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Evaluation of Plasma Biomarkers to Understand the Biology and Heterogeneity of Treatment Effect in Lower Tidal Volume Ventilation Facilitated by Extracorporeal CO_2 Removal in Acute Hypoxemic Respiratory Failure: A Secondary Analysis of the REST Trial

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OBJECTIVES: In patients with acute hypoxemic respiratory failure (AHRF), the use of lower tidal volume ventilation facilitated by veno-venous extracorporeal CO_2 removal (vv-ECCO$_2$R) does not improve clinical outcomes. The primary objective of this analysis was to evaluate for differences in indices of systemic inflammation and ventilator-induced lung injury between patients treated with lower tidal volume ventilation facilitated by vv-ECCO$_2$R and standard care. Secondary objectives included an evaluation for heterogeneity of treatment effect.

DESIGN: Substudy of a randomized clinical trial.

SETTING: Nine U.K. ICUs.

PATIENTS: Moderate-to-severe AHRF (Pa_{O_2}: F_{IO_2} < 150mmHg [20ka]).

INTERVENTION: Plasma samples obtained at baseline and day 3.

MEASUREMENTS AND MAIN RESULTS: The primary outcome was day 3 C-reactive protein (CRP). Clinical outcomes included 90-day mortality and ventilator-free days (VFD) until day 28. Exploratory analyses included an evaluation of plasma indices of lung injury, inflammation, and heterogeneity of treatment effect (HTE). Seventy-nine patients were enrolled, and 69 patients had paired plasma samples taken at baseline and day 3. There was no difference in day 3 plasma CRP (intervention 138.6 [70.4, 189.4] vs. standard care 113.0 [62.7, 233.8] mg/L; $p = 0.72$). Between baseline and day 3, there was a greater increase in plasma interleukin-18 in patients that received intervention compared with those that received standard care (Δ 337.7 [−128.9, 738.9] vs. 6.4 [−457.2, 6.4] pg/mL $p = 0.05$). In patients with high interleukin-18, allocation to intervention was associated with increased VFDs ($p = 0.03$). Similarly in patients with a hyperinflammatory phenotype, the intervention was independently associated with increased VFDs ($p < 0.01$) and decreased 90-day mortality ($p = 0.01$).

CONCLUSIONS: In patients with moderate-to-severe AHRF, lower tidal volume ventilation, facilitated by vv-ECCO$_2$R, was not associated with a difference in day 3 plasma CRP, but was associated with an increase in plasma interleukin-18 between baseline and day 3. Baseline plasma interleukin-18 and inflammatory phenotypes may identify subgroups of patients with moderate-to-severe AHRF that benefit from lower tidal volume ventilation facilitated by vv-ECCO$_2$R.

TRIAL REGISTRATION: NCT02654327

KEYWORDS: acute hypoxemic respiratory failure; veno-venous; extracorporeal CO_2 removal; heterogeneity of treatment effect; Acute Respiratory Distress Syndrome; phenotypes

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KEY POINTS

Question: In patients with moderate-to-severe acute hypoxemic respiratory failure (AHRF), does lower tidal volume ventilation, facilitated by veno-venous extracorporeal CO_2 removal (vv-ECCO$_2$R), reduce systemic markers of lung injury and inflammation when compared with standard care? Furthermore, is there HTE based on inflammatory phenotypes?

Findings: This was a planned sub-study of the REST trial, which was a multicenter, randomized controlled clinical trial that compared lower tidal volume ventilation, facilitated by vv-ECCO$_2$R (intervention), with standard care. There was no difference in day 3 plasma C-reactive protein between treatment groups, however plasma interleukin-18 had a greater increase between baseline and day 3 in patients that received the intervention than those who received standard care, while there was heterogeneity of treatment effect identified whereby patients with either a high interleukin-18 or a hyperinflammatory phenotype appeared to benefit from the intervention.

Meaning: These findings suggest that, in comparison to standard care, a treatment strategy that includes lower tidal volume ventilation, facilitated by vv-ECCO$_2$R, may have a pro-inflammatory effect in patients with moderate-to-severe AHRF, although there is a heterogenous treatment effect based on baseline plasma interleukin-18 and inflammatory phenotype.

Acute hypoxemic respiratory failure (AHRF) is one of the most common causes for patient admission to the ICU, and up to 67% of these patients will experience the acute respiratory distress syndrome (ARDS) (1, 2). ARDS is associated with significant morbidity, mortality, and healthcare resource burden (3, 4). Patients with AHRF and ARDS are at increased risk of ventilator-induced lung injury (VILI) because of reduced aerated lung volume. This results in the tidal volume being distributed over a smaller lung area, causing excessive alveolar stretch and increased alveolar pressure within the ventilated regions (5). Lung protective ventilation, whereby tidal volumes are limited to 6–8 mL/kg predicted body weight (PBW), has been shown to be associated with less pulmonary and systemic inflammation in patients both with and without ARDS (6–8). However, it is unknown whether further reductions in tidal volume are associated with less pulmonary and systemic inflammation.

The protective ventilation with veno-venous lung assist in respiratory failure (REST) trial evaluated whether lower tidal volume ventilation, targeting tidal volumes of 3 mL/kg PBW and facilitated by veno-venous extracorporeal CO_2 removal (vv-ECCO$_2$R), was associated with reduced mortality when compared with standard care in patients with moderate-to-severe AHRF. The trial was discontinued early because of futility and feasibility, and did not demonstrate a difference in 90-day mortality between the treatment arms (9). Despite these results, it is plausible that lower tidal volume ventilation reduces VILI and systemic inflammation when compared with standard care (10). It has previously been demonstrated that interleukin-6 is reduced with lung protective ventilation (6, 11). C-reactive protein (CRP) is downstream of interleukin-6 activity and is reflective of systemic inflammation (12), and plasma CRP at 48 hr is associated with outcome from ARDS (13). This suggests that CRP can be used as a surrogate biomarker for interleukin-6 activity and VILI.

In contrast to the reduction in inflammation associated with lower tidal volume ventilation, extracorporeal circuits can cause an increase in the plasma concentration of pro-inflammatory cytokines (14, 15) and cause hemolysis (16, 17). Cell-free hemoglobin can act as a mediator of tissue injury in both ARDS and sepsis (18, 19), and it is feasible that both hemolysis and the pro-inflammatory effect of the extracorporeal circuit may offset the potential benefits of lower tidal volume ventilation when facilitated by an extracorporeal circuit.

Heterogeneity in patient populations is likely to affect the results of clinical trials. This concept has been demonstrated in ARDS, where clinical trials that originally showed no treatment effect have been reevaluated based on patient phenotype and heterogeneity of treatment effect (HTE) for the intervention was identified (20–22). Similar effects have been demonstrated when patients are stratified using plasma interleukin-18 (23).

The aim of this study was to evaluate systemic indices of lung injury, inflammation, hemolysis and HTE, in a subgroup of patients recruited to the REST trial (9).

MATERIALS AND METHODS

Study Design

This was a planned substudy of the REST trial (9). In brief, the REST trial was a randomized, controlled clinical trial that was conducted in 51 adult, general ICUs within the United Kingdom. Patients were eligible for enrollment if they were within 48 hr of onset of an acute and potentially reversible cause of hypoxemic respiratory failure (defined as a ratio of the Po_2 in arterial blood to the FiO_2 [PF] ratio < 150 mm Hg [20kPa]) although receiving invasive mechanical ventilation with a positive end-expiratory pressure (PEEP) of at least 5 cm H_2O. In patients randomized to receive intervention, vv-$ECCO_2R$ was commenced via a percutaneous catheter inserted into a central vein. IV heparin was commenced as systemic anticoagulation to prevent circuit thrombosis and sweep gas flow of 10L/min was commenced to maximize CO_2 removal. Tidal volume reduction was performed in decrements, aiming for a tidal volume less than or equal to 3 mL/kg PBW. The intervention was intended to be continued for at least 48 hr, and for a maximum of 7 days. Patients who were randomized to standard care were recommended to receive mechanical ventilation with a tidal volume 6 mL/kg PBW, and PEEP was titrated according to the ARDSNet protocol (24).

This substudy was conducted at nine sites that enrolled patients within the REST trial. Patients were eligible for the substudy if they were enrolled into the REST trial, and additional written, informed consent from patients, or agreement from their surrogates were obtained in keeping with regional regulations. Enrollment into the substudy did not affect the conduct of the REST trial. Following enrollment into the substudy, plasma samples were obtained at baseline and on the third day following randomization and administration of the intervention. Samples were stored at –80°C until analyzed. The protocol for the REST trial has been previously published (25), and the protocol for this substudy is included in the **supplementary appendix** (http://links.lww.com/CCX/B487). Ethical approval for this sub-study was provided alongside the REST trial by research ethics committees in United Kingdom, Wales, Northern Ireland (16/SC/089; approval date September 9, 2016), and Scotland (16/SS/048; approval date November 18, 2016). All procedures were followed in accordance with the ethical approvals and with the Helsinki Declaration of 1975.

Outcome Measures

The primary outcome was plasma CRP at day 3, selected as a downstream biomarker of interleukin-6 activity and therefore reflective of systemic inflammation (12), which was measured in the clinical biochemistry laboratory of the Belfast Health and Social Care Trust. Exploratory outcomes included the change from baseline to day 3 in plasma angiopoietin-2, interleukin-6, -8, -18, soluble receptor for advanced glycation end-products (sRAGE), surfactant protein-D, soluble suppression of tumerogenicity-2, and soluble tumor necrosis factor receptor-1 (sTNFR1). These analytes were measured using enzyme linked immunoassay according to the manufacturer's instructions (R&D systems, Bio-Techne Ltd. United Kingdom) (23, 26). As a metric of hemolysis plasma-free hemoglobin was measured (16) using colorimetric assay according to the manufacturer's instructions (MAK 115, Merck). Clinical outcomes evaluated in this analysis included mortality status at day 28, day 90, and ventilator-free days (VFDs) (defined as the number of days from the time of initiating unassisted breathing to day 28 after randomization).

Statistical Methods

Based on a reduction in systemic biomarkers of inflammation of up to 60% in patients receiving lower tidal volume ventilation compared with higher tidal volume ventilation (6, 10), a minimum sample size of 48 was calculated to provide 80% power to detect a 60% reduction in plasma CRP at day 3 based on a mean value of 135 (±100) mg/L (27). The minimum sample size was increased to 26 patients per group to account for loss to follow-up. Approval was obtained for recruitment of patients above the minimum sample size for the duration of the REST trial. Descriptive statistics included calculation of proportions for categorical variables and median interquartile range for continuous variables. The population of included patients was grouped by randomization allocation generated as part of the REST trial. The statistical difference between these groups was analyzed using chi-square test for categorical variables and Mann-Whitney *U* test for continuous variables. The a priori analysis plan was to evaluate for a difference between baseline and day 3 of indices of lung injury, inflammation and hemolysis

between patients allocated to intervention and to standard care. This was compared between treatment groups using Mann-Whitney *U* test. Kruskal-Wallis test or chi-square test were used to compare patients from the full REST trial cohort based on their inclusion in this substudy. As an exploratory substudy from a large randomized controlled trial, no adjustment was made for multiple comparisons (28). Within a post hoc analysis, the response to both treatment strategies was evaluated in patients with high baseline plateau pressure (defined as a plateau pressure ≥ 28 cm H_2O) (29), patients with high driving pressure (defined as plateau pressure – PEEP ≥ 15 cm H_2O) (30), and in patients with ARDS at study entry. These analyses were performed using JASP v0.18.3 (https://jasp-stats.org/).

Patients were split into phenotypes based upon baseline plasma interleukin-18, dichotomizing the population using a threshold of greater than or equal to 800 pg/mL (high interleukin-18) which has previously been associated with increased risk of mortality in patients with ARDS (23). Patients were also allocated into the hyperinflammatory and hypoinflammatory phenotypes previously described in ARDS using an established parsimonious model incorporating interleukin-8, sTNFR1, bicarbonate, and vasopressor use (31), using a cutoff threshold of greater than or equal to 0.5 probability for allocation to the hyperinflammatory phenotype. Hyper/hypoinflammatory phenotype was allocated irrespective of the presence of ARDS. Within phenotyping schema (interleukin-18 high/low, hypo/hyperinflammatory), statistical differences were analyzed using Fisher exact test for categorical variables and Mann-Whitney *U* test for continuous variables. To test for interactions between treatment and phenotype assignment, logistic regression was used for mortality (at day 28 and day 90) and Poisson regression was used for VFDs at day 28. For multivariate regression, age and acute physiology and chronic health evaluation II were included as biologically relevant covariates known to influence clinical outcome in similar studies (23). No evaluation of model fit for effect was performed. Where multivariate regression demonstrated a significant treatment interaction for phenotype/treatment group pairs, regression was repeated and modeled on continuous data rather than dichotomous phenotype membership (i.e., numerical interleukin-18 concentration and probability of hyperinflammatory phenotype allocation from parsimonious model). Regression model predictions were used to generate plots demonstrating these interactions. All phenotype analyses were post hoc in design and were performed using R Software (version 4.3.3, R Project for Statistical Computing, Vienna, Austria).

RESULTS

Seventy-nine patients from nine ICUs were enrolled in this substudy of the REST trial. Baseline samples were available for 75 patients, and 69 patients with correctly timed and paired baseline and day 3 samples were included in the final analysis (**Fig. 1**).

The baseline characteristics of the 75 patients with baseline samples are presented in **Table 1**. Baseline driving pressure was lower in patients allocated to intervention (15.0 [13.0, 18.0] vs. 18.0 [14.0, 21.5] cm H_2O; $p = 0.04$), whereas the baseline ratio of PF ratio was higher in patients allocated to intervention (170 [144, 207] vs. 145 [124, 163] kPa; $p = 0.003$). Patients in the substudy received lower PEEP and had a higher baseline PF ratio than patients who were recruited to the REST trial but were not included in this sub-study (**Table S1**, http://links.lww.com/CCX/B487).

Effect of Lower Tidal Volume Ventilation, Facilitated by vv-ECCO$_2$R, on Plasma Indices of Inflammation and Lung Injury

Baseline indices of inflammation and lung injury were similar between both groups in patients with paired samples ($n = 69$). There was no difference in day 3 CRP between intervention and standard care (138.6 [70.4, 189.4] vs. 113.0 [62.7, 233.8] mg/L; $p = 0.72$). Similarly, there were no differences in other day 3 indices of lung injury and inflammation. However, patients allocated to intervention had a greater increase in plasma interleukin-18 between baseline and day 3 than patients allocated to standard care (Δ 337.7 [–128.9, 738.9]] vs. 6.4 [–457.2, 6.4] pg/mL; $p = 0.05$) (**Table 2**). The effect of vv-ECCO2R and lower tidal volume ventilation in clinical phenotypes of AHRF is reported in the **supplementary file** (http://links.lww.com/CCX/B487).

Exploratory Analysis to Evaluate the Effect of Interleukin-18 Phenotype on Treatment Effect

The baseline characteristics and outcome data from the 75 patients with baseline samples, stratified by

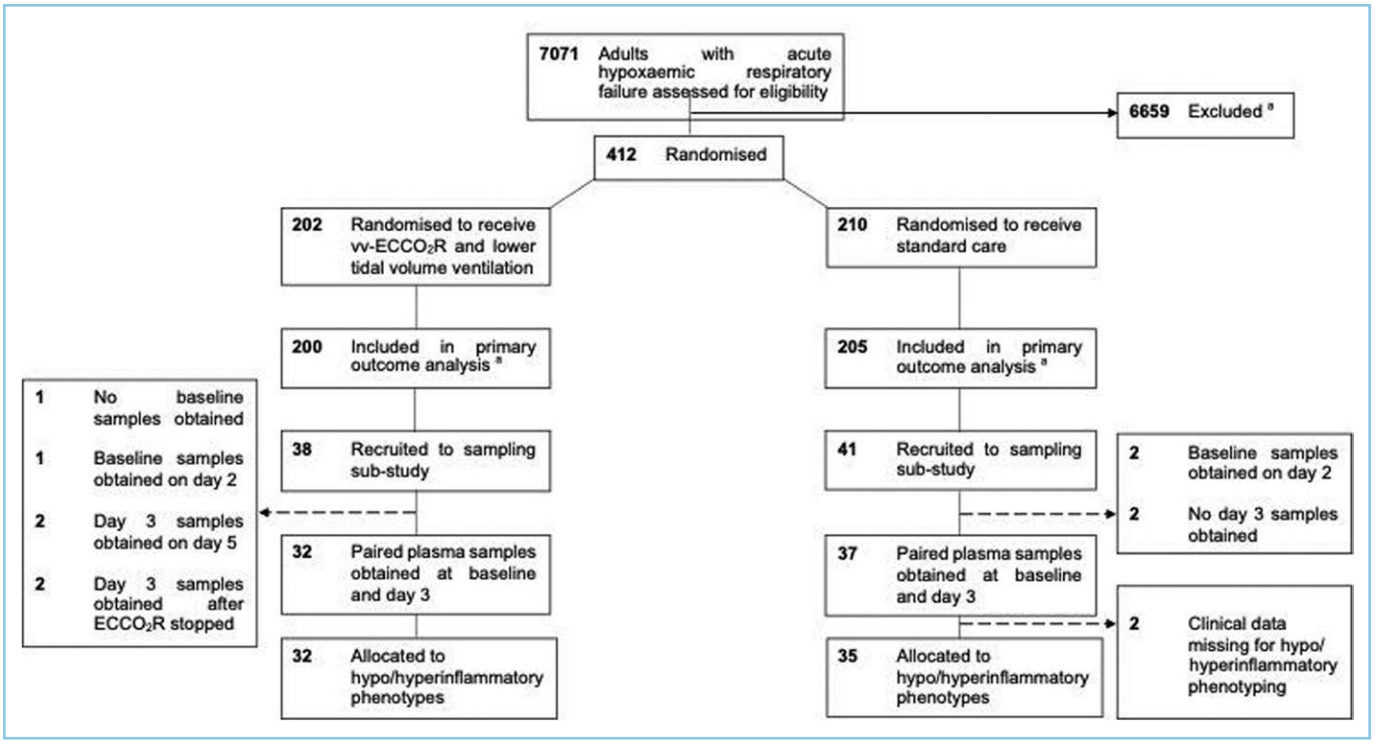

Figure 1. Flow of patients in the REST trial and REST trial sub-study.

interleukin-18 phenotype, are presented in **Table S5** (http://links.lww.com/CCX/B487). Of these, 35 (46.7%) patients had low baseline plasma interleukin-18 (< 800 pg/mL) and 43 (57.3%) had high baseline plasma interleukin-18 (≥ 800 pg/mL), categorized using previously established thresholds (23). There was no difference between patients with high interleukin-18 and low interleukin-18 in 28-day (high interleukin-18: 39% vs. low interleukin-18: 28%, p = 0.50) or 90-day mortality (41% vs. 28%, p = 0.30), nor VFDs (4 [0, 19] vs. 10 [0, 18], p = 0.30) (Table S5, http://links.lww.com/CCX/B487).

Among the 69 patients with paired samples at baseline and day 3, patients with high interleukin-18 demonstrated a significant reduction in plasma sRAGE between baseline and day 3 (Δ −99 [−235, −11] vs. 0 [−63, 65] pg/mL; $p < 0.01$) (**Table S6**, http://links.lww.com/CCX/B487). Patients with high baseline interleukin-18 had a modest reduction in plasma interleukin-18 by day 3, whereas those with low interleukin-18 at baseline had an increase (Δ −39 [−1,097, 358] vs. 242 [−6.0, 552] pg/mL; p = 0.04) (Table S6, http://links.lww.com/CCX/B487). Within patients allocated to standard care, those with high baseline interleukin-18 demonstrated a greater reduction in sRAGE (Δ −104 [−255.8, −36.4] vs. 0 [−47.6, 94.1] pg/mL; p = 0.005) and interleukin-18 (Δ −400.2 [−1096.9, 223.5] vs. 217.1 [−52.5, 296.0] pg/mL; p = 0.02) than patients with low baseline interleukin-18 (**Table S7**, http://links.lww.com/CCX/B487).

There was no difference in 28- or 90-day mortality, or VFDs, between intervention and standard care when patients were grouped by baseline high/low interleukin-18 (**Table S8a–c**, http://links.lww.com/CCX/B487). Logistic regression showed no treatment interaction with the high interleukin-18 phenotype for either 28- or 90-day mortality. In multivariate Poisson regression, a significant treatment interaction for VFDs was observed (p = 0.03) (**Table S9**, http://links.lww.com/CCX/B487). When interleukin-18 was modeled as a continuous variable in multivariate Poisson regression there was also a significant treatment interaction ($p < 0.01$) (**Fig. 2**).

Exploratory Analysis to Evaluate for Hypo/Hyperinflammatory Phenotype on Treatment Effect

Of the 75 patients with baseline samples, two patients in the standard of care group could not be allocated to the hypoinflammatory and hyperinflammatory

TABLE 1.
Baseline Characteristics of Included Patients

Characteristic	Intervention $n = 36$	Standard Care $n = 39$	p
Age (yr)	59 (48, 67)	64 (50, 70)	0.57
Male	24 (66.7)	24 (61.5)	0.61
Dependency prior to hospital, *n* (%)			0.55
Able to live without assistance	29 (80.6)	34 (87.2)	
Major assistance	0	1 (2.6)	
Minor assistance	5 (13.9)	3 (7.7)	
Acute Physiology and Chronic Health Evaluation II score	18 (14, 23)	20 (17, 23)	0.31
Sequential Organ Failure Assessment score[a]	9 (7, 11)	9 (7, 12)	0.88
Presence of acute respiratory disease syndrome, *n* (%)[b]	15 (41.7)	22 (56.4)	0.20
ICU admission diagnostic criteria, *n* (%)[c]			
Respiratory	26 (72.2)	26 (66.7)	0.60
Cardiovascular	7 (19.4)	11 (28.2)	0.38
Sepsis	8 (22.2)	10 (25.6)	0.73
Gastrointestinal/hepatology	8 (22.2)	4 (10.3)	0.16
Renal	6 (16.7)	4 (10.3)	0.42
Toxicology	4 (11.1)	2 (5.1)	0.34
Other	6 (16.7)	8 (22.2)	0.67
Mode of ventilation, *n* (%)			0.55
Mandatory	27 (75.0)	29 (74.4)	
Mixed	7 (19.4)	7 (17.9)	
Spontaneous	1 (2.8)	0	
Other	1 (2.8)	2 (5.1)	
Mechanical ventilation data			
Tidal volume (mL/kg predicted body weight)[d]	6.4 (5.8, 8.1)	6.5 (5.9, 7.5)	0.76
Respiratory rate (breaths/min)[e]	22.0 (19.8, 24.3)	24.0 (20.0, 26.8)	0.22
Positive end-expiratory pressure (cm H_2O)[e]	10.0 (8.0, 10.0)	10.0 (7.3, 10.0)	0.55
Plateau pressure (cm H_2O)[f]	25 (22.0, 27.5)	27 (23.5, 31.0)	0.09
Driving pressure (cm H_2O)[f]	15.0 (13.0, 18.0)	18.0 (14.0, 21.5)	0.04
Pao_2 of arterial oxygen to Fio_2 ratio (closest to randomization) (mm Hg)	170 (144, 207)	145 (124, 163)	0.003
Adjunctive therapies, *n* (%)			
Neuromuscular blockade	15 (41.7)	21 (53.8)	0.24
Prone positioning	3 (8.3)	5 (12.8)	0.50
Inhaled nitric oxide	2 (5.6)	3 (7.7)	0.58

Data presented as median (interquartile range) unless otherwise stated.
[a]Data missing in $n = 9$ (intervention $n = 2$; standard care $n = 7$);
[b]Data missing in $n = 2$ (intervention = 1; standard care = 1);
[c]Patients may have had more than one admission diagnostic criteria;
[d]Data missing in $n = 2$ (standard care);
[e]Data missing in $n = 1$ (standard care); and
[f]Data missing in $n = 13$ (intervention = 1; standard care = 12).

TABLE 2.
Change in Plasma Indices of Inflammation and Lung Injury Between Baseline and Day 3

	Intervention ($n = 32$)		Standard Care ($n = 37$)		
Plasma Index	**Baseline**	**Day 3**	**Baseline**	**Day 3**	***p*[a]**
C-reactive protein (mg/L)	213.7 (41.9, 339.1)	138.6 (70.4, 189.4)	204.6 (122.6, 320.4)	113.0 (62.7, 233.8)	0.88
Surfactant protein-D (ng/mL)	19.0 (5.8, 38.8)	35.4 (9.4, 57.6)	17.9 (6.7, 62.6)	32.0 (11.9, 62.0)	0.35
Soluble receptor for advanced glycation end-products (pg/mL)	298.4 (190.9, 520.8)	196.9 (114.3, 374.0)	200.6 (138.6, 400.5)	177.8 (93.1, 294.7)	0.53
Angoiopoietin-2 (ng/mL)	6.5 (2.6, 8.9)	2.3 (1.3, 4.4)	4.4 (1.9, 8.6)	2.5 (1.4, 4.6)	0.72
Soluble suppression of tumerogenicity-2 (ng/mL)	262.6 (176.9, 686.4)	286.4 (147.8, 624.9)	403.7 (176.9, 686.4)	404.6 (222.5, 839.0)	0.61
Interleukin-6 (pg/mL)	52.6 (18.6, 116.7)	42.5 (14.8, 133.9)	57.8 (9.4, 189.2)	26.5 (13.9, 67.5)	0.28
Interleukin-8 (pg/mL)	32.3 (32.3, 65.6)	44.3 (14.8, 133.9)	32.3 (32.3, 69.8)	32.3 (32.3, 54.6)	0.11
Interleukin-18 (pg/mL)	1023.9 (536.9, 1770.0)	1236.0 (494.0, 2065.6)	880.6 (447.8, 1765.3)	826.3 (535.4, 1234.3)	0.05
Soluble tumor necrosis factor receptor-1 (ng/mL)	3.8 (2.3, 8.9)	4.2 (2.8, 9.7_	213.7 (141.9, 339.1)	4.4 (2.2, 8.0)	0.55

Data presented as median (interquartile range) and analyzed using Mann-Whitney *U* test.
[a]*p* values represent the between group comparison for Δ between baseline and day 3.

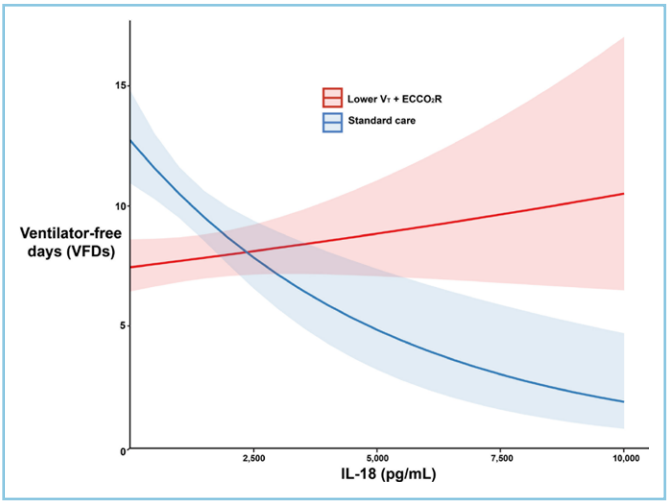

Figure 2. Predicted ventilator-free days by treatment group and plasma interleukin-18 concentration. As interleukin-18 increases, multivariate Poisson regression predicts increasing (improved) ventilator-free days (VFDs) in the treatment group (lower tidal volume + extracorporeal CO_2 removal). Conversely, VFDs decrease (disimprove) in the standard care group (*p* for interaction < 0.01). *Shaded areas* represent 95% CIs. $ECCO_2R$ = extracorporeal CO2 removal, V_T = tidal volume.

phenotypes because of missing clinical data (plasma bicarbonate and vasopressor use) (Fig. 1). Baseline characteristics and outcome data for the 73 patients included in this analysis are presented in **Table S11** (http://links.lww.com/CCX/B487); 54 (74.0%) patients were classified as hypoinflammatory and 19 (26.0%) patients were classified as hyperinflammatory. Between hyper/hypoinflammatory phenotypes there was no difference in clinical outcomes (Table S11, http://links.lww.com/CCX/B487). Similarly, within the hyper/hypoinflammatory phenotypes, there was no difference in 28- or 90-day mortality, nor VFDs, between patients allocated to intervention or standard care (Tables S9c, http://links.lww.com/CCX/B487).

Patients classified as hyperinflammatory had a greater reduction in sRAGE between baseline and day 3 than patients with a hypoinflammatory phenotype (Δ −126 [−429, −26] vs. −32 [−97, 34]; $p = 0.01$) (**Table S11**, http://links.lww.com/CCX/B487). In patients with the hypoinflammatory phenotype, there was a greater increase in plasma interleukin-18

between baseline and day 3 in patients allocated to intervention (Δ 277.5 [–7.0, 634.5] vs. 10.2 [–302.8, 260.2] pg/mL; $p = 0.05$) than those patients allocated to standard care (**Table S12**, http://links.lww.com/CCX/B487). Furthermore, within patients allocated to intervention, patients characterized as hyperinflammatory had a reduction in sTNFR1 (Δ –1.4 [-6.7, 1.0] vs. 0.8 [–0.5, 2.3] ng/mL; $p = 0.05$), and a greater reduction in CRP (Δ –125.0 [–173.1, –94.6] vs. –55.6 [–123.9, 44.5] mg/ L; $p = 0.05$) than patients with a hypoinflammatory phenotype. In patients allocated to standard care, those with a hyperinflammatory phenotype had a greater reduction in sRAGE than patients classified as hypoinflammatory between baseline and day 3 (Δ –236.0 [–784.2, –110.5] vs. –16.7 [–92.2, 50.2] pg/mL; $p = 0.03$) (**Table S13**, http://links.lww.com/CCX/B487). In multivariable regression, there was a significant treatment interaction for hypo/hyperinflammatory phenotype for mortality at day 28 (p for interaction = 0.02) and day 90 mortality (p for interaction = 0.01) (**Table S14**, http://links.lww.com/CCX/B487). Furthermore, there was also a significant treatment interaction for hypo/hyperinflammatory phenotype for VFDs (p for interaction < 0.01).

When probability of assignment to either hypo/hyperinflammatory phenotype was modeled on a continuous scale (31), there was a significant treatment interaction for both 90-day mortality (p for interaction = 0.05) (**Fig. 3**) and VFDs (p for interaction < 0.01) (**Fig. 4**).

Evaluation of Hemolysis Between Treatment Groups

There was no difference in baseline plasma-free hemoglobin between patients allocated to lower tidal volume ventilation facilitated by vv-ECCO$_2$R or standard care (42.5 [35.9] vs. 39.2 [33.7] mg/dL; $p = 0.80$). Furthermore, treatment strategy did not affect the change in plasma-free hemoglobin from baseline to day 3 (intervention 0.15 [20.9] vs. standard care –1.02 [22.2] mg/dL; $p = 0.75$).

DISCUSSION

In patients with moderate-to-severe AHRF, the use of lower tidal volume ventilation, facilitated by vv-ECCO$_2$R, did not result in significant changes in day

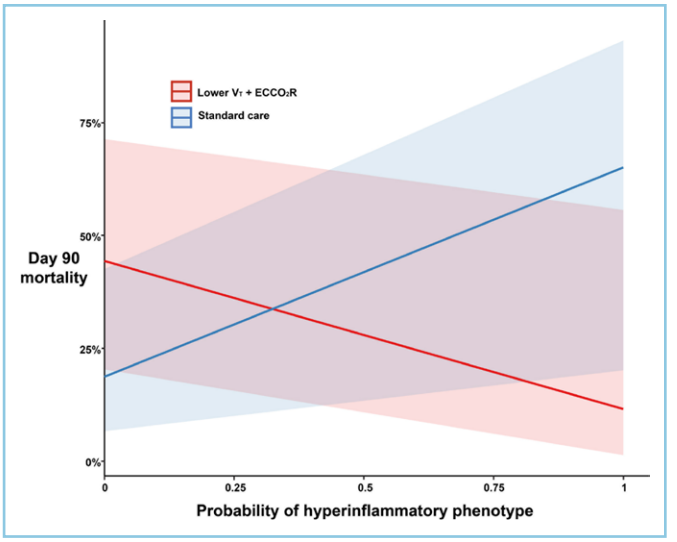

Figure 3. Predicted 90-day mortality by treatment group and strength of hypo/hyperinflammatory phenotype membership. As the strength of hyperinflammatory class membership increases (probability tends toward 1), multivariate logistic regression predicts decreased 90-day mortality in the treatment group (lower tidal volume + extracorporeal CO_2 removal [ECCO$_2$R]). Conversely, mortality increases with increased strength of hyperinflammatory class membership in the standard care group (p for interaction = 0.05). *Shaded areas* represent 95% CIs. V_T = tidal volume.

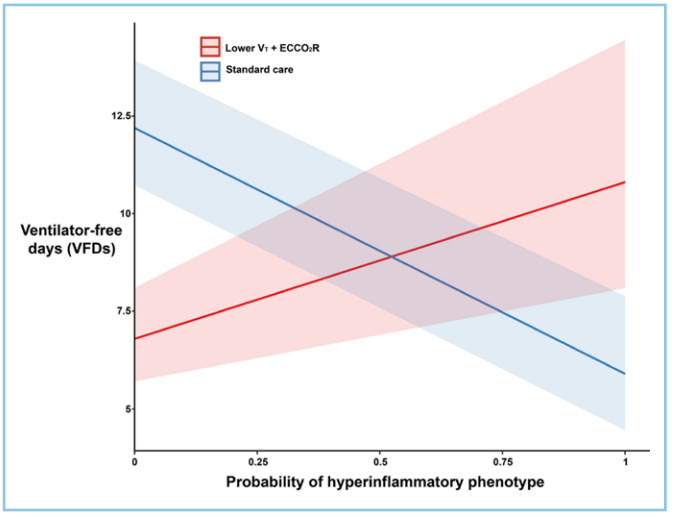

Figure 4. Predicted ventilator-free days by treatment group and strength of hypo/hyperinflammatory phenotype membership. As the strength of hyperinflammatory class membership increases (probability tends toward 1), multivariate logistic regression predicts increased (improved) ventilator-free days (VFDs) in the treatment group (lower tidal volume + extracorporeal CO_2 removal [ECCO$_2$R]). Conversely, VFDs decrease (disimprove) with increased strength of hyperinflammatory class membership in the standard care group (p for interaction < 0.01). *Shaded areas* represent 95% CIs. V_T = tidal volume.

3 plasma CRP compared to standard care. However, patients receiving the intervention showed an increase in plasma interleukin-18 levels from baseline to day 3. Furthermore, in an evaluation for heterogeneity of treatment, the findings in this study suggest that subgroups with high interleukin-18 and a hyperinflammatory phenotype may respond better to lower tidal volume ventilation.

The temporal increase in plasma interleukin-18 in patients allocated to intervention, who were less hypoxemic at baseline, offers a plausible mechanism to explain the results of the REST trial, with previous data demonstrating that a temporal increase in plasma interleukin-18 is associated with increased mortality in patients with ARDS (32). Interleukin-18 is a downstream product of inflammasome activation (33) that itself has broad proinflammatory effects that include increasing interferon-γ production (34). Although it is plausible that the increase in plasma interleukin-18 reflects extracorporeal circuit-induced inflammasome activation, this would be in contrast to the effect observed with other extracorporeal devices (35, 36). When patients in this analysis were phenotyped by baseline plasma interleukin-18 (high interleukin-18 ≥ 800 pg/mL, low interleukin-18 < 800 pg/mL), those with high interleukin-18 at baseline, had numerically greater 28-day mortality, 90-day mortality, and fewer VFDs. Although these results did not reach statistical significance, they align with previous studies (23, 32). In multivariable Poisson regression analysis (Fig. 2), allocation to intervention was associated with an increase in VFDs in patients with higher baseline interleukin-18, whereas in patients allocated to standard care higher interleukin-18 was associated with lower VFDs. This suggests that patients with higher baseline inflammasome activation may benefit more from the intervention strategy, although the interleukin-18 threshold for benefit appears to be much higher than 800 pg/mL previously used to phenotype patients, although there was no observed reduction in indices of inflammation or lung injury in this group. This implies only a small subset of patients might have experienced positive effects. Furthermore, patients with high interleukin-18 had higher baseline plasma sRAGE, which has previously been shown to be associated with increased mortality, and to undergo greater temporal reduction in patients receiving lower tidal volume ventilation (7). In contrast, the results in the subgroup of patients with high interleukin-18 in this study suggest that patients have a greater reduction in sRAGE when receiving standard care. The mechanisms underpinning this are uncertain, although it may reflect that the use of vv-$ECCO_2R$ was proinflammatory, and that this effect offset the benefits from lower tidal volume ventilation.

When categorized by inflammatory phenotype, the majority of patients were classified as hypoinflammatory (74%), consistent with previous studies of patients with ARDS. At baseline, patients with a hyperinflammatory exhibited higher tidal volumes, plateau and driving pressures, which differs from previous studies. No significant differences in mortality were seen between the hypoinflammatory and hyperinflammatory groups. However, multivariate analysis revealed HTE, with hyperinflammatory patients appearing to benefit more from lower tidal volume ventilation facilitated by vv-$ECCO_2R$, whereas patients with a hypoinflammatory phenotype had improved outcomes with standard care. This difference may be because of reduced VILI in the hyperinflammatory group, while the hypoinflammatory group might have been negatively affected by the intervention. However, although patients with a hyperinflammatory phenotype had a temporal reduction in plasma sTNFR1, and greater reductions in CRP when allocated to intervention, they also had a greater reduction in sRAGE when allocated to standard care. These results may reflect type 1/2 errors given the small number of patients in these subgroup analyses. However, they also offer support the hypothesis that the use of lower tidal volume ventilation with vv-$ECCO_2R$ in patients with higher baseline inflammation may have an effect that modulates the reduction in type 1 alveolar epithelial cell injury that sRAGE represents (7). Future studies using extracorporeal devices to modify mechanical ventilation should consider evaluation for HTE by inflammatory phenotype.

There are important limitations to consider when evaluating this study. The small sample size in this substudy is an important limitation when interpreting the findings with regards to HTE. Although there was a broader range of biological indices evaluated, in a greater number of patients than previously reported (10), there remain a limited number of patients from which to evaluate the effect of lower tidal volume ventilation compared with standard care. This limits the generalizability of these findings. Plasma CRP was

selected as the primary outcome for this study because it is downstream of interleukin-6 signaling (12) and is therefore reflective of systemic inflammation, whereas plasma CRP at 48 hr is associated with outcome from ARDS (13). Lower tidal volume ventilation is associated with temporal reductions in plasma cytokines (including interleukin-6, interleukin-8, and sTNFR1) (6, 11), and these changes may reflect a reduction VILI. The inflammatory response to extracorporeal circuits is complex and includes proinflammatory effects that modulate coagulation, endothelial cell, and leukocyte function (37). Although plasma interleukin-6 has been shown to decrease by 48 hr in patients receiving lower tidal volume ventilation facilitated by $ECCO_2R$ (10), it is plausible that plasma CRP is not a reliable index of VILI in the context of extracorporeal circuits because of their proinflammatory effects.

In summary, lower tidal volume ventilation facilitated by vv-ECCO2R was associated with an increase in plasma interleukin-18, and differential treatment responses were observed based on interleukin-18 levels and inflammatory phenotypes. These results provide a potential explanation for the lack of overall clinical benefit observed in the REST trial.

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