Distinct profiles of host responses between plasma and lower respiratory tract during acute respiratory failure

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To the Editor:

Subphenotypes derived from plasma levels of host response biomarkers can predict patient-centred outcomes during acute respiratory failure (ARF) [1, 2]. However, it is not well studied whether plasma-defined ARF subphenotypes reflect biological processes in the lungs or whether they capture extrapulmonary processes [3]. Limited evidence suggests that plasma-derived subphenotypes may not represent lower respiratory tract (LRT) processes [4]. Given the implications of subphenotyping for future precision medicine approaches [3], there is an urgent need to define whether blood-based stratification captures LRT heterogeneity.

We prospectively investigated 207 patients with ARF, either from non-coronavirus disease 2019 (COVID-19) aetiologies (n=126) or COVID-19 pneumonia (n=81), receiving invasive mechanical ventilation in UPMC Presbyterian/Shadyside intensive care units. Following informed consent, we simultaneously collected plasma and endotracheal aspirate (ETA) samples with a standardised protocol [5] at a median of 1 and 4 days post-intubation for non-COVID and COVID-19 subjects, respectively. For non-COVID ETA samples, we performed a two-fold dilution with Sputasol (Thermo Scientific), followed by centrifugation (375×*g* for 5 min) and mixing supernatant with PBS to a final 20-fold dilution. Due to biosafety regulations, we inactivated COVID-19 samples with four-fold dilution in DNA/RNA Shield (Zymo Research), followed by centrifugation and mixing supernatant with PBS to a final 20-fold dilution. In plasma and diluted supernatants, we measured 10 host response biomarkers with a custom Luminex assay (R&D Systems) [1, 6]. In ETA supernatants, we used Pierce bicinchoninic acid assay to quantify total protein concentration and a colorimetric assay for urea (Abcam). We analysed raw ETA biomarker values, as well as normalised values by total protein or urea concentration in each sample to account for variable dilution in sample acquisition. We classified patients into a hyper- *versus* hypo-inflammatory subphenotype with a parsimonious logistic regression model based on plasma levels of angiopoietin (Ang)-2, procalcitonin, soluble tumour necrosis factor receptor (sTNFR)1 and bicarbonate [1]. In sensitivity analyses, we examined subphenotypic classifications by two other published models [7, 8].

We first analysed data from 126 patients with (non-COVID) ARF of different clinical categories: 1) acute respiratory distress syndrome (ARDS; n=30), at-risk for ARDS (n=54; 66.7% due to pneumonia), congestive heart failure (CHF; n=9) or patients intubated for airway protection (airway controls; n=33). ARF subjects had similar age and sex distributions in the four clinical categories (median age 57.3 years; 60.3% men), but significant differences in 30-day mortality (ARDS 20.0%, at-risk for ARDS 18.5%, CHF 11.1% and airway controls 9.1%). Most ARF patients (88.1%) received volume-controlled breaths with similar tidal volumes between clinical categories (median 6.7 $mL \cdot kg^{-1}$ ideal body weight), but patients with ARDS received higher positive end-expiratory pressures (PEEP; median 10 cm) compared to the other categories (median 5 cm, $p<0.01$). In pairwise comparisons, ARDS patients had significantly higher plasma levels of all 10 biomarkers compared to airway controls ($p<0.01$). However, only four ETA biomarkers (soluble receptor of advanced glycation end-products (sRAGE) (figure 1a), soluble suppressor of tumorigenicity (sST)2, procalcitonin and fractalkine (all $p<0.01$)) were higher in ARDS patients compared to airway controls. For respiratory mechanics end-points, sRAGE and sST2 were the only ETA biomarkers significantly correlated with peak inspiratory pressures, whereas ETA sRAGE was also significantly correlated with PEEP (all $p<0.05$ adjusted for multiple comparisons) among all ARF patients.

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Current plasma-based subphenotyping approaches in acute respiratory failure represent host responses at a systemic level but do not capture important differences in lower respiratory tract biology https://bit.ly/40kTdDG

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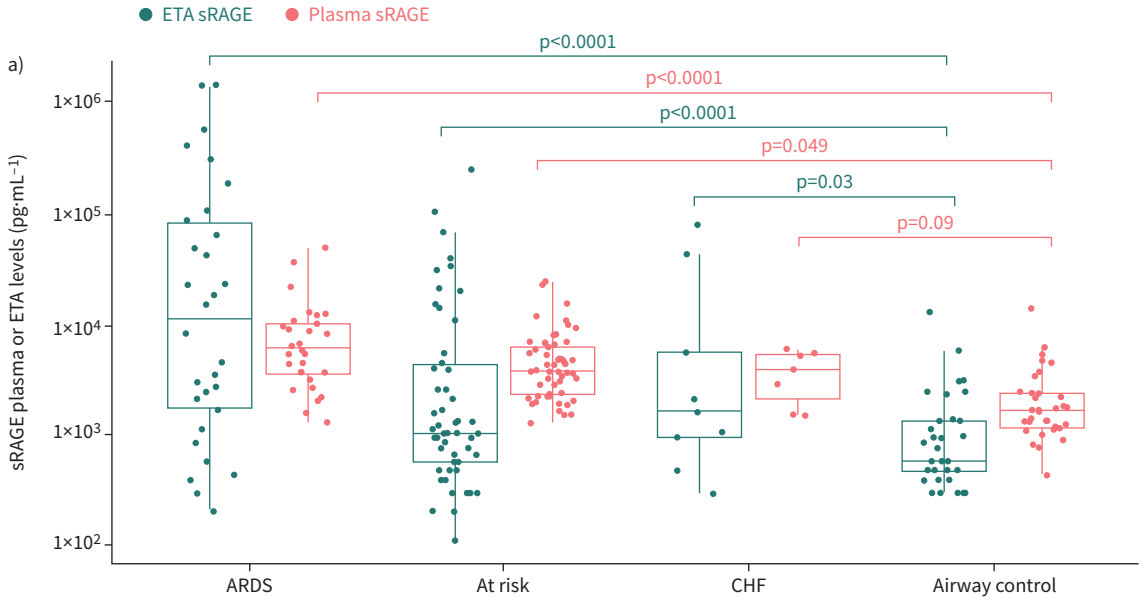
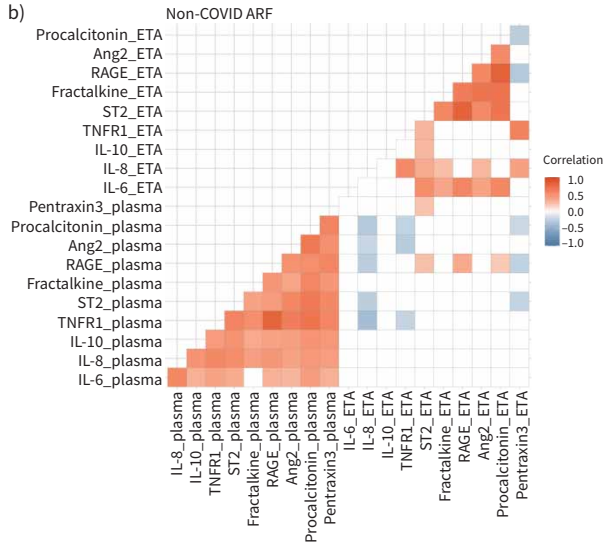
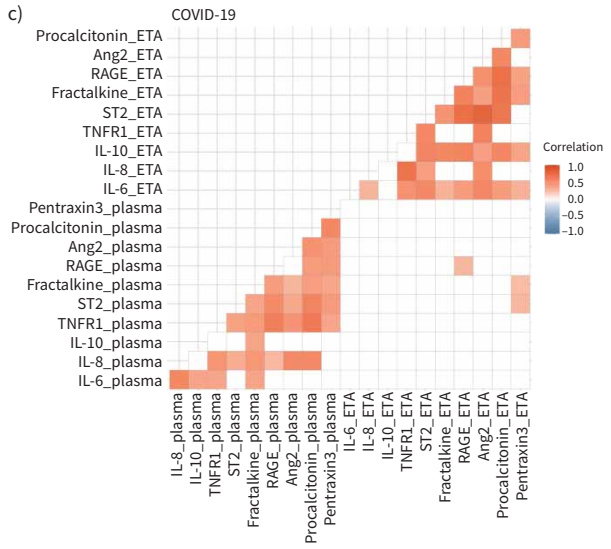

a)
ETA sRAGE
Plasma sRAGE
p<0.0001
p<0.0001
p<0.0001
p=0.049
p=0.03
p=0.09
sRAGE plasma or ETA levels (pg·mL⁻¹)
1×10⁶
1×10⁵
1×10⁴
1×10³
1×10²
ARDS
At risk
CHF
Airway control


b)
Non-COVID ARF
Procalcitonin_ETA
Ang2_ETA
RAGE_ETA
Fractalkine_ETA
ST2_ETA
TNFR1_ETA
IL-10_ETA
IL-8_ETA
IL-6_ETA
Pentraxin3_plasma
Procalcitonin_plasma
Ang2_plasma
RAGE_plasma
Fractalkine_plasma
ST2_plasma
TNFR1_plasma
IL-10_plasma
IL-8_plasma
IL-6_plasma
Correlation
1.0
0.5
0.0
−0.5
−1.0


c)
COVID-19
Procalcitonin_ETA
Ang2_ETA
RAGE_ETA
Fractalkine_ETA
ST2_ETA
TNFR1_ETA
IL-10_ETA
IL-8_ETA
IL-6_ETA
Pentraxin3_plasma
Procalcitonin_plasma
Ang2_plasma
RAGE_plasma
Fractalkine_plasma
ST2_plasma
TNFR1_plasma
IL-10_plasma
IL-8_plasma
IL-6_plasma
Correlation
1.0
0.5
0.0
−0.5
−1.0

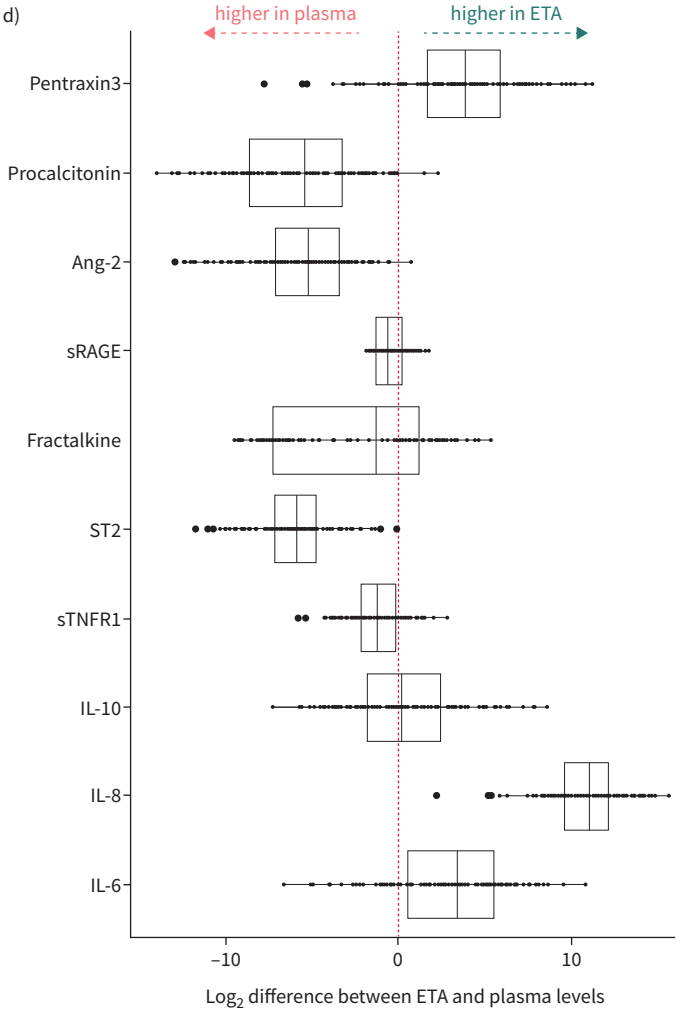

d)
higher in plasma
higher in ETA
Pentraxin3
Procalcitonin
Ang-2
sRAGE
Fractalkine
ST2
sTNFR1
IL-10
IL-8
IL-6
−10
0
10
Log2 difference between ETA and plasma levels

FIGURE 1 Lower respiratory tract biomarker levels discriminate types of acute respiratory failure (ARF) and constitute distinct host-response profiles compared to plasma biomarkers. a) Plasma and endotracheal aspirate (ETA) soluble receptor for advanced glycation end-products (sRAGE) discriminate ARF clinical categories (acute respiratory distress syndrome (ARDS), at-risk for ARDS, congestive heart failure (CHF) or airway controls (patients intubated for airway protection)). CHF and airway controls were included as a control group with expected low levels of lower respiratory tract inflammation compared to subjects with lung injury (ARDS and at-risk for ARDS). b and c) Depicted correlograms represent pairwise correlations of plasma and ETA biomarkers (raw) with Pearson's correlation test, adjusted for multiple comparisons with the Benjamini–Hochberg method. Significant correlations (positive in red and negative in blue) are shown. Results were similar when we analysed total protein and urea-normalised ETA biomarker values. d) Comparison of $\log_2$ differences in ETA-plasma biomarker ratios (estimated by the following equation: biomarker ratio=$\log_2$(biomarker_ETA/urea_ETA)/(biomarker_plasma/urea_plasma)). Pentraxin-3, interleukin (IL)-8 and IL-6 were significantly enriched in ETA samples, whereas procalcitonin, angiotensin (Ang)-2 and soluble suppressor of tumorigenicity (sST)2 were enriched in plasma samples. We did not obtain analysable urea values in coronavirus disease 2019 (COVID-19) subjects due to interference of the DNA/RNA Shield solution with the colorimetric urea assays. sTNFR: soluble tumour necrosis factor receptor.

Clinical diagnosis of pneumonia (43%) was not associated with differential distribution of ETA biomarkers, whereas among subjects with available LRT specimen cultures within 48 h of the ETA sample (71%), patients with any organismal growth in cultures (69%) had higher ETA levels of sTNFR1 and interleukin (IL)-8 ($p=0.02$ and $p=0.05$, respectively), but lower levels of sRAGE ($p=0.02$) compared to patients with no growth in LRT cultures.

We then evaluated plasma-based subphenotypes in non-COVID ARF subjects and compared clinical and biomarker variables by subphenotype. Hyperinflammatory patients (25 (21%) out of 119 subjects with available data for subphenotype assignments) had higher temperature, lower haemoglobin, higher creatinine, lower pH and higher incidence of shock compared to hypoinflammatory patients (all $p<0.05$), but no difference in respiratory mechanics (peak and plateau pressures) or gas exchange (arterial oxygen tension/inspiratory oxygen fraction and ventilatory ratios). Similarly, we found that hyperinflammatory subjects had higher plasma levels for all seven biomarkers not included in the parsimonious predictive model (*i.e.* IL-6, IL-8, IL-10, sST2, fractalkine, sRAGE and pentraxin-3; all $p<0.05$), but no significant differences for ETA biomarkers. Therefore, we found no significant differences in LRT clinical or biomarker variables when comparing inflammatory phenotypes.

We then focused on the relationships between the systemic and respiratory biomarkers to determine intra- and inter-compartment associations by examining pairwise correlations for plasma and ETA biomarker levels. In these comparisons, we found stronger intra-compartment compared to inter-compartment correlations (figure 1b). For example, IL-6 in ETA samples was significantly correlated with other ETA biomarkers (*e.g.* sRAGE and procalcitonin in ETA), but not with plasma IL-6. Plasma biomarkers had the strongest positive intra-compartment correlations (median $r=0.45$), followed by ETA biomarkers (median $r=0.24$), whereas ETA–plasma correlations were weak (median $r=-0.05$) and mostly nonsignificant (86% of comparisons). Plasma sRAGE was the biomarker with the most significant correlations with ETA biomarkers (positively with sST2, sRAGE and procalcitonin, and negatively with IL-8 and pentraxin-3). In an exploratory analysis comparing raw ETA and plasma biomarker levels ratios (figure 1d), we found that IL-6, IL-8 and pentraxin-3 were more abundant in ETA samples, whereas procalcitonin, sST2 and Ang-2 had markedly higher levels in plasma, confirming published comparative data between airspace and plasma values for IL-6, IL-8 and Ang-2 [9].

We then validated our findings in the COVID-19 cohort. Similar to non-COVID ARF, hyperinflammatory COVID-19 patients (10 (13%) out of 78) had findings consistent with worse extrapulmonary dysfunction, such as higher creatinine and worse leukocytosis ($p<0.01$), but no difference in respiratory physiology parameters (mechanics or gas exchange). Consistent with the clinical variable difference pattern between subphenotypes, we found that hyperinflammatory COVID-19 patients had significantly higher plasma biomarker levels for five of seven biomarkers not included in the predictive model (IL-8, sST2, fractalkine, sRAGE and pentraxin-3), but no difference in ETA biomarkers. The ETA and plasma compartments had significant, intra-compartment positive correlations (figure 1c; median $r=0.41$ and 0.37 for ETA and plasma, respectively), with no difference in strength of correlations between compartments. In contrast, inter-compartment correlations were weak (median $r=0.045$, $p<0.0001$ for correlation coefficient comparisons *versus* ETA or plasma compartments) and rarely significant (3%), which recapitulates our findings in non-COVID ARF. Furthermore, sRAGE was the sole biomarker whose values were significantly correlated between the two compartments. Sensitivity analyses with subphenotypic classifications derived by alternative predictive models or by using protein- and urea-normalised ETA biomarker values provided similar results (data not shown).

Our systematic examination of the LRT and the systemic circulation in two temporally independent datasets of mechanically ventilated patients suggests distinct compartmentalisation of host-response profiles. Plasma-derived classification into hyper- *versus* hypoinflammatory subphenotypes provided meaningful differences in plasma biomarker levels and extrapulmonary organ dysfunction, but showed no differences in ETA biomarkers or respiratory physiology parameters in either non-COVID-19 or COVID-19 subjects. These results were robust to alternative subphenotypic classifications or adjustments for normalisation of ETA biomarker values.

Therefore, our findings suggest that plasma-based subphenotypes may reflect extrapulmonary pathophysiology rather than LRT heterogeneity. Notably, a recent secondary analysis with biomarker measurements in bronchoalveolar lavage fluid (BALF) from ARDS patients demonstrated that plasma-based subphenotypes did not display significant differences in LRT biologic profiles and were only related to nonpulmonary organ dysfunction [10]. In our cohort, although some ETA biomarkers showed proof-of-concept validity by discriminating aetiology of ARF (figure 1a), especially for sRAGE, sST2, procalcitonin and fractalkine, we propose that further investigation of the LRT is needed to identify and define biomarkers that more fully capture respiratory heterogeneity during ARF. Despite these intriguing findings, interpretation must be cautious as our biomarker panel may be insufficient to detect important blood–lung relationships, which should be the focus of future investigations. Furthermore, we did not have invasive bronchoscopic samples for study of the distal airspaces [11]. It is also important to note that all LRT sampling is subject to variability due to spatial heterogeneity and dilution effects and that there may be selection bias due to safety/tolerability of bronchoscopy in severely hypoxaemic or haemodynamically unstable patients. Thus, noninvasive ETA sampling provides potential benefit by offering a cost-effective and safe option for serial study of LRT host responses in mechanically ventilated patients, similar to noninvasive sampling for clinical microbiological diagnosis in suspected pneumonia [12].

In summary, our findings suggest that current plasma-based subphenotyping approaches represent several dimensions of host response at a systemic level, but may not capture important differences in LRT biomarkers. We recommend focused study of LRT biological processes in ARF [2, 3], with validation of noninvasive ETA specimens as surrogates for BALF, to uncover unappreciated sources of clinical heterogeneity.

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