ADDITIONAL FILE 1

Post-hoc Mediation Analysis of two biomarkers, and Survival in Acute Respiratory Distress Syndrome

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APPENDICES

Appendix 1. Exclusion criteria

Exclusion criteria of the LIVE study [1]:

- mechanical ventilation for more than 7 consecutive days in the past 30 days;
- a previous history of acute respiratory distress syndrome (ARDS) in the previous month;
- intracranial hypertension;
- a body-mass index higher than 40 kg/m²;
- chronic respiratory diseases requiring long-term oxygen therapy or respiratory assistance;
- allogeneic bone-marrow transplantation;
- metastatic cancer;
- extensive burns;
- liver cirrhosis with Child Pugh class C;
- bronchopleural fistula;
- pulmonary fibrosis;
- pregnant patients;
- patients who were moribund, or facing end of life;
- patients under tutelage;
- and patients already enrolled in another interventional ARDS study.

As in the per-protocol analysis of the LIVE study, we only excluded patients misclassified in the intervention group (i.e., the personalized strategy) because misclassified

patients of the control group (i.e., the standard strategy) were not misaligned with the mechanical ventilation strategy, which by definition was not related to lung morphology.

Appendix 2. Measurements

Radiographic assessment of lung edema (RALE) score [2]

RALE score was calculated as the summed products of the consolidation and density scores for each radiograph quadrant (upper/lower right quadrants, upper/lower left quadrants), with a maximal score for each quadrant of 12 and a maximum total score of 48. The consolidation score is based on the extent of alveolar opacities in each quadrant (none: 0 points; < 25%: 1 point; 25%-50%: 2 points; 50%-75%: 3 points; > 75%: 4 points), and the density of alveolar opacities in each quadrant is scored as hazy (1 point), moderate (2 points), or dense (3 points). Two investigators independently scored all chest radiographs, with an excellent between observer agreement for the total RALE score (Lin concordance correlation coefficient ρ of 0.978) [3].

Soluble receptor for advanced glycation end-products (sRAGE) [4]

The soluble form of the receptor for advanced glycation end-products (RAGE), composed of the extracellular domain of RAGE, is a marker of lung alveolar type I cell injury [5]. Plasma sRAGE was measured in duplicate using enzyme-linked immunosorbent assay kits (RAGE Quantikine, R&D Systems, Minneapolis, MN, USA). The personnel responsible for performing sRAGE assays had no knowledge of the clinical data or of the randomization group.

Appendix 3. Statistical analyses

The standardized mean difference is the difference in the mean values divided by their pooled standard deviations.

Group-based trajectory modeling (GBTM) [6]

The group-based trajectory modeling is a finite mixture-based model for longitudinal data where patients in the same group have the same trajectory. This model assumed that our population was composed of a mixture of distinct groups defined by their trajectory. These trajectories were summarized by a finite set of different polynomial functions of time and determined by maximum likelihood estimation. This estimation was performed with an EM algorithm which is an iterative method consisting of the Expectation step (E-step) and the Maximization step (M-step). To perform patient clustering using GBTM, it was necessary to have at least one baseline measurement and an additional follow-up measurement. For sRAGE, patients had a median of 5 measurements [IQR 5 – 6], while for the RALE score, the median was 2 measurements [IQR 2 – 3]) per patient.

We estimated models using up to eight groups under the assumption that interpreting more than eight groups would be clinically irrelevant. We allowed all groups to have one (for the RALE score) to three degrees (cubic terms, for sRAGE) based on expert opinion (NM) and visual inspection (i.e., the shape of data) [7]. The optimal number of groups were based on several fit statistics. First, we used the lower values of information criteria indicating a better balance between goodness-of-fit and complexity (AIC and BIC). Second, we retained the only model with an average posterior probability of assignment (i.e., entropy) greater than 0.7 for each group. Third, only models with an adequate sample size in each group (i.e., more than five patients for each group) were retained [7, 8].

After selecting the number of groups, one of the aims was to find different traits between each group, using variables not included for modeling. We described the characteristics of the patients within each group without performing any statistical tests, because of the small sample size and to avoid alpha risk inflation.

The advantage of this method was twofold: on the one hand, we could estimate trajectories even with missing values; and on the other, we obtained an imputed database through individual estimated trajectory groups. After modeling, individuals were no longer summarized by longitudinal measures, but by trajectory groups of sRAGE and RALE scores.

Least absolute shrinkage and selection operator (LASSO) [9]

We used high-dimensional data with more variables than patients. Hence, we were looking for a compromise between enough features for a correct prediction and a reasonable number of features to consider. Therefore, we set up a LASSO regression model. This model allowed the selection of variables by shrinking down to zero coefficients for variables nonrelated to the outcome. Our outcomes for this regression were sRAGE and RALE score trajectory group membership. The features with non-zero coefficient were selected based on a tuning parameter called λ. This parameter achieved a standard error of the minimum mean square error. The following features were included:

- *patient characteristics:* age, sex, body mass index, predicted body weight, comorbidities, indication for intensive care unit (ICU) admission, cause and onset of ARDS, lung morphology, position, ventilation strategy and center;
- *baseline respiratory variables:* non-invasive ventilation, ventilation mode, onset of ventilation, tidal volume, respiratory rate, peak pressure (P_{max}), plateau pressure (P_{plat}), positive-end respiratory pressure (PEEP), driving pressure, FiO_2, PaO_2, PaO_2 / FiO_2, SpO_2, $PaCO_2$;

- *laboratory values at inclusion:* arterial blood pH, plasma bicarbonate, serum lactate, creatinine, urea, leucocyte count, platelets, prothrombin ratio, kalemia, natremia, bilirubin;
- *clinical settings at inclusion:* systolic and diastolic blood pressure, heart rate, temperature, SAPS II score, SOFA score, Glasgow coma scale, diuresis;
- *baseline treatments:* analgesic, antibiotic, corticosteroid, hypnotic, neuromuscular blocking agent, vasopressor or inotrope;
- need for renal replacement therapy at inclusion;
- *measurement at end of study:* ventilator-free days (VFDs) at day 28, ICU length of stay, 90-day vital status, time to death, tracheostomy, Confusion Assessment Method (CAM) - ICU scale, Medical Research Council (MRC) scale, systolic blood pressure, heart rate, temperature.

To avoid model overfitting, the data were iteratively divided into a training set to train the model and a test set to evaluate the predictive ability of the model. We used thus a cross-validation approach with 10 blocks, since there are no tests that would enable us to determine the best λ.

Causal mediation analysis [10]

Causal effects

The estimations of causal effects were based on the counterfactual framework [11, 12]. A counterfactual outcome is an individual's outcome value that would be observed when exposed to a certain exposure value. The direct effect was conceived as the effect of the mechanical ventilation strategy on 90-day survival at a fixed level of the mediators (sRAGE or RALE score); the indirect effect was conceived as the effect on 90-day survival of changes in the mechanical ventilation strategy which operated through mediator levels; the total effect

represented the overall effect of the mechanical ventilation strategy; the proportion mediated assessed the extent to which the total effect of the mechanical ventilation strategy on 90-day survival operated through the mediator. However, we used this proportion only when direct and indirect effects were in the same direction. A priori <5% was considered as a small proportion mediated, 5 to 20% as a moderate proportion mediated, and >20% as a large proportion mediated. Complementary mediation means that the indirect and direct effects point at the same direction. Competitive mediation, on the other hand, means that the indirect and direct effects point in opposite directions [13].

Let T be the mechanical ventilation strategy (C for the control group, or I for the intervention group), Y the survival status at day 90 (alive or dead), Z the vector of our K = 2 mediators and M^k the mediator of interest (sRAGE or RALE score). We denoted by W^k the complement of M^k in Z, which is the other mediator not of direct interest.

The average indirect effect mediated by M^k was defined by [14]:

$$\delta^k(t) = \mathbb{E}\left[Y\left(t, M^k(I), W^k(t)\right)\right] - \mathbb{E}\left[Y\left(t, M^k(C), W^k(t)\right)\right]$$

The average joint indirect effect, which is the indirect effect mediated by all the mediators, was defined by:

$$\delta^Z(t) = \mathbb{E}\left[Y\left(t, Z(I)\right)\right] - \mathbb{E}\left[Y\left(t, Z(C)\right)\right]$$

The direct effect was defined by:

$$\zeta(t_{sRAGE}, t_{RALE}) = \mathbb{E}\left[Y\left(I, M^{sRAGE}(t_{sRAGE}), M^{RALE}(t_{RALE})\right)\right] - \mathbb{E}\left[Y\left(C, M^{sRAGE}(t_{sRAGE}), M^{RALE}(t_{RALE})\right)\right]$$

The total effect was defined by:

$$\tau = \mathbb{E}\left[Y\left(I, Z(I)\right)\right] - \mathbb{E}\left[Y\left(C, Z(C)\right)\right]$$

The proportion mediated by M^k:

$$PM^k(t) = \frac{\delta^k}{\tau}$$

Assumptions

We made several assumptions following Vanderweele and Vansteelandt [15]:

No unmeasured confounding (known as the Sequential Ignorability Multiple Mediators Assumption [14]): there were (1) no unmeasured mechanical ventilation strategy – survival status at day 90 confounder, (2) no unmeasured sRAGE trajectory groups – survival status at day 90 confounder, (3) no unmeasured RALE score trajectory groups – survival status at day 90 confounder, (4) no unmeasured mechanical ventilation strategy – sRAGE trajectory groups confounder, (5) no unmeasured mechanical ventilation strategy – RALE score trajectory groups confounder, and (6) no measured or unmeasured sRAGE or RALE score trajectory groups – survival status at day 90 confounder affected by the mechanical ventilation strategy. Assumptions (1), (4), and (5) were verified because the mechanical ventilation strategy was randomized. Indeed, although the mechanical ventilation strategy was randomized, assumptions (2), (3), and (6) could not be verified because randomization of individuals to levels of exposure did not ensure that individuals had been randomized to levels of the mediators. The measured baseline covariates included in the models should be sufficient to control for confounding in these relationships. When there are more than one mediator, it is important to consider all these mediators. Indeed, they may be correlated or they may influence one another, violating the assumption that there were no confounders of the mediator-outcome effect that should be affected by the exposure. Therefore, we did not perform two separate analyses (i.e., one with each mediator) but we included the two mediators within the same analysis.

Temporal ordering: the mechanical ventilation strategy preceded the survival status at day 90, the mediators preceded temporally the survival status at day 90, and mechanical ventilation strategy preceded the mediators.

Positivity assumption: there was a probability greater than zero of being assigned to each of the treatment levels. The same positivity assumption is required for the mediators.

Consistency assumption (known as the Stable Unit Treatment Value Assumption (SUTVA) [16]): the observed outcome for each treated patient was equal to their outcome if they had received the treatment, and the observed outcome for each untreated patient was equal to their outcome if they had not been treated. The same consistency assumption is required for the mediators.

Exchangeability: the outcome distribution in the treated and the untreated would be the same if both groups had received the same treatment level. The same exchangeability assumption is required for the mediators.

Mediators and outcome

We obtained three groups from the group-based trajectory modeling. We removed the first dummy of every group such that only n-1 dummies remained. This avoided multicollinearity issues in models. After selection of baseline covariates (i.e., potential confounders), we checked models for multicollinearity and normality of residuals. We used the variance inflation factor to look for potential multicollinearity between covariates. A value > 2 was considered to be collinear.

Multiple imputation-chained equations [17]

First, we inspected the missing pattern and measured the total number of missing values. We assumed that missing values were missing at random (i.e., the probability for a datum to be missing did not depend on its true value but only on the observed values).

Second, we performed multiple imputation. We did 63 database imputations because there were 63% missing values and 20 iterations, including the following variables to be as congenial as possible:

- *patient characteristics:* age, sex, body mass index, comorbidities, indication for ICU admission, cause and onset of ARDS, lung morphology, position, ventilation strategy and study center;
- *baseline respiratory variables:* non-invasive ventilation, ventilation mode, respiratory rate, PaO2 / FiO2 ratio, SpO2;
- *clinical settings at inclusion:* SAPS II score;
- *baseline treatments:* corticosteroid, antibiotic, analgesic, vasopressor or inotrope;
- *RALE score* at day 0, 2 and 3, and trajectory groups;
- *Plasma sRAGE level* at day 0, 1, 2, 3, 4 and 6, and trajectory groups;
- *measurement at end of study:* VFDs at day 28, 90-day vital status, time to death.

Third, we estimated the causal effects and fitted an equal-effects model, assuming that the true effects were homogeneous.

Appendix 4. Packages of R [18]

We used several packages:

`readxl` to read Excel files.

Wickham H, Bryan J. readxl: Read Excel Files. 2023 R package version 1.4.2. Available from: https://CRAN.R-project.org/package=readxl.

`Tidyverse` for data visualization and manipulation .

Wickham H, Averick M, Bryan J, et al. Welcome to the tidyverse. *J Open Source Soft*. 2019; 4(43):1686. Doi:10.21105/joss.01686.

`fastDummies` to create dummy variables.

Kaplan J. fastDummies: Fast Creation of Dummy (Binary) Columns and Rows from Categorical Variables. 2020 R package version 1.6.3. Available from: https://CRAN.R-project.org/package=fastDummies.

`Tableone` to construct "Table 1" (i.e., patient baseline characteristics) and produce the standardized mean differences (SMD).

Yoshida K, Bartel A. tableone: Create 'Table 1' to Describe Baseline Characteristics with or without Propensity Score Weights. 2022 R package version 0.13.2. Available from: https://CRAN.R-project.org/package=tableone.

`Gbmt` for the group-based trajectory modeling.

Magrini A. gbmt: Group-Based Multivariate Trajectory Modeling. 2022 R package version 0.1.3. Available from: https://CRAN.R-project.org/package=gbmt.

`Glmnet` for the LASSO regression analysis.

Friedman J, Tibshirani R, Hastie T. Regularization Paths for Generalized Linear Models via Coordinate Descent. *J Stat Soft*. 2010; 33(1):1–22. Doi:10.18637/jss.v033.i01.

Tay JK, Narasimhan B, Hastie T. Elastic Net Regularization Paths for All Generalized Linear Models. *J Stat Soft*. 2023; 106(1):1-31. Doi:10.18637/jss.v106.i01.

`performance` and `see` to check regression models.

Lüdecke D, Ben-Shachar MS, Patil I, Waggoner P, Makowski D. performance: An R package for assessment, comparison and testing of statistical models. *J Open Source Soft.* 2021; 6(60):3139. Doi:10.21105/joss.03139.

Lüdecke D; Patil I, Ben-Shachar MS, Wiernik M, Waggoner P, Makowski D. see: An R Package for Visualizing Statistical Models. *J Open Source Soft*. 2021; 6(64):3393. Doi:10.21105/joss.03393.

`multimediate` to causal mediation analysis.

Jérolon A. multimediate: Causal mediation analysis in presence of multiple mediators uncausally related. 2023 R package version 0.0.0.9000. Available from: https://github.com/AllanJe/multimediate.

`Mice` to analyze missing values and impute them.

Van Buuren S, Groothuis-Oudshoorn K. mice: Multivariate Imputation by Chained Equations in R. *J Stat Soft*. 2011; 45(3):1-67. Doi:10.18637/jss.v045.i03.

`metafor` to aggregate causal effects.

Viechtbauer W. Conducting meta-analyses in R with the metafor package. *J Stat Soft*. 2010; 36(3):1-48. Doi:10.18637/jss.v036.i03.

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TABLES

Table S1. Characteristics of the included and non-included LIVE populations [1]

	Included patients (n = 115)	Non-included patients (n = 285)	SMD
Age, years	58.72 ± 16	63.24 ± 14.6	**.30**
Sex			.07
Female	31 (27)	68 (23.9)	
Male	84 (73)	217 (76.1)	
BMI, kg/m^2	26.1 ± 5	26.33 ± 5.1	.05
Comorbidities			
COPD	11 (9.6)	29 (10.2)	.02
Hematologic neoplasm	6 (5.2)	8 (2.8)	**.12**
Other	79 (68.7)	189 (66.3)	.05
None	27 (23.5)	74 (26)	.06
Indication for ICU admission			**.45**
Acute metabolic disorders	4 (3.5)	6 (2.1)	
Acute respiratory failure	52 (45.2)	123 (43.2)	
Coma	8 (7)	16 (5.6)	
Hemorrhagic shock	1 (0.9)	16 (5.6)	
Septic shock	14 (12.2)	60 (21.1)	
Intra-abdominal sepsis	16 (13.9)	18 (6.3)	
Traumatic injuries	6 (5.2)	15 (5.3)	
Elective surgery	10 (8.7)	19 (6.7)	
Urgent surgery	4 (3.5)	12 (4.2)	
Cause of ARDS			**.13**
Pulmonary	79 (68.7)	213 (74.7)	
Extrapulmonary	36 (31.3)	72 (25.3)	
Lung morphology			.08
Focal ARDS	35 (30.4)	98 (34.4)	
Non-focal ARDS	80 (69.6)	187 (65.6)	
Position			.08
> 30°	106 (92.2)	251 (90)	
Dorsal	9 (7.8)	34 (10)	
Invasive mechanical ventilation			**.17**
Pressure-support ventilation	8 (7)	9 (3.2)	
Controlled ventilation	107 (93)	276 (96.8)	
Baseline respiratory variables			
PaO_2 / FiO_2, mmHg	114 [83 – 159]	113 [83 – 146]	.08
P_{plat}, cmH_2O	22 [20 – 28]	24 [21 – 28]	**.14**

	Included patients (n = 115)	Non-included patients (n = 285)	SMD
Driving pressure[a], cmH_2O	12 [10 – 15]	14 [11 – 18]	**.46**
Need for RRT	3/114 (2.6)	23/284 (8.1)	**.24**
Laboratory values			
Arterial blood pH	7.33 ± 0.1	7.31 ± 0.1	**.21**
$PaCO_2$, mmHg	44.5 ± 10.1	46.4 ± 11.2	**.18**
Serum lactate, mmol/L	1.7 [1.2 – 2.6]	1.8 [1.1 – 3.2]	.03
Plasma bicarbonate, mmol/L	23.6 ± 4.9	22.8 ± 4.8	**.15**
Baseline treatment at enrolment			
Antibiotic	101 (87.8)	259 (91.5)	**.12**
Corticosteroids	28 (24.3)	63 (22.2)	.05
Vasopressors or inotropes	66 (57.4)	182 (64.5)	**.15**
Hypnotic	87 (75.7)	256 (89.8)	**.38**
NMBA	94 (81.7)	221 (78.1)	.09
Baseline severity illness			
McCabe score[b]			**.17**
0: non-fatal disease	71/111 (64)	192/276 (69.6)	
1: ultimately fatal disease	37/111 (33.3)	72/276 (26.1)	
2: rapidly fatal disease	3/111 (2.7)	12/276 (4.3)	
SAPS II[c]	48.6 ± 16.8	52.4 ± 16	**.23**
SOFA	8.8 ± 3.6	9.6 ± 3.8	**.22**
Outcomes			
Death at day 90	32 (27.8)	77 (27)	.02
Ventilator-free days at day 28[d]	18 [0 – 25]	18 [0 – 23]	.06
At discharge			
Tracheostomy	15/113 (13.3)	28/277 (10.1)	**.10**
Delirium[e]	5/83 (6)	27/181 (14.9)	**.29**

Data are presented as number (percentage), number/total number (percentage) if there were missing values, mean ± SD (standard deviation) or median [IQR] (interquartile range). Percentages may not total 100 because of rounding. Boldface indicates unbalanced groups (i.e., standardized mean difference (SMD) >.10).

ARDS acute respiratory distress syndrome, *BMI* body mass index (calculated as weight in kilograms divided by height in meters squared), *COPD* chronic obstructive pulmonary disease, *ICU* intensive care unit, *NMBA* neuromuscular blocking agent, $PaCO_2$ partial pressure of arterial carbon dioxide, PaO_2 / FiO_2 ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen, *PBW* predicted body weight, P_{plat} end-inspiratory plateau pressure, *RRT* renal replacement therapy, *SOFA* Sequential Organ Failure Assessment score

[a] Driving pressure was calculated as P_{plat} (end-respiratory plateau pressure) minus PEEP (positive-end respiratory pressure) [19]

[b] The McCabe score is a subjective score of underlying illness severity classifying patients according to a prognosis of rapidly fatal (< 1 year), ultimately fatal (1-4 years), and nonfatal (> 5 years) [20]

[c] The Simplified Acute Physiology Score II (SAPS II) is based on 17 variables; scores range from 0 to 163, with higher scores indicating more severe disease [21]

[d] Ventilator-free days was defined as the number of days from the last day of mechanical ventilation to day 28

[e] Delirium was assessed by the CAM-ICU [22]. There were several missing values (32 for the included patients, 104 for the non-included patients)

Table S2. Characteristics of the three patient groups based on plasma sRAGE trajectories (N = 100 patients)

	Group 1 (n = 28)	Group 2 (n = 36)	Group 3 (n = 36)
Death at day 90	8 (28.6)	10 (27.8)	13 (36.1)
Ventilator-free days at day 28[a]	19 [0 – 25]	20 [0 – 24]	17 [0 – 24]
Length of stay in ICU, days	8.5 [5 – 16.3]	13.0 [9 – 25.3]	14.5 [6 – 26.8]
Age, years	60.2 ± 15.9	70 ± 16.7	58.4 ± 15.7
Sex			
Female	5 (17.9)	13 (36.1)	8 (22.2)
Male	23 (82.1)	23 (63.9)	28 (77.8)
BMI, kg/m^2	27 ± 5	24.2 ± 4.5	26.2 ± 4.5
Comorbidities			
COPD	1 (3.6)	3 (8.3)	3 (8.3)
Hematologic neoplasm	1 (3.6)	3 (8.3)	2 (5.6)
Other	23 (82.1)	27 (75)	24 (66.7)
None	3 (10.7)	7 (19.4)	10 (27.8)
Indication for ICU admission			
Acute metabolic disorders	1 (3.6)	0 (0)	3 (8.3)
Acute respiratory failure	13 (46.4)	15 (41.7)	16 (44.4)
Coma	2 (7.1)	3 (8.3)	3 (8.3)
Hemorrhagic shock	0 (0)	1 (2.8)	1 (2.8)
Septic shock	5 (17.9)	5 (13.9)	3 (8.3)
Intra-abdominal sepsis	3 (10.7)	3 (8.3)	7 (19.4)
Traumatic injuries	2 (7.1)	3 (8.3)	0 (0)
Elective surgery	1 (3.6)	5 (13.9)	1 (2.8)
Urgent surgery	1 (3.6)	1 (2.8)	2 (5.7)
Cause of ARDS			
Pulmonary	25 (89.3)	25 (69.4)	17 (47.2)
Extrapulmonary	3 (10.7)	11 (30.6)	19 (52.8)
Lung morphology			
Focal ARDS	9 (32.1)	5 (13.9)	21 (58.3)
Non-focal ARDS	19 (67.9)	31 (86.1)	15 (41.7)
Position			
> 30°	27 (96.4)	33 (91.7)	32 (88.9)
Dorsal	1 (3.6)	3 (8.3)	4 (11.1)
Invasive mechanical ventilation			
Pressure-support ventilation	0 (0)	5 (13.9)	3 (8.3)
Controlled ventilation	28 (100)	31 (86.1)	33 (91.7)
Baseline respiratory variables			

	Group 1 (n = 28)	Group 2 (n = 36)	Group 3 (n = 36)
PaO_2 / FiO_2, mmHg	103 [83 – 154]	115 [88 – 161]	117 [72 – 147]
P_{plat}, cmH_2O	24 [21 – 27]	21 [18 – 28]	22 [21 – 25]
Driving pressure[b], cmH_2O	12 [10 – 17]	12 [10 – 15]	11 [10 – 12]
Need for RRT	1 (3.6)	0 (0)	2/35 (5.7)
Laboratory values			
Arterial blood pH	7.34 ± 0.1	7.35 ± 0.1	7.33 ± 0.1
$PaCO_2$, mmHg	45.0 ± 10.7	44.7 ± 9.7	42.4 ± 9.3
Serum lactate, mmol/L	1.4 [1.2 – 2.5]	1.4 [1.1 – 2]	2.1 [1.4 – 3.3]
Plasma bicarbonate, mmol/L	24.3 ± 5.2	24.4 ± 4.6	22.0 ± 5.1
Baseline treatment at enrolment			
Antibiotic	25 (89.3)	33 (91.7)	30 (83.3)
Corticosteroids	2 (7.1)	11 (30.6)	9 (25)
Vasopressors or inotropes	15 (53.6)	23 (63.9)	23 (63.9)
Hypnotic	20 (71.4)	26 (72.2)	30 (83.3)
NMBA	22 (78.6)	30 (83.3)	30 (83.3)
Baseline severity illness			
McCabe score[c]			
0: non-fatal disease	22 (78.6)	16 (44.4)	21/32 (65.6)
1: ultimately fatal disease	5 (17.9)	18 (50)	11/32 (34.4)
2: rapidly fatal disease	1 (3.6)	2 (5.6)	0/32 (0)
SAPS II[d]	48.1 ± 17.9	46.0 ± 15	53.5 ± 15.5
SOFA	9.1 ± 3.9	8.4 ± 3.2	9.6 ± 3.5
Centers			
Clermont-Ferrand	14 (50)	17 (47.2)	21 (58.3)
Montpellier	4 (14.3)	11 (30.6)	10 (27.8)
Nîmes	10 (35.7)	8 (22.2)	5 (13.9)
At discharge			
Tracheostomy	2 (7.1)	5 (13.9)	5/35 (14.3)
Delirium[e]	1/20 (5)	1/28 (3.6)	0/23 (0)

Data are presented as number (percentage), number/total number (percentage) if there were missing values, mean ± SD (standard deviation) or median [IQR] (interquartile range). Percentages may not total 100 because of rounding.

ARDS acute respiratory distress syndrome, *BMI* body mass index (calculated as weight in kilograms divided by height in meters squared), *COPD* chronic obstructive pulmonary disease, *ICU* intensive care unit, *NMBA* neuromuscular blocking agent, $PaCO_2$ partial pressure of arterial carbon dioxide, PaO_2/FiO_2 ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen, *PBW* predicted body weight, P_{plat} end-inspiratory plateau pressure, *RALE* radiographic assessment of lung edema, *RRT* renal replacement therapy, *SOFA* Sequential Organ Failure Assessment score

[a] Ventilator-free days was defined as the number of days from the last day of mechanical ventilation to day 28

[b] Driving pressure was calculated as P_{plat} (end-respiratory plateau pressure) minus PEEP (positive-end respiratory pressure) [19]

[c] The McCabe score is a subjective score of underlying illness severity classifying patients according to a prognosis of rapidly fatal (< 1 year), ultimately fatal (1-4 years), and nonfatal (> 5 years) [20]

[d] The Simplified Acute Physiology Score II (SAPS II) is based on 17 variables; scores range from 0 to 163, with higher scores indicating more severe disease [21]

[e] Delirium was assessed by the CAM-ICU [22]. There were several missing values (8 for the first trajectory, 8 for the second trajectory and 13 for the third trajectory)

Table S3. Characteristics of the three patient groups based on RALE score trajectories (N = 82 patients)

	Group 1 (n = 28)	Group 2 (n = 21)	Group 3 (n = 33)
Death at day 90	7 (25)	5 (23.8)	10 (30.3)
Ventilator-free days at day 28[a]	15 [0 – 23]	18 [0 – 25]	23 [15 – 26]
Length of stay in ICU, days	16.0 [9 – 25.3]	16.0 [11 – 30]	9.0 [5 – 19]
Age, years	65.7 ± 13.4	57.2 ± 16.4	56.7 ± 17.1
Sex			
Female	13 (46.4)	6 (28.6)	4 (12.1)
Male	15 (53.6)	15 (71.4)	29 (87.9)
BMI, kg/m^2	25.3 ± 4.5	28.4 ± 5.3	23.9 ± 4
Comorbidities			
COPD	2 (7.1)	1 (4.8)	3 (9.1)
Hematologic neoplasm	1 (3.6)	4 (19.1)	0 (0)
Other	20 (71.4)	14 (66.7)	26 (78.8)
None	6 (21.4)	4 (19.1)	6 (18.2)
Indication for ICU admission			
Acute metabolic disorders	0 (0)	0 (0)	3 (9.1)
Acute respiratory failure	12 (42.9)	9 (42.9)	16 (48.5)
Coma	5 (17.9)	0 (0)	2 (6.1)
Hemorrhagic shock	0 (0)	0 (0)	1 (3)
Septic shock	3 (10.7)	2 (9.5)	4 (12.1)
Intra-abdominal sepsis	4 (14.3)	5 (23.8)	3 (9.1)
Traumatic injuries	1 (3.6)	1 (4.8)	1 (3)
Elective surgery	3 (10.7)	3 (14.3)	1 (3)
Urgent surgery	0 (0)	1 (4.8)	2 (6.1)
Cause of ARDS			
Pulmonary	18 (64.3)	14 (66.7)	20 (60.1)
Extrapulmonary	10 (35.7)	7 (33.3)	13 (39.4)
Lung morphology			
Focal ARDS	10 (35.7)	6 (28.6)	12 (36.4)
Non-focal ARDS	18 (64.3)	15 (71.4)	21 (63.6)
Position			
> 30°	27 (96.4)	19 (90.5)	28 (84.8)
Dorsal	1 (3.6)	2 (9.5)	5 (15.2)
Invasive mechanical ventilation			
Pressure-support ventilation	5 (17.9)	1 (4.8)	1 (3)
Controlled ventilation	23 (82.1)	20 (95.2)	32 (97)
Baseline respiratory variables			

	Group 1 (n = 28)	Group 2 (n = 21)	Group 3 (n = 33)
PaO_2 / FiO_2, mmHg	117 [85 – 161]	104 [88 – 134]	138 [84 – 168]
P_{plat}, cmH_2O	23 [18 – 28]	24 [21 – 28]	20 [19 – 22]
Driving pressure[b], cmH_2O	12 [10 – 16]	13 [10 – 17]	10 [9 – 12]
Need for RRT	1 (3.6)	0 (0)	0/32 (0)
Laboratory values			
Arterial blood pH	7.34 ± 0.1	7.34 ± 0.1	7.35 ± 0.1
PaCO2, mmHg	42.1 ± 11.8	44.6 ± 7.4	43.4 ± 9.8
Serum lactate, mmol/L	1.5 [1.3 – 2.8]	1.5 [1 – 3.2]	1.5 [1.2 – 2.4]
Plasma bicarbonate, mmol/L	22.5 ± 4.2	24.2 ± 4.8	23.4 ± 4.1
Baseline treatment at enrolment			
Antibiotic	25 (89.3)	18 (85.7)	30 (90.9)
Corticosteroids	6 (21.4)	4 (19.1)	9 (21.2)
Vasopressors or inotropes	18 (64.3)	10 (47.6)	18 (54.5)
Hypnotic	20 (71.4)	14 (66.7)	24 (72.7)
NMBA	22 (78.6)	18 (85.7)	25 (75.8)
Baseline severity illness			
McCabe score[c]			
0: non-fatal disease	16/26 (61.5)	12/20 (60)	18/32 (56.3)
1: ultimately fatal disease	9/26 (34.6)	8/20 (40)	13/32 (40.6)
2: rapidly fatal disease	1/26 (3.9)	0/20 (0)	1/32 (3.1)
SAPS II[d]	50.8 ± 15.6	55.6 ± 17.4	44.1 ± 15.8
SOFA	9.7 ± 4.1	8.4 ± 3.4	8.3 ± 3.1
Centers			
Clermont-Ferrand	15 (53.6)	20 (95.2)	16 (48.5)
Montpellier	9 (32.1)	1 (4.8)	15 (45.5)
Nîmes	4 (14.3)	0 (0)	2 (6.1)
At discharge			
Tracheostomy	5 (17.9)	2/20 (10)	2 (6.1)
Delirium[e]	1/21 (4.8)	0/16 (0)	1/25 (4)

Data are presented as number (percentage), number/total number (percentage) if there were missing values, mean ± SD (standard deviation) or median [IQR] (interquartile range). Percentages may not total 100 because of rounding.

ARDS acute respiratory distress syndrome, *BMI* body mass index (calculated as weight in kilograms divided by height in meters squared), *COPD* chronic obstructive pulmonary disease, *ICU* intensive care unit, *NMBA* neuromuscular blocking agent, $PaCO_2$ partial pressure of arterial carbon dioxide, PaO_2 / FiO_2 ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen, *PBW* predicted body weight, P_{plat} end-inspiratory plateau pressure, *RALE* radiographic assessment of lung edema, *RRT* renal replacement therapy, *SOFA* Sequential Organ Failure Assessment score

[a] Ventilator-free days was defined as the number of days from the last day of mechanical ventilation to day 28

[b] Driving pressure was calculated as P_{plat} (end-respiratory plateau pressure) minus PEEP (positive-end respiratory pressure) [19]

[c] The McCabe score is a subjective score of underlying illness severity classifying patients according to a prognosis of rapidly fatal (< 1 year), ultimately fatal (1-4 years), and nonfatal (> 5 years) [20]

[d] The Simplified Acute Physiology Score II (SAPS II) is based on 17 variables; scores range from 0 to 163, with higher scores indicating more severe disease [21]

[e] Delirium was assessed by the CAM-ICU [22]. There were several missing values (7 for the first trajectory, 5 for the second trajectory and 8 for the third trajectory)

Table S4. Causal mediation analysis in complete cases (N = 81 patients)

Causal effect	Estimation [95% CI]	*P* Value
ACME sRAGE Trajectory – Group 2[a]	0.01 [-0.03 ; 0.07]	.25
PM[b] sRAGE Trajectory – Group 2	-	-
ACME sRAGE Trajectory – Group 3[a]	0.01 [-0.04 ; 0.07]	.46
PM[b] sRAGE Trajectory – Group 3	-	-
ACME RALE Trajectory – Group 2[a]	0.02 [-0.05 ; 0.09]	.37
PM[b] RALE Trajectory – Group 2	-	-
ACME RALE Trajectory – Group 3[a]	-0.00 [-0.04 ; 0.03]	.38
PM[b] RALE Trajectory – Group 3	-	-
ACME Joint	0.04 [-0.06 ; 0.14]	.41
PM[b] Joint	-	-
ADE	-0.17 [-0.35 ; 0.01]	.05
Total effect	-0.14 [-0.32 ; 0.05]	.13

Boldface indicates statistical significant (i.e., *P* value <.05).

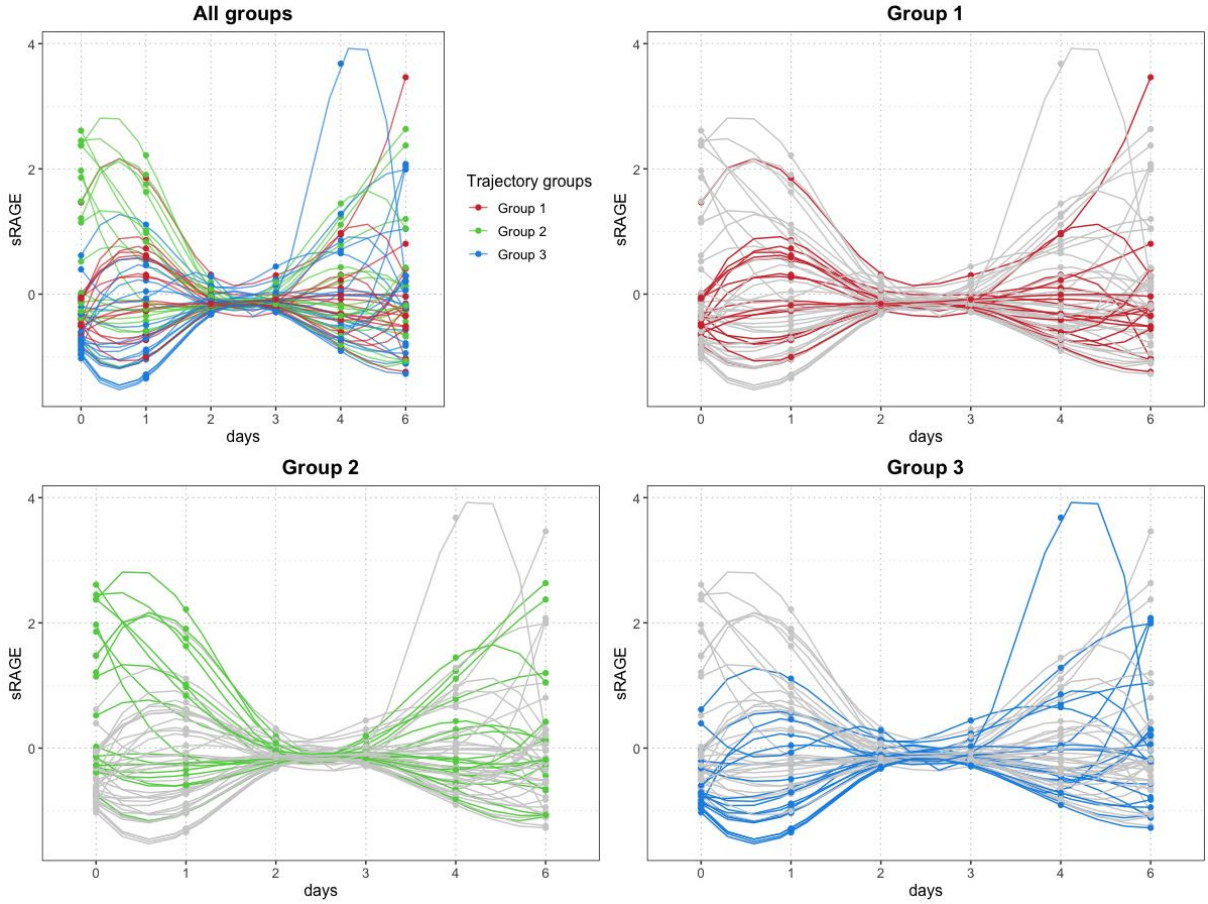
ACME average causal mediation effect, *ADE* average direct effect, *CI* confidence interval, *PM* proportion mediated, *RALE* radiographic assessment of lung edema, *sRAGE* soluble receptor for advanced glycation end-products

[a] The first trajectory group was the reference group

[b] The proportion mediated was reported only when direct and indirect effects were in the same direction and statistically significant

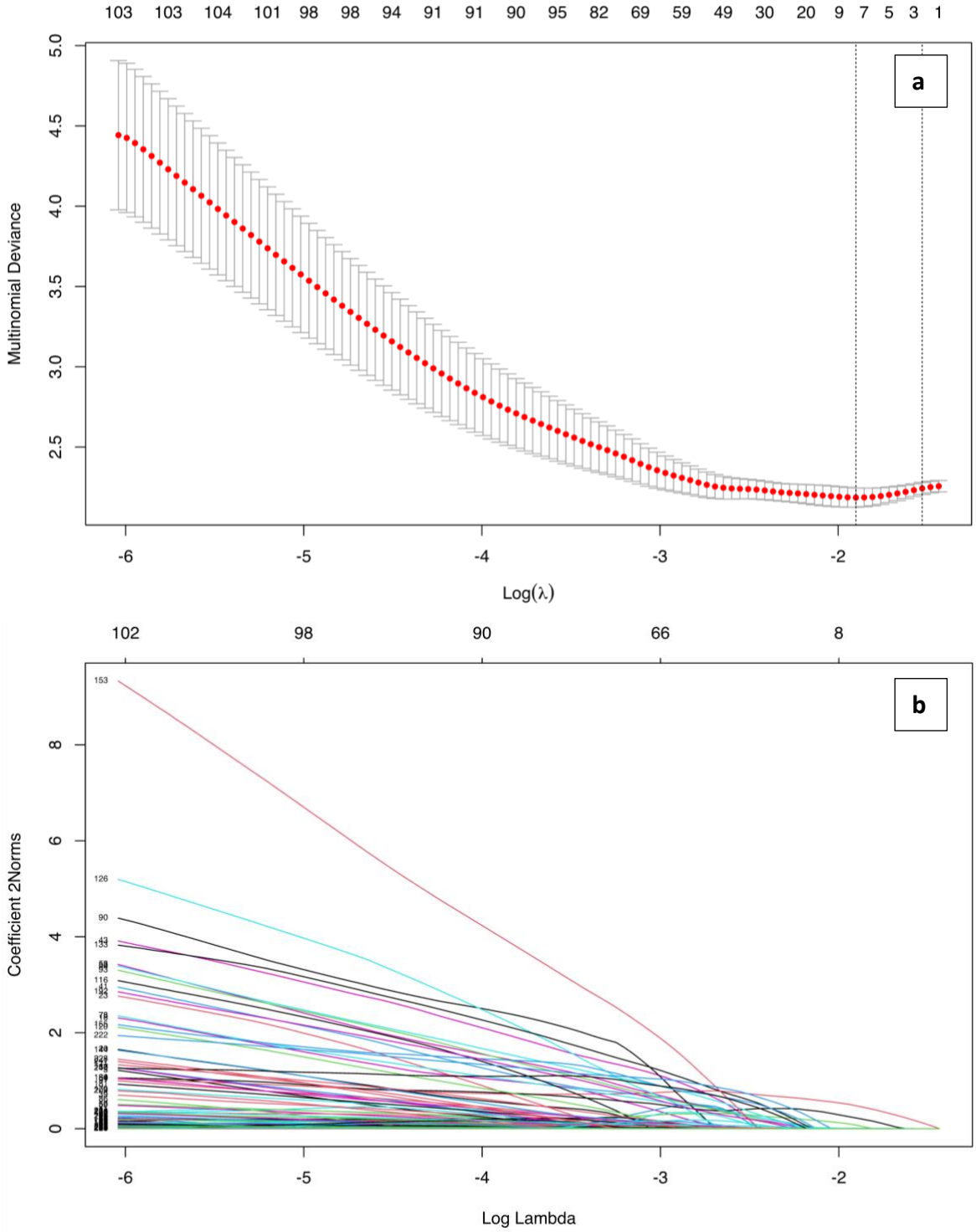
FIGURES

Figure S1. Parallel coordinate plot for sRAGE trajectories



Each point represent a single individual and each color represent an individual's membership of a trajectory group. The variables (i.e., sRAGE) were standardized (i.e., subtract mean and divide by the standard deviation) and a spline interpolation were used. sRAGE was measured six times: at baseline and on days 1, 2, 3, 4, and 6.
sRAGE soluble receptor for advanced glycation end-products

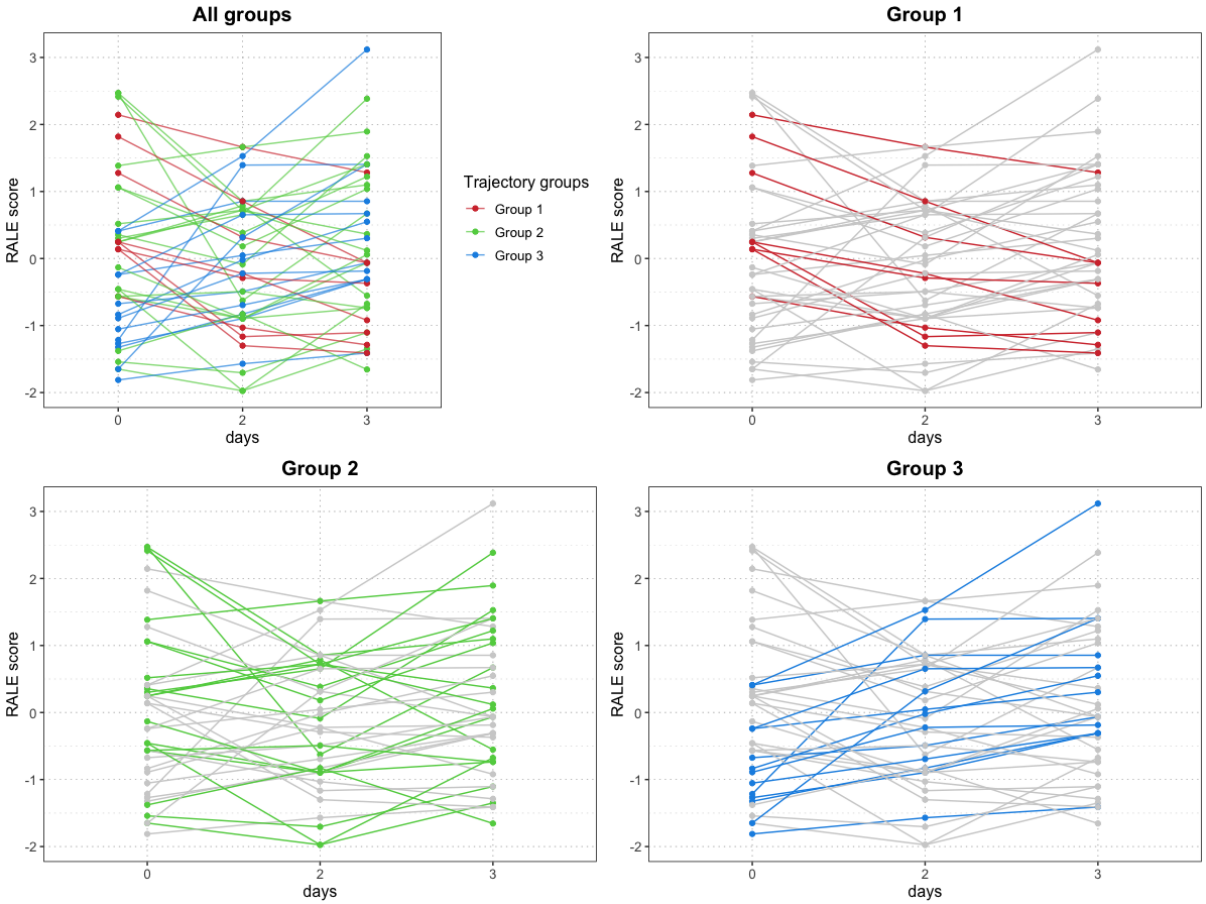
Figure S2. Identification of features as independent predictors of the sRAGE trajectory using LASSO regression analysis



(a) The multinomial deviance (y-axis) was plotted versus log(λ) (lower x-axis). The upper x-axis represents the average number of predictors. Dotted vertical lines were drawn at the optimal values using the minimum mean square error and 1 standard error of the minimum criteria. The tuning parameter (λ) was selected in the LASSO model via 10-fold cross-validation based on minimum criteria and was 0.217. (b) LASSO coefficient profiles of the 151 features (y-axis) were plotted against Log Lambda (λ) sequence. Two features with nonzero coefficients were retained.

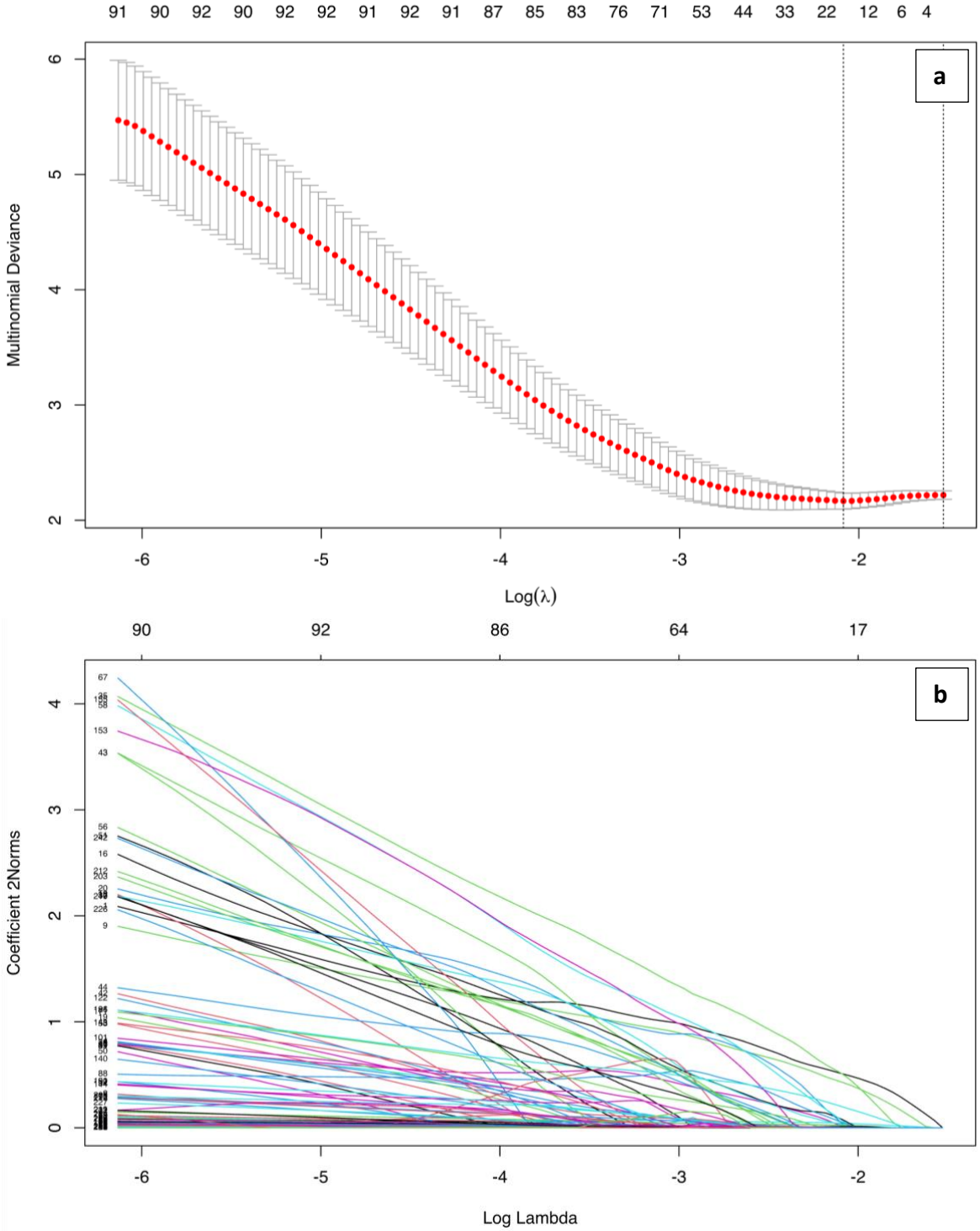
LASSO least absolute shrinkage and selection operator, *sRAGE* soluble receptor for advanced glycation end-products

Figure S3. Parallel coordinate plot for RALE score trajectories



Each point represent a single individual and each color represent an individual's membership of a trajectory group. The variables (i.e., the RALE score) were standardized (i.e., subtract mean and divide by the standard deviation). The RALE score was measured three times: at baseline, on day 2, and on day 3.
RALE radiographic assessment of lung edema

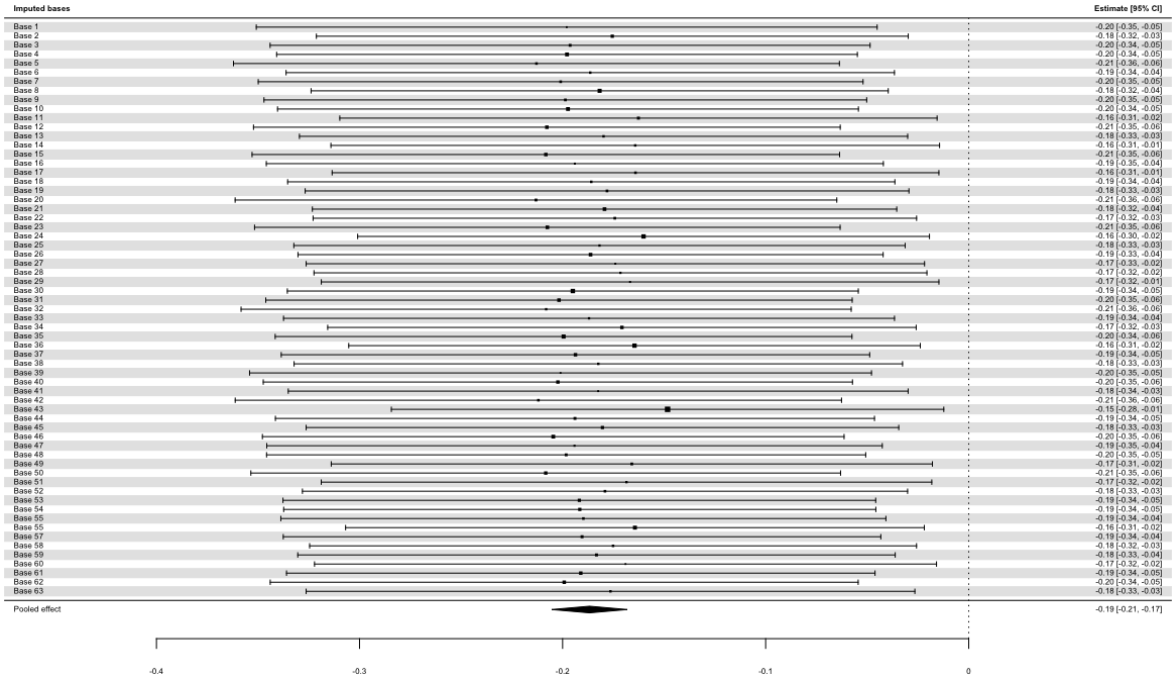
Figure S4. Identification of features as independent predictors of the RALE score trajectory using LASSO regression analysis



(a) The multinomial deviance (y-axis) was plotted versus log(λ) (lower x-axis). The upper x-axis represents the average number of predictors. Dotted vertical lines were drawn at the optimal values using the minimum mean square error and 1 standard error of the minimum criteria. The tuning parameter (λ) was selected in the LASSO model via 10-fold cross-validation based on minimum criteria and was 0.217. (b) LASSO coefficient profiles of the 151 features (y-axis) were plotted against Log Lambda (λ) sequence. One feature with nonzero coefficient was retained.

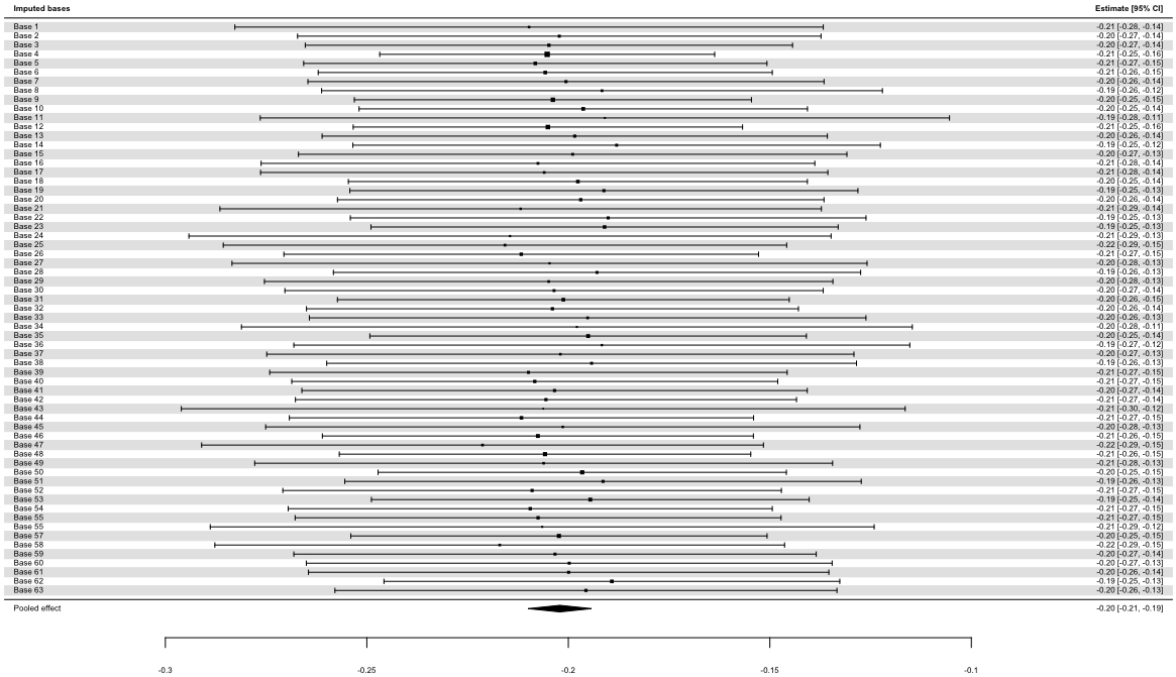
LASSO least absolute shrinkage and selection operator, *RALE* radiographic assessment of lung edema

Figure S5. Forest plot of imputed bases for the direct effect in causal mediation analysis



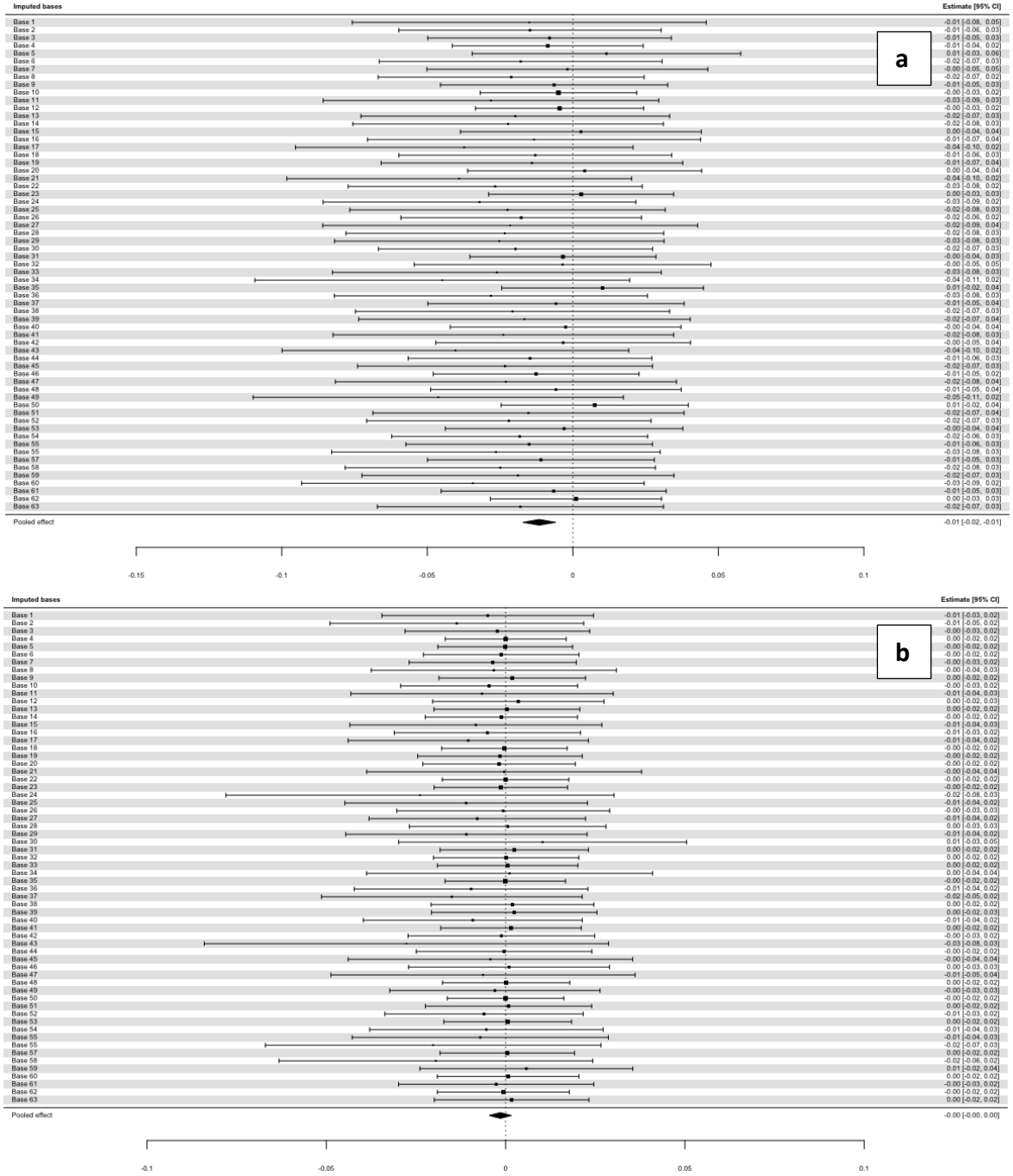
When the line crosses the vertical axis at 0, the estimate is not statistically significant. The diamond shows the summary estimate with the center of the polygon corresponding to the estimate and the left/right edges indicating the confidence interval limits.
CI confidence interval

Figure S6. Forest plot of imputed bases for the total effect in causal mediation analysis



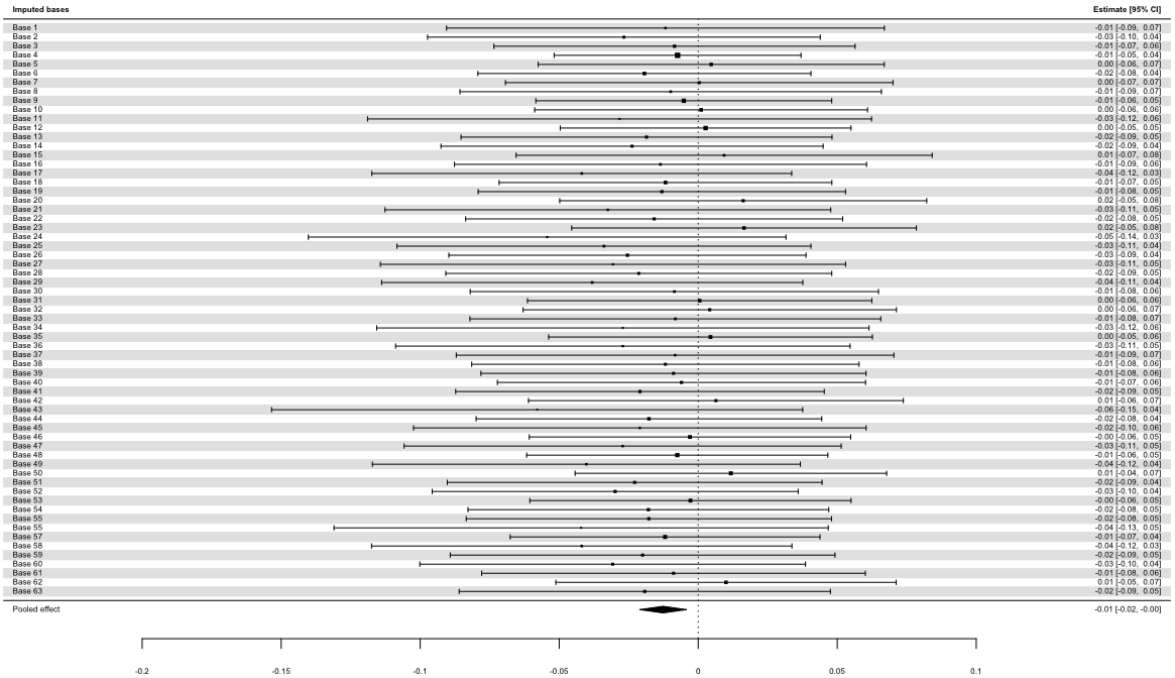
When the line crosses the vertical axis at 0, the estimate is not statistically significant. The diamond shows the summary estimate with the center of the polygon corresponding to the estimate and the left/right edges indicating the confidence interval limits.
CI confidence interval

Figure S7. Forest plot of imputed bases for the indirect effect of the RALE score in causal mediation analysis



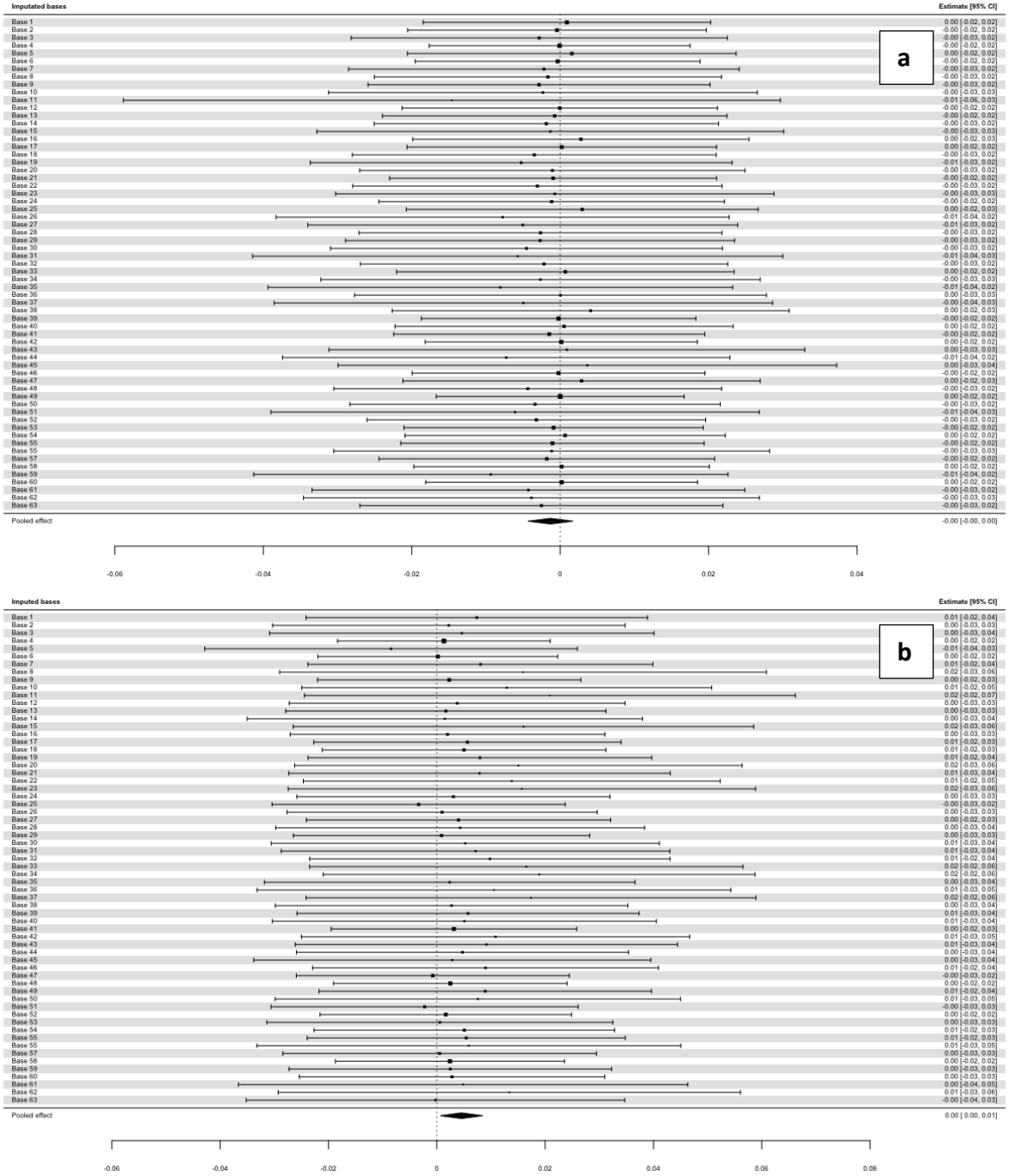
When the line crosses the vertical axis at 0, the estimate is not statistically significant. The diamond shows the summary estimate with the center of the polygon corresponding to the estimate and the left/right edges indicating the confidence interval limits. The first trajectory group was the reference group, the second trajectory group is shown in (a) and the third trajectory group in (b).
CI confidence interval

Figure S8. Forest plot of imputed bases for the indirect effect of plasma sRAGE and the RALE score in the causal mediation analysis



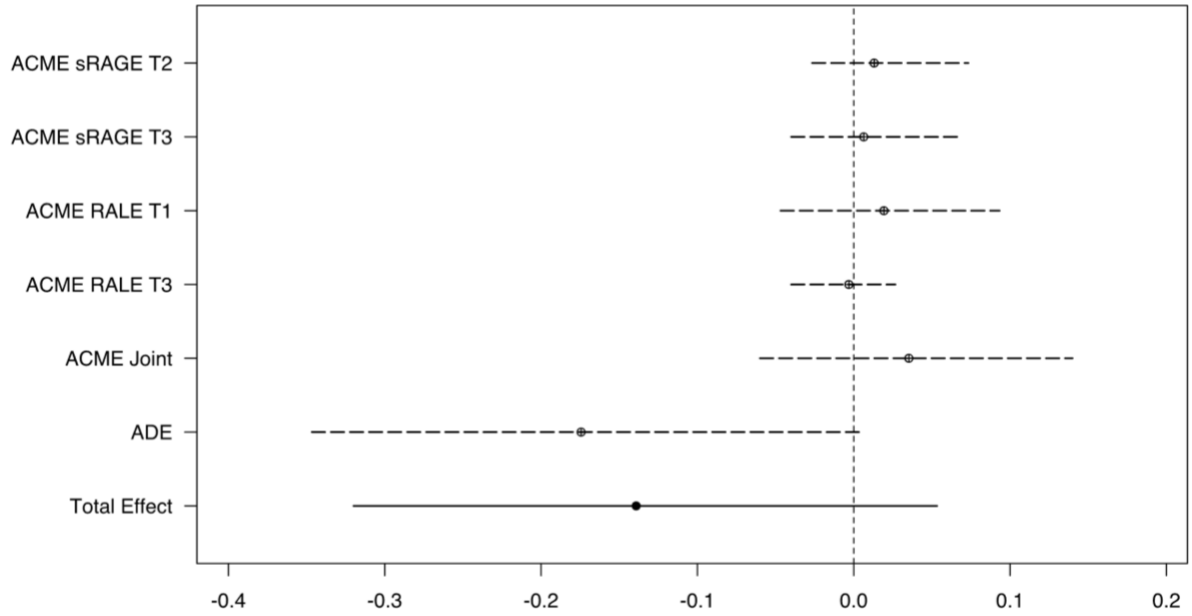
When the line crosses the vertical axis at 0, the estimate is not statistically significant. The diamond shows the summary estimate with the center of the polygon corresponding to the estimate and the left/right edges indicating the confidence interval limits.
CI confidence interval

Figure S9. Forest plot of imputed bases for the indirect effect of plasma sRAGE in causal mediation analysis



When the line crosses the vertical axis at 0, the estimate is not statistically significant. The diamond shows the summary estimate with the center of the polygon corresponding to the estimate and the left/right edges indicating the confidence interval limits. The first trajectory group was the reference group, the second trajectory group is shown in (A) and the third trajectory group in (B).
CI confidence interval

Figure S10. Causal mediation analysis in complete cases (N = 81 patients)



Circles represent estimates and lines 95% confidence intervals. When the line crosses the vertical axis at 0, the estimate is not statistically significant. The first trajectory groups were the reference group.
ACME average causal mediation effect, *ADE* average direct effect, *RALE* radiographic assessment of lung edema, *sRAGE* soluble receptor for advanced glycation end-products, *T* trajectory group