 25 minutes at room temperature. Protect from light with aluminum foil.
23) Stop the reaction by adding 100 µL of stop solution. Mix solutions in wells by gently swirling plate and gently tapping; should turn yellow. If green, mix solutions in wells until yellow.

24) Read the plate within 30 minutes after addition of stop solution at 450 nm.

3b. Cytokine and chemokine measurement - BioPlex® multiplex immunoassay protocol: The Bio-Plex® multiplex immunoassay is a magnetic bead-based flow cytometric platform that is built on Luminex® xMAP™ technology. To prepare experimental samples, frozen biobanked plasma aliquots were passively thawed to room temperature and diluted four-fold in assay buffer. To prepare a magnetic capture bead mixture, bead stock solution (20x) was vortexed for 30 seconds and diluted 20-fold in assay buffer. The preparation of standards involved first adding 500 µL of standard diluent to each stock vial of lyophilized standard, which contained known analyte concentrations. The reconstituted standard was vortexed and placed on ice for 30 minutes. Thereafter, we prepared a four-fold serial dilution series of eight total standards. After preparation of samples, capture beads, and standards, each immunoassay was carried out on a 96-well plate. First, we added standards to eight wells, assay buffer to one well ('blank' well to measure background fluorescence), and experimental samples to the remaining wells (75 µL/well). Next, we added 25 µL of capture bead mixture to all wells. The plate was sealed, placed on a shaker for two hours (800 rpm), and incubated at 4°C overnight. The next day, solution was removed and the plate was washed by magnetic separation with the Bio-Plex Pro™ wash station (200 µL buffer, 3 cycles). Biotinylated detection antibody stock solution (10x) was diluted 10-fold in assay buffer and added to each well (25 µL), followed by two-hour incubation on a shaker (800 rpm), solution removal, and three magnetic separation washes. Next, streptavidin-phycoerythrin stock solution (100x) was diluted 100-fold, incubated in each well (50 µL) for 40 minutes on a shaker (500 rpm), removed, and the plate was washed again. Finally, after addition of reading buffer (100 µL) and ten-minute incubation on a shaker (800 rpm), the plate was read by a Luminex 200™ dual-laser detection instrument. Data acquisition was set to a 50 bead count minimum per analyte per well. Data was processed and presented with Bio-Plex Manager™ software.

3c. Quantification of BMPR2 mRNA expression: After obtaining and thawing biobanked blood mononuclear cell samples, RNA was purified and extracted with the RNAeasy kit (Qiagen, Redwood City, CA). RNA was then reverse transcribed using Superscript III reverse transcriptase (Invitrogen, Waltham, MA), in accordance with manufacturer instructions. BMPR2 expression levels were then measured by real-time PCR using pre-verified Assays-On-Demand TaqMan primer/probe sets (Applied Biosystems, Foster City, CA), in accordance with manufacturer instructions. The delta-delta CT relative quantitation method was used to quantify BMPR2 expression, which was normalized to the GAPDH housekeeping gene.
**4a. UK validation cohort A.** We obtained data from idiopathic PAH patients treated at Hammersmith Hospital (Imperial College Health Care System; London, UK) (N=75) who had been part of a previously published multicenter plasma proteome analysis. In this observational cohort study, subjects were enrolled from October 1, 2002 to June 30, 2011. The diagnostic criteria for IPAH/HPAH over the course of this study were stable - mPAP>25 mmHg and PCWP<15 (and PVR>3 Woods) at rest with exclusion of known associated diseases according to contemporary international consensus. Patient outcomes (deaths and transplantations) were captured from NHS Digital and a structured query language database which was linked to electronic medical record data at Royal Hammersmith Hospital. Patient survival and transplantation status was censored on May 15, 2014.

At routine clinical visits, peripheral venous blood was collected from non-fasting patients into EDTA vacutainer tubes (BD Biosciences, Oxford, UK). Samples were immediately put on ice, processed to plasma within 30 minutes of collection, and then stored at -80°C until later acquired for assay runs. NE and 1128 other proteins were measured by SOMAscan version 3 assay (Somalogic, Boulder, CO), though only NE data was acquired for our validation analyses. Quality control included the use of bridging samples across all batches, to minimize between-experiment variation.

**4b. UK validation cohort B.** Patients with prevalent idiopathic or heritable pulmonary arterial hypertension aged 18-65 years (N=357) from the UK National Cohort Study of Idiopathic and Heritable Pulmonary Arterial Hypertension (clinicaltrials.gov NCT01907295) were recruited between February 19, 2014 and November 6, 2018 at 10 centres: University of Cambridge (Cambridge, UK), Freeman Hospital (Newcastle, UK), Golden Jubilee National Hospital (Glasgow, UK), Imperial College Healthcare (London, UK), Royal Papworth Hospital (Cambridge, UK), Royal Brompton Hospital (London, UK), Royal Free London Hospital (London, UK), Royal Hallamshire Hospital (Sheffield, UK), Royal United Hospitals NHS Foundation Trust (Bath, UK), and Great Ormond Street Hospital (London, UK) (collaborators listed in supplemental acknowledgements, section IX). The diagnostic criteria for IPAH/HPAH over the course of this study have been stable - raised mPAP>25 mmHg with PCWP<15 (and PVR>3 Woods) at rest with exclusion of known associated diseases according to contemporary international consensus. Patient outcomes (deaths and transplantations) were captured from NHS Digital with censoring on March 14, 2020. Age- and sex-matched healthy controls without cardiovascular or respiratory diseases (n=70) were recruited over the same period from the same centres.

Peripheral venous blood samples were collected into EDTA vacutainer tubes (BD Biosciences, Oxford, UK), immediately put on ice, processed to plasma within 30 minutes of collection, and then stored at -80°C for later assays as previously described. All samples were obtained with informed consent and research ethics committee approval. Patients were sampled in the non-fasting state at their routine clinical appointment visits. The plasma samples underwent one freeze-thaw cycle to aliquot 120 µL for assay.

Proteomic analysis was performed using the SOMAscan version 4 assay (Somalogic Inc. Boulder, CO, USA) and technicians were blinded to patient status. 4349 somamers targeting 4152 unique proteins were included for analysis following removal of non-human/non-protein aptamers and quality control to select only those with stable measurements defined as <20% coefficient of variance in the repeated pooled plasma assay controls. Relative fluorescence units were log10 transformed to normalise protein levels, then corrected for the first two principal components by linear regression to correct for population stratification or sample quality differences. Finally, protein levels were standardised to the healthy control levels for ease of interpretation of results and comparability of proteins.
e-Appendix 5. Pulmonary artery endothelial cell (PAEC) studies: supplemental methods

Upon quantifying endogenous NE and elafin levels in PAH, we conducted studies in cultured PAECs to assess how the NE/elafin balance impacts homeostasis. PAECs were harvested from explanted lungs of end-stage PAH transplant recipients (n=3) and control transplant donors (n=3), which were obtained through the Pulmonary Hypertension Breakthrough Initiative (PHBI) – a multicenter network of lung transplant centers (https://www.ipahresearch.org/services.html) including the University of Michigan (Ann Arbor, MI), Allegheny General Hospital (Pittsburgh, PA), Vanderbilt University Medical Center (Nashville, TN), the Cleveland Clinic (Cleveland, OH), Baylor College of Medicine (Houston, TX), Stanford University (Stanford, CA), and University of Alabama Birmingham (Birmingham, AL) (collaborators listed in supplemental acknowledgements, section X). The PHBI is funded by the Cardiovascular Medical Research and Education Fund (CMREF) and the NIH (R24-HL123767). The control samples were from donors not found to have a suitable recipient, although these lungs were deemed to meet physiologic acceptability standards.

PAECs were harvested from small pulmonary arteries (<1 mm) then cultured in EC media (Sciencell, Carlsbad, CA) as previously described, and cells from passage four to six were used to evaluate effects of elastase and elafin on apoptosis and angiogenesis. Apoptosis was measured via the caspase 3/7 assay, as previously detailed. Following cell seeding (5,000/well), growth (90% confluence), and growth arrest (12-hour starvation), cells were exposed to various treatment conditions for 24 hours: (i) unopposed human leukocyte-derived elastase (400 ng/mL) (Sigma-Aldrich, St. Louis, MO), (ii) elastase plus recombinant elafin (Proteo-Biotech-AG, Kiel, Germany) at escalating doses (50–800 ng/mL)(e-Table 1), or (iii) untreated. Cells were then incubated with Luciferase Reagent (Promega, Madison, WI) and caspase activity was measured by detecting luminescence (BioTek Synergy H1 Reader, Winooski, VT). Angiogenesis was assessed by tube formation assay, as previously described.

Cells were seeded (10,000 cells/well) on growth factor-reduced Matrigel (Cultrex BME, Gaithersburg, MD), then exposed to the aforementioned NE and elafin treatment conditions. At four hours, tube numbers for each condition were quantitated by light microscopy.
e-Appendix 6. Statistical analysis: supplemental methods

Analyses were performed in the R software environment (version 3.5.1) using packages from the Comprehensive R Archive Network (CRAN). Missing data was uncommonly encountered and omitted from analyses (no imputation). The number of subjects with missing data is indicated in either the body or legend of each individual data table when pertinent.

6a. Comparison of NE and elafin in PAH vs healthy controls.

Mann-Whitney U (Wilcoxon rank-sum) test: The ‘wilcox.test’ function from the ‘stats’ R package (version 3.5.1) was applied to perform the non-parametric Wilcoxon rank sum test of two unpaired independent groups (biomarkers in PAH vs controls, females vs males). The null hypothesis was that the two groups had a similar variable distribution, while the alternative was that the groups differed.

Analysis of receiver operating characteristic (ROC) curves: ROC curves were plotted and analyzed to assess the PAH discriminatory power of NE and elafin. The ‘pROC’ (version 1.13.0) and ‘plotROC’ (version 2.2.1) R packages were employed. Areas under ROC curves were calculated (with 95% confidence intervals determined from 2,000 stratified bootstrap replicates). Ideal discrimination thresholds were identified by way of the Youden’s J statistic, which gives equal weight to sensitivity and specificity.

6b. Analysis of NE and elafin relationships with baseline clinical features.

Spearman’s correlation test: We assessed NE and elafin correlations with age, BMI, six-minute walk distance, NT-proBNP, DLCO, echo metrics, and hemodynamic indices. The ‘cor.test’ function from the ‘stats’ R package (version 3.5.1) was used to evaluate Spearman’s rho statistic (non-parametric ranked-based measure of association). The null hypothesis was no association between variables, while the two-sided alternative was a direct or inverse relationship. Based on this two-sided hypothesis, p-values were computed with the AS 89 algorithm with Edgeworth series approximation.

Kruskall-Wallis test: The ‘kruskal.test’ function from the ‘stats’ R package (version 3.5.1) was utilized to perform non-parametric comparison a NE or elafin across ethnicity/racial groups, PAH subtypes, and background therapy categories. The null hypothesis was that all groups had a similar variable distribution, while the alternative was that at least one group distribution differed from others.

Post-hoc Dunn’s method: The ‘dunn.test’ function from the ‘dunn.test’ R package (version 1.3.5) was applied after the Kruskal-Wallis test, to compute the Dunn’s test for stochastic dominance. Results were reported for all possible pairwise group comparisons, to determine which individual groups differed from each other. The Benjamini-Hochberg method was implemented to adjust p-values for multiple comparisons.

Cuzick test: The ‘cuzickTest’ function from the ‘PMCMRplus’ R package (version 1.4.1) was used to implement the Cuzick’s extension of the Wilcoxon rank sum test, which assessed for trend in data across three or more ordinal groups (NYHA classes, number of background therapies). The two-tailed null hypothesis was no trend in either direction across the ordered groups. P-values were determined by way of Monte Carlo simulation (1,000 replicates).

Median regression analysis: The ‘rq’ function from the ‘quantreg’ R package (version 5.36) was employed to relate NE and elafin to continuous PAH severity metrics. Models were fit invoking a variant of the Barrodale and Roberts simplex algorithm. Regression was fit at the median. In each model, a clinical severity metric (6MWD, NT-proBNP, DLCO, TAPSE, mPAP, PVR, or RAP) served as the dependent variable while NE and elafin were assessed as predictors with age, sex, and BMI included as adjustment covariates. For predictor regression coefficients, standard errors were computed by the ‘nid’ method (local linearity was presumed about the median, and a Huber sandwich estimate of the asymptotic covariance matrix was computed by estimating local sparsity at each sample).

6c. Evaluation of NE and elafin as prognostic biomarkers.

Kaplan-Meier survival analysis: The ‘survfit’ function from the ‘survival’ R package (version 2.42-6) was used obtain Kaplan-Meier estimates of survival probability. Kaplan-Meier curves
were plotted via the ‘ggsurvplot’ function in the ‘survminer’ R package (version 0.4.3). Subjects were followed until death, transplantation, or last encounter prior to data lock (right censored). To compare survival curves across analysis groups via log-rank test, the ‘survdiff’ function was applied from the ‘survival’ R package.

**Univariate analysis of NE and elafin in relation to mortality risk:** Kaplan-Meier analysis of survival according to biomarker quartile indicated a non-linear relationship between NE and outcome. Therefore, we could not satisfy the assumption of linearity between predictor and log-hazard required for a Cox proportional hazards model. To sidestep this issue and investigate non-linear associations between each biomarker and the outcome, we fitted Cox regression models with cubic spline functions. Cubic regression splines are non-parametric and flexible tools that can uncover otherwise hidden relationships, produce visibly smooth curves, and are easy to interpret. In contrast to global polynomial regression procedures in which the fitted function at a given value is impacted by data values far from that point (the ‘non-locality’ drawback), cubic spline regression fits a piecewise continuous model by fitting functions to data intervals partitioned by points called knots. The piecewise splines are flexible smooth cubic polynomial functions, where the location and number of knots can vary. Natural (restricted) splines are cubic splines with the additional constraint that they are linear in the tails of the outermost knots, as polynomial functions tend to be erratic at the boundaries of the data. In general, with a greater number of knots the flexibility of a cubic spline model increases at the cost of potential overfitting.

To investigate univariate non-linear relationships between each biomarker and the outcome, we fitted Cox regression models with a natural cubic spline function by employing the ‘cph’ and ‘rcs’ functions from the ‘rms’ R package (version 6.2). To confirm that relationships were indeed non-linear, we implemented analysis of variance using the ‘anova’ function from the ‘stats’ R package. The number of knots in each univariate model was determined on the basis of the Akaike information criterion (AIC). Bootstrapped cubic spline model estimates of mortality risk (hazard ratio and 95% CI bounds) were obtained across the range of measured biomarker levels. For each biomarker, we identified the prognostic threshold beyond which mortality risk remained significantly increased (biomarker level at which the lower bound of the 95% CI crossed above a hazard ratio of 1 = point of reliable worsening).

**Multivariable Cox proportional hazards regression models:** The prognostic NE threshold (identified above) was subsequently entered into a multivariable Cox regression model to evaluate whether it predicted mortality risk after adjustment for the effects of other known clinical predictors. To fit these multivariable Cox proportional hazards models, we employed the ‘coxph’ function from the ‘survival’ R package. In a model which adjusted for clinical predictors, covariates were selected *a priori* on the basis of demonstrating significant predictive value in prior PAH registry studies: age (≥60 years), sex (male), etiology (CTD-APAH or PoPH), incident PAH), NYHA class (III/IV), NT-proBNP (>1400 pg/mL), and right atrial pressure (≥14 mmHg). Prostacyclin treatment status was also included as a covariate, since we demonstrated that prostacyclin initiation was associated with decreases in NE and elafin levels over time. These clinical predictors were also used to define subgroups for sensitivity analyses.

**Analysis of the incremental prognostic value of NE relative to established PAH risk scores:** Three different validated PAH risk scores were used to stratify study patients into low, intermediate, and high risk groups, including the Registry to Evaluate Early and Long-Term PAH Disease Management (REVEAL) risk calculator version 2.0 9, the French Pulmonary Hypertension Registry (FPHR) approach 10, and the Comparative Prospective Registry of Newly Initiated Therapies for Pulmonary Hypertension (COMPERA) algorithm 11. These scores and risk stratifications were determined as shown below. To evaluate the incremental prognostic value added by NE to these PAH risk scores, Cox regression models were fit with NE and each risk score as independent predictors (scores were stratified by risk group). The performance of these NE + risk score models for prediction of 5-year death or transplant was compared to that of nested risk score-only models via likelihood ratio test.
## REVEAL 2.0 risk stratification

| PAH subtype          | CTD-PAH (+1), HPAH (+2), PoPH (+3) |
|----------------------|-------------------------------------|
| Demographics         | Male age >60 (+2)                   |
| Comorbidities        | Renal insufficiency- eGFR <60 (+1)  |
| Functional class     | FC I (-1), FC III (+1), FC IV (+2)  |
| Vitals               | Systolic BP <110 (+1), Heart rate >96 (+1) |
| Hospitalization      | Any hospitalization in preceding 6 mos (+1) |
| 6MWD                 | ≥440 meters (-2), 320 to <440 m (-1), <165 m (+1) |
| NT-proBNP            | <300 pg/ml (-2), >1100 pg/mL (+2)   |
| Echocardiogram       | Pericardial effusion present (+1)   |
| DLCO                 | <40% of predicted (+1)              |
| Hemodynamics         | RAP >20 mmHg (+1), PVR <5 Wood units (-1) |

Starting from a baseline of 6 points, sum total points from all clinical variable categories

### Risk groups
- Low (score 0-6)
- Intermediate (score 7-8)
- High (score ≥9)

## FPHR risk stratification

| Clinical variable          | Low-risk criteria          | Determine number of low-risk criteria met |
|----------------------------|---------------------------|------------------------------------------|
| Functional class           | Functional class I or II   |                                          |
| 6MWD                       | ≥ 440 meters              |                                          |
| Right atrial pressure      | < 8 mmHg                  |                                          |
| Cardiac index              | ≥2.5 L min⁻¹ m⁻²          |                                          |

### Risk groups
- Low (3 or 4 criteria met)
- Intermediate (1 or 2 criteria)
- High (0 criteria)

## COMPERA risk stratification

| Clinical variable | Low risk (1 point) | Intermediate risk (2 points) | High risk (3 points) | Calculate average score = sum of points across variables / number of available variables, then round to nearest integer |
|-------------------|--------------------|------------------------------|----------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| Functional class  | I/II               | III                          | IV                   |                                                                                                                                 |
| 6MWD (meters)     | >440               | 165-440                      | <165                 |                                                                                                                                 |
| NT-proBNP (pg/mL) | <300               | 300-1400                     | >1400                |                                                                                                                                 |
| Right atrial pressure (mmHg) | <8                 | 8-14                         | >14                  |                                                                                                                                 |
| Cardiac index (L min⁻¹ m⁻²) | ≥2.5           | 2.0-2.4                      | <2.0                 |                                                                                                                                 |
| SvO2 (%)           | >65                | 60-65                        | <60                  |                                                                                                                                 |

### Risk groups
- Low (average score= 1)
- Intermediate (average score= 2)
- High (average score= 3)

### Investigating whether NE offers prognostic information independent of other prognostic inflammatory biomarkers:

Same-day measurements of 48 cytokines, chemokines, and growth factors were available from most patients in the Stanford PAH cohort (n=220). First, we established which measured cytokines had prognostic significance in this cohort. Univariate Cox regression models were fitted with cubic splines (see methodology above) to evaluate the relationship between 14 of the measured cytokines and mortality risk. The 14 analyzed cytokines (IL-1α, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12, IL-13, TNF-α, HGF, CXCL10, CXCL12, CCL2, and VEGF) were those which have been implicated in PAH survival by prior published studies. Of these 14 biomarkers, 8 achieved significance in univariate analysis, as highlighted by e-Table 9 (IL-1β, IL-2, IL-6, IL-10, IL-12, TNF-α, HGF, and VEGF). Each marker exhibited a non-linear relationship with the outcome (determined by analysis of variance of cubic spline model), and we identified a prognostic threshold beyond which mortality risk remained significantly increased (same method as described for NE analysis above). Next, the 8 inflammatory biomarker thresholds with univariate significance were analyzed in multivariable Cox models that adjusted for age, sex, PAH subtype, incident vs
prevalent PAH status, right atrial pressure, functional class, and prostacyclin treatment. Of the 8 markers, 4 maintained prognostic significance after adjustment for clinical parameters (IL-1β, IL-6, IL-10, and TNF-α), as also highlighted in e-Table 9. Finally, we evaluated whether our previously determined NE prognostic threshold (168.5 ng/mL) is associated with mortality risk after adjustment for the effects of the 4 cytokines identified as independent predictors of survival. To do so, another multivariable Cox regression model was fitted with NE, IL-1β, IL-6, IL-10, TNF-α, age, sex, PAH subtype, and incident vs prevalent PAH status.

6d. Relating NE and elafin to clinical features over time

Linear mixed effects models: The 'lmer' function from the 'lme4' R package (version 1.1-17) was used to fit linear mixed effects regression models that related biomarker changes to clinical variables over time. Model estimates were chosen to optimize the log-likelihood criterion. A random effect was introduced for subject in all models, which accounted for baseline differences across patients. To evaluate for significant biomarker changes from baseline to follow-up for the cohort, models were fit to each biomarker as functions of time (fixed effect) and the random effect. Significance (p-value) was determined by applying analysis of variance (ANOVA) to compare this model to a null model (only random effect). To determine if biomarker changes were associated with clinical variables over time, we again used ANOVA to compare two models that were fit to the biomarker: model fit as function of time and the clinical variable (separate fixed effects) vs. model fit as function of the time-clinical variable interaction alone. P-values reflected the significance of this interaction term and indicated if the clinical variable associated with biomarker changes.

6e. Identification of differentially expressed cytokines in setting of increased NE

Significance analysis of microarrays (SAM): SAM is a statistical technique which can be used find individual features in high-dimensional biological data (i.e. specific genes in a gene expression profiling study, or proteins within a measured proteomic panel) that are significantly associated with a clinical variable (including a diagnosis category, treatment, outcome, etc.). The 'samr' function in the 'samr' R package (version 3.0) was applied to associate cytokines with NE upregulation. NE upregulation was defined as NE >134.8 ng/mL, the ideal cut-off derived in receiver operating characteristic analysis of NE's ability to discriminate PAH from health (see Figure 1C, main manuscript). The 'two class unpaired' response type and the Mann-Whitney U test statistic was implemented in SAM. Analysis was false discovery rate (FDR) controlled for multiple testing, and the significance level was set at FDR <0.05.
e-Appendix 7. Supplemental Acknowledgements

7a. UK National Cohort Study of Idiopathic and Heritable PAH

Funding is provided by the National Institute of Health Research BioResource (NIHRBR) Rare Diseases study, the Imperial NIHR Clinical Research Facility and the Biomedical Research Centres at Imperial College Healthcare NHS Trust, Cambridge Biomedical Research Centre, Guy's and St Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust and the Sheffield NIHR Clinical Research Facility award to Sheffield Teaching Hospitals Foundation NHS Trust. The UK National Cohort of Idiopathic and Heritable PAH is supported by the NIHRBR; the BHF (SP/12/12/29836) and the UK Medical Research Council (MR/K020919/1).

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e-Appendix 8.  Supplemental References

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