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ABSTRACT

Purpose: Benefit from convalescent plasma therapy for coronavirus disease 2019 (COVID-19) has been inconsistent in randomized clinical trials (RCTs) involving critically ill patients. As COVID-19 patients are immunologically heterogeneous, we hypothesized that immunologically similar COVID-19 subphenotypes may differ in their treatment responses to convalescent plasma and explain inconsistent findings between RCTs.

Methods: We tested this hypothesis in a sub-study involving 1239 patients, by measuring 26 biomarkers (cytokines, chemokines, endothelial biomarkers) within the Randomized, Embedded, Multifactorial, Adaptive Platform Trial for Community-Acquired Pneumonia (REMAP-CAP) that assigned 2097 critically ill COVID-19 patients to either high titer convalescent plasma or usual care. Primary outcome was organ support free days at 21-days (OSFD-21).

Results: Unsupervised analyses identified three subphenotypes/endotypes. In contrast to the more homogeneous subphenotype-2 (N=128 patients, 10.3%; with elevated type i and type ii effector immune responses) and subphenotype-3 (N=241, 19.5%; with exaggerated inflammation), the subphenotype-1 had variable biomarker patterns (N=870 patients, 70.2%). Subphenotypes-2, and -3 had worse outcomes, and subphenotype-1 had better outcomes with convalescent plasma therapy compared with usual care (median (IQR) OSFD-21 in convalescent plasma vs usual care were 0 (-1, 21) vs 10(-1, to 21) in subphenotype-2; 1.5 (-1, 21) vs 12 (-1, to 21) in suphenotype-3 and 0 (-1, 21) vs 0(-1, to 21) in subphenotype-1; test for between subphenotype differences in treatment effects p=0.008).

Conclusions: We report three COVID-19 subphenotypes, among critically ill adults, with differential treatment effects to ABO-compatible convalescent plasma therapy. Differences in subphenotype prevalence between RCT populations probably explain inconsistent results with COVID-19 immunotherapies.

Key words: Precision medicine; sub-phenotypes; convalescent plasma

Take home message:

We report three COVID-19 subphenotypes with differences in treatment response to ABO-compatible high-titer convalescent plasma therapy among critically ill adults, participating in a large international randomized clinical trial.

Our findings support the hypothesis that passive immunotherapy in critically ill adults with COVID-19 could be enhanced with patient selection based on host immune response characteristics.

Introduction

The coronavirus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) causes COVID-19 (coronavirus disease 2019), an acute illness affecting pulmonary and extrapulmonary organs[1]. COVID-19 patients requiring hospitalization (moderate-to-severe disease) have significant viral load in the respiratory tract[2] and/or detectable viral RNA in blood[3]. Therefore, in moderate-to-severe COVID-19, antiviral therapies (either passive immunotherapy or antiviral medications) are considered potential treatment options[4]. The benefit of passive immunotherapy with convalescent plasma (blood product containing SARS-CoV-2–specific polyclonal antibodies) reported in cohort studies have not been observed in RCTs, with guidelines recommending against use of convalescent plasma outside of RCTs[5].

It is well recognized that in hospitalized patients, COVID-19 is an immunologically heterogenous illness[6-13]. It is also recognized that immunological heterogeneity in COVID-19 patients is observable at protein level i.e., differences in cytokine, chemokines, and other biomarker profiles[8, 13]. Abnormal immune responses persist throughout critical illness in COVID-19 patients[13]. Thus, we hypothesized that differences in immune responses will manifest as subphenotypes and may be associated with subphenotype differences in treatment effect to convalescent plasma therapy (also known as heterogeneity of treatment effect (HTE)[14]) on OSFD-21 (primary outcome of pandemic appendix to REMAP-CAP Trial)[15] and on hospital mortality (outcome of interest).

We tested these hypotheses in a biological sampling sub-study conducted in the UK within the immunoglobulin domain of the REMAP-CAP Trial, which randomized 2097 patients with severe COVID-19 to two units of high-titer convalescent plasma or usual care, and found no overall benefit with convalescent plasma therapy in critically ill COVID-19 patients[15]. Informed by previous work on protein biomarkers[6-13, 16], we explored whether differences

in treatment effect (HTE[14] on OSFD-21 and mortality outcomes) was detectable between COVID-19 subphenotypes, that were identified based on unsupervised analyses of changes in the CXC family of chemokines (CXCL1, CXCL5, CXCL8, CXCL9, CXCL10, CXCL11), the CC family of chemokines (CCL3, CCL4, CCL11, CCL17, CCL20), transforming growth factor-beta 1(TGF-β1), vascular endothelial growth factor (VEGF), interleukins (IL-6, IL-2, IL-4, IL-5, IL-10), interferons (IFN-α2, IFN-β, IFN-λ1, IFN-y), granulocyte monocyte colony stimulating factor (GM-CSF), soluble tumor necrosis factor receptor-1 (sTNF-R1), angiopoietin-2, intercellular adhesion molecule-1 (ICAM-1), A proliferation-inducing ligand (APRIL, TNFSF13) and B cell-activating factor (BAFF, TNFSF13B)[17]. We selected these biomarkers a priori i.e., before our primary RCT results were available.

Methods

Study design

Briefly, REMAP-CAP trial is an international, multicenter, open-label adaptive platform designed to determine the best treatment strategies for patients with severe pneumonia in both settings during the pandemic and outside the pandemic[18]. This trial's design, eligibility criteria, and results regarding glucocorticoids[19], anticoagulants[20, 21], antivirals[22], interleukin-6 (IL-6) receptor antagonists[23], antiplatelet therapy, and immunoglobulin domain convalescent plasma[15] for treatment of COVID-19 have been reported previously.

Study population

Our study population consisted of critically ill adult patients (>18 years old) with microbiologically confirmed COVID-19, randomized to receive 2 units of high-titer, ABO-compatible convalescent plasma (total volume approximately 550 mL ± 150 mL) within 48 hours of randomization or no convalescent plasma, between March 9, 2020, and January 18, 2021[15], who had a baseline blood sample collected after consent, and before

administration of allocated intervention (convalescent plasma vs no convalescent plasma (usual care)).

Biomarker measurements

Serum was separated from whole blood by centrifugation (1300g for 10 minutes at room temperature) and stored in 200 µl aliquots at −80°C until analyses. Two custom 14-plex Legendplex™ (BioLegend) bead-based multiplex assay was used to measure a priori selected biomarkers described in introduction section, as per manufacturer's instructions (e-Methods-1). SARS-CoV-2 immunoglobulin G (IgG) antibody against spike was measured using enzyme-linked immunosorbent assay (ELISA), as reported previously[2, 24]. Viral loads and strains in the respiratory tract were measured as described previously [2] (eMethods-2).

Data analyses

We describe the study cohort characteristics (overall and by randomized allocation status) in terms of age, sex, body mass index (BMI), pre-existing chronic health conditions defined using the Acute Physiology and Chronic Health Evaluation II (APACHE-II) score, SARS-CoV-2 antibody status, viral loads in respiratory tract, respiratory support status, concomitant COVID-19 therapy use, and allocation status.

Before biomarker analyses, two proteins (IL-2 and TGF-β1) with more than 20% missing data were excluded from the dataset (eFigure-1a-b). We then used Gibbs sampler-based left-censored missing value imputation approach (GSIMP)[25], which considers the lower limit of detection calculated in the LEGENDplex™ Data Analysis Software Suite. We checked data for batch effects and did not observe any batch effects (eFigure-1c-d). Thus, the final analytic dataset consisted of 26 biomarkers. The following analyses were performed in R statistical environment[26]. First, we assessed biomarker differences by SARS-CoV-2 antibody status and by hospital mortality, as differences in immune responses may be associated with

antibody status and clinical outcomes. Second, we assessed biomarker differences by allocation status (convalescent plasma vs usual care), to check for baseline biomarker imbalances by randomization status, as any imbalances will need to be accounted in the subsequent unsupervised analyses. Third, we used the agglomerative hierarchical clustering method with WARD2 linkage function on $\log_{10}$ transformed data. [27]. Additional details are reported in online supplement (eMethods-3).

Finally, we explored the associations between sub-populations, allocation status and outcomes, with regression models incorporating robust standard errors using Stata 15.1[28]. We report the association between the outcome of OSFD-21 and subphenotypes and by allocation to convalescent plasma using ordered logistic regression models to relate our sub-study results to the primary outcome in our original publication[15]. OSFD-21 is an ordinal scale outcome, where mortality is given a score of -1 and among survivors OSFD are calculated up to day 21, such that a higher number represents faster recovery[15]. We also report the associations between hospital mortality and SARS-CoV-2 antibody status in the overall cohort, by subphenotypes and by allocation to convalescent plasma using logistic regression models. We report unadjusted analyses as testing associations between subphenotypes and treatment effects of convalescent plasma is equivalent to performing a subgroup analyses with moderate sized RCT data, where the additional value of baseline prognostic covariates adjustment needs careful consideration, due to risk of alpha error[29]. After the regression models, we used post estimation commands and test of heterogeneity for differences in treatment effects.

Results

Study cohort and clinical characteristics: Amongst the 2097 participants randomized to a COVID-19 immunoglobulin domain within the REMAP-CAP Trial, 1023 were assigned to convalescent plasma and 868 to usual care in the UK[15]. Our report is based on data from

1239 participants (737/1023 (72%) assigned to convalescent plasma and 502/868 (57.8%) assigned to usual care) from the UK, who had baseline blood samples after consent but before intervention (Table-1). Clinical characteristics of our sub-study cohort were similar to the overall trial population[15]. Importantly, clinical characteristics were similar between patients assigned to convalescent plasma and usual care, enabling us to analyze this UK sub-population as a cohort (eTable-1).

The study cohort had a median (IQR) age of 61 (52, 70) years, 408 (32.9%) were females and median (IQR) APACHE II score was 13 (8, 19). Typing of the specific SARS-CoV-2 variant was successful in 56% of cases; of which wild type and B.1.1.7 variants represented 61.1% and 38.9%, respectively. SARS-CoV-2 antibodies (seropositive) were detected at baseline in 846 (68.3%) patients. Nearly all (98.7%) patients required either invasive or non-invasive respiratory support, 94.1% received low dose corticosteroids and 35.7% received remdesivir. The overall cohort had a hospital mortality of 35.9%. Seronegative patients had higher hospital mortality compared to seropositive patients (odds ratio (OR) (95% confidence interval (CI)) of 2.05 (1.58-2.65; $p<0.001$), which is consistent with the literature and explained by the deficient or delayed humoral immunity in severe COVID-19[30].

Unsupervised clustering identified three subphenotypes

We found that a three subphenotype model (Figure-1a; Figure-1b; eFigure-2) optimally explained our biomarker data (eFigure-3). Subphenotype-1 was most common (70.2%; n=870/1239), followed by subphenotype-3 (19.5%; 241/1239) and subphenotype-2 (10.3%;128/1239) (Table-1). The top 10 contributing proteins to principal component-1 (PC1) and PC2 were biomarkers determining subphenotype-2 (IL-4, IFN-α2, GM-CSF, IFN-γ, IL-5) and subphenotype-3 (CXCL8, CCL4, IL-6, CCL20, CCL3) allocations (Figure-1c). We observed striking differences between these subphenotypes on biomarker changes (Figure-

1d), with biologically plausible correlations between biomarkers (Figure-1e) and differences in average biomarker concentrations (Figure-1f).

Immunologically, subphenotype-1 patients had variable levels of pro-inflammatory chemokines involved in leukocyte trafficking (CXCL9, CXCL10, CXCL11)[31], immune activating cytokines (IL-10[32]), interferons[33] (IFN- λ1, IFN-β), TNF family biomarkers (APRIL, BAFF, sTNF-R1), and endothelial biomarkers of COVID-19 severity (ICAM-1[34], angiopoietin-2[35]). Taken together, subphenotype-1 represents a dysregulated immune state with biomarkers strongly associated with severe COVID-19[6, 13], without a dominant effector or co-regulated immune responses.

In contrast, subphenotype-2 appears homogeneous on biomarker patterns, with elevated levels of IL-4, and IL-5 (known to polarize naïve T cells into T-helper 2 (Th2), enable selective B cell class switch, and macrophage activation)[36], elevated levels of interferons (IFN-γ, IFN-α2) dysregulated in severe COVID-19[33], and high GM-CSF levels with a central role in endothelial injury, Th17 T cell response[37], neutrophil recruitment, and thrombosis seen in COVID-19[38, 39] (Figure-1a). Taken together, subphenotype-2 resembles the *mixed* immune response pattern reported previously in COVID-19[13].

Subphenotype-3 also appears more homogeneous compared to subphenotype-1 on biomarker patterns, with elevated pro-inflammatory cytokines such as IL-6 (with prognostic[6, 8, 13]/therapeutic relevance[40]), and elevated chemokines indicating leukocyte recruitment / activation[41] (Figure-1a). Taken together, subphenotype-3 represents a heightened early innate immune response state[42].

Clinical features of subphenotypes

These subphenotypes were broadly similar in-terms of age, sex, prevalence of comorbidities, illness severity, types of respiratory support, and prevalence of immunosuppression. SARS-CoV-2 wild-type infections were detected in a third of patients within each of the three subphenotypes. Compared to other subphenotypes, subphenotype-3 had the highest proportion of SARS-CoV-2 B.1.1.7 variant and the highest B.1.1.7 variant viral load. Compared to seronegative patients, seropositive patients had lower viral loads for all variants, in all three subphenotypes (eFigure-4). There were no differences between subphenotypes in terms of glucocorticoid and remdesivir therapy (Table-1).

Biomarker associations between subphenotypes and serology status

Subphenotype-2 had the highest proportion of seropositive patients (74.2%) (Table-1). In the overall cohort, compared to seronegative, seropositive patients had significantly higher CXCL8, IL-5, CCL3 and CCL4, and lower levels IFN-λ1, CXCL10, IL-10, IL-6, IFN-α2, CXCL11, and Angiopoietin-2 (Figure-2a; eFigure-5). Serology status did not segregate patients on principal component analyses (PCA; eFigure-6a). In subphenotype-1, the seropositive – seronegative comparison highlighted a pattern similar to the overall cohort (Figure-2b). There were no significant biomarker differences between seropositive and seronegative patients within subphenotype-2 (Figure-2c). In addition, seropositive subphenotype-1 patients had higher levels of CCL20, while seropositive subphenotype-3 patients had lower levels of CCL20 (Figure-2d). Individual biomarker differences by serology status are shown in Figure-2e-h.

Biomarker associations between subphenotypes and hospital mortality

Hospital mortality differed between subphenotypes (p=0.03, Chi square test), with highest mortality observed in subphenotype-1 (38.7%), and the lowest hospital mortality in

subphenotype-2 (30.1%). In all three subphenotypes, seronegative patients had a higher (and importantly similar) hospital mortality compared to seropositive patients (Table-1).

In the overall cohort, compared to survivors, non-survivors had significantly higher levels of CXCL10, CXCL9, IL-10, sTNF-RI, IL-6, angiopoietin-2, CCL20 and lower levels of CCL3 and CCL4 (Figure-3a; eFigure-7). Mortality did not segregate patients on PCA (eFigure-6b). In subphenotype-1, and subphenotype-3, the comparison of survivors versus non-survivors had a biomarker pattern similar to the overall cohort (Figure-3b-d). Although the volcano plot appears to show no biomarker differences between survivors versus non-survivors in subphenotype-2 (Figure-3c), non-survivors in this cohort had higher IL-6, CXCL-10, and angiopoietin-2 (Figure-3e-h), which is consistent with pattern seen in the overall cohort.

Association between subphenotypes and treatment effect of convalescent plasma

Within each subphenotype there was no difference in baseline biomarkers between subjects allocated to convalescent plasma or usual care (eFigure-8), and allocation status does not segregate patients on PCA (eFigure-6c).

The overall treatment effect of convalescent plasma compared to usual care for OSFD-21 was OR (95%CI) of 0.91 (0.74-1.11)), which is consistent with our original RCT result[15] (OR (95%CI) 0.97 (0.82 to 1.14)). The overall treatment effect of convalescent plasma compared to usual care for mortality was OR (95%CI) 1.01 (0.80-1.29)), which was also consistent with our original RCT result[15] with OR (95%CI) 1.04 (0.85 to 1.27)[15].

There were no major differences in the main baseline prognostic clinical characteristics[43] (age, sex, BMI, comorbidities, and need for mechanical ventilation) by allocation status, within each subphenotype (eTable-2). In subphenotype-1 the median (IQR) OSFD-21 was 0 (-1, 21) in convalescent plasma and 0(-1, to 21) in the usual care arm. In subphenotype-2 and -3, the

usual care had higher OSFD-21 compared to convalescent plasma arm (subphenotype-2 (OSFD-21 median (IQR) 0 (-1, 21) in convalescent plasma vs 10(-1, to 21) in usual care) and subphenotype-3 (OSFD-21 median (IQR) 1.5 (-1, 21) in convalescent plasma vs 12 (-1, to 21) in usual care). The corresponding odds ratio differed by subphenotype (test of heterogeneity; p=0.008 (Figure-4).

In subphenotype-1 the hospital mortality in the convalescent plasma group was lower than usual care group (37.6% vs 40.5%). In contrast, in subphenotype-2 and in subphenotype-3 the hospital mortality in the convalescent plasma group was higher than usual care (subphenotype-2 = 35.4% vs 24.1% and subphenotype-3 = 34.1% vs 29.7%). The corresponding odds ratio differed by subphenotype (test of heterogeneity; p=0.02) (eFigure-9).

Association between serology status of subphenotypes and treatment effect of convalescent plasma

In our main trial publication[15] the treatment effect of convalescent plasma did not meaningfully vary in prespecified serology status subgroup. Consistent with the main trial result, the treatment effect on mortality did not vary by serology status of the subphenotypes (p = 0.69, for 3-way interaction test between allocation to convalescent plasma, serology status and subphenotype). It could be that our sub-study is underpowered to assess this sub-group within a sub-group effect (i.e., serology status within subphenotypes). Of note, only in subphenotype-1, seronegative patients who received convalescent plasma had lower mortality, compared to seronegative patients who received usual care (eFigure-10). As serology is a prognostic covariate, our sensitivity analyses including serology status as a covariate in two additional regression models (for OSFD-21 and for mortality) were consistent with the main findings.

Discussion

We report the largest biomarker study conducted within an RCT in critically ill COVID-19 patients. We highlight three distinct subphenotypes based on biomarker profiles within critically ill COVID-19 patients who had similar clinical features, but with differences in clinical outcomes and in treatment effect estimates for OSFD-21 and hospital mortality. Compared to subphenotype-1, mortality was lower despite higher inflammation in suphenotype-2 and in subphenotype-3. Our observations, if validated, favor avoiding convalescent plasma therapy in subphenotype-2 and -3.

Our subphenotypes have biological plausibility. The median (IQR) IL-6 levels in our cohort was 62.2 (23.8-290.6) pg/mL, which is consistent with literature[44]. The negative association between IL-10, CXCL-10, and IL-6 with seropositive status in COVID-19 have been reported previously[6], explaining the prognostic utility of this biomarker triad. Biomarker differences between seropositive and seronegative patients in our study represent altered interferon responses[33], and compromised humoral immunity[12, 13, 30] in critically ill COVID-19 patients. Prognostic associations with many of these biomarkers have been reported previously in acute respiratory distress syndrome[45].

As we are unable to assess potential molecular mechanisms, we propose the following hypotheses as to why convalescent plasma therapy could theoretically worsen outcomes in the more proinflammatory subphenotypes[46-49]. High-affinity antibodies present in convalescent plasma elicit SARS-CoV-2 neutralization[46-48]. However, the low affinity antibodies present either in donor plasma or forming in recipients following convalescent plasma administration, could activate proinflammatory pathways[49], worsening outcomes. Presence of autoantibodies reported in COVID-19 patients[50, 51], may be present in convalescent plasma, which could worsen outcomes in the more proinflammatory

subphenotypes. Although a rare event in our primary trial[15], convalescent plasma is a blood product that can cause transfusion related adverse events.

Our sampled population appears representative of the overall RCT publication[15]. Our findings also highlight the value of enriching trial populations[52]. Although, our findings are likely to be widely applicable to moderately or severe COVID-19 patients, our primary RCT was not powered to detect subgroup effects. We neither have non-COVID controls nor validation cohorts. Research blood sampling was not possible outside the UK, and not every patient enrolled in the UK had sampling. As our RCT was conducted early on the pandemic, SARS-CoV-2 vaccination may alter the prevalence of reported subphenotypes.

Our results have clinical utility for the following reasons. Our findings support the hypothesis that immunotherapy in COVID-19 could be useful with better patient selection based on host immune response characteristics. It is feasible to determine subphenotype-2 and -3, where we observed a harm signal by measuring a limited biomarker set based on discriminant value (such as IL-6, CCL3 and IL-8 based our data in Figure-1). Lower overall mortality in the more inflammatory subphenotypes in our cohort support the notion that strong prognostic association to cytokines such as IL-6 in mild COVID-19[8], may be masked by complex cytokine networks or hubs in severe inflammatory illnesses[53] observed with severe COVID-19, highlighting the futility of measuring single cytokines as value added biomarkers, for informing treatment decisions.

Our novel findings highlight future research questions. A key next step is to study the molecular mechanisms underpinning these subphenotypes, to consider them as COVID-19 endotypes[52]. A related research question is whether these subphenotypes are associated with HTE to other immunomodulators. It is important to determine whether these subphenotypes are identifiable in non-critically ill COVID-19 patients and whether they have

HTE to immunotherapies, as our study focused on critically ill COVID-19. In any viral pandemic, as convalescent plasma will be a potential treatment, understanding the mechanisms for harm may lead to better selection of donor plasma in the future.

Conclusions

We report three COVID-19 subphenotypes with differences in treatment response to ABO-compatible high-titer convalescent plasma therapy among critically ill adults, participating in a RCT. Given the distinct immunological mechanisms, these *subphenotypes* could be termed *endotypes*. These findings support the hypothesis that benefits of immunotherapy in COVID-19 could be enhanced with patient selection based on host immune response characteristics.

Legends

Table-1: Clinical characteristics of overall cohort and by subphenotypes

Acute physiology and chronic health evaluation (APACHE-II) score measures the severity of illness based on age, medical history, and physiological variables. Scores range from 0 to 71; higher numbers represent greater risk of death. The median score of 12 is typical for critically ill COVID-19 patients[15]. Immunosuppression treatment refers to recent chemotherapy, radiation, high-dose, or long-term glucocorticoid treatment.

Figure-1: Unsupervised clustering of 26 protein biomarkers identifies 3 sub-subphenotypes of critically ill COVID-19 patients.

a, *Heatmap displaying the agglomerative hierarchal clustering identified 3 subphenotypes. Each row is a patient (N=1239) and each column a biomarker. Each cell is coloured by the scaled log10 transformed protein levels (high=red, low=blue). Rows are annotated by; Subphenotype (subphenotype-1=blue, subphenotype-2=orange, subphenotype-3=red); Allocation of convalescent plasma (Yes=dark blue and No=orange); serology (positive=pink and negative=navy) and hospital mortality (alive=blue and deceased=red).* ***b,*** *Principal component analysis (PCA) of the same 26 protein biomarkers coloured by subphenotype. Subphenotype-1=blue, subphenotype-2=orange and subphenotype-3=red. Columns are annotated by protein biomarker signature; A=sky blue, B=light green, C=light red.* ***c,*** *Top 10 contributing variables to principal component (PC) PC1 and PC2. Arrows are coloured based on their respective protein contribution to variation from low (blue) to high (red).* ***d,*** *Box, and whisker plots of Log2 fold change of protein biomarkers normalised to median of subphenotype-1 and grouped by protein signature (A-B). Boxes are coloured by subphenotype. The bottom border of box represents the 25th percentile; line bisecting the box represents median; upper border of the box is the 75th percentile. The whiskers represent extreme 1.5 times the 75th (highest) and 25th (lowest) values.* ***e,*** *Circos plots of each patient*

subphenotype represent Spearman correlations between each protein biomarker. Only correlations of an adjusted p value <0.001 are shown. Positive and negative correlations are coloured by red and blue, respectively. The strength of the correlation is depicted by the strength of the colour. Proteins are grouped into the three signatures; A = sky blue (representing biomarkers associated with dysregulated COVID-19 immune responses), B = light green (representing Type ii, Type i and altered interferon responses), C = light red (Co-regulated innate immune responses with chemokines and cytokines associated with leukocyte migration and activation). Subphenotype-1 had the weakest positive correlations between the biomarkers evaluated. In subphenotype-2, all 26 biomarkers were positively correlated, consistent with the mixed immune response pattern[54]. In subphenotype-3, CXCL8 was negatively correlated with CXCL9, CXCL10, IFN-γ, and IFN-α2, as previously reported in COVID-19 [55]. ***f,*** *Summary radar plot of the 26 protein biomarkers. Medians of the log10 transformed values of each protein by subphenotype are plotted. Lines are coloured by subphenotype, subphenotype-1 = blue, subphenotype-2 = orange, subphenotype-3 = red.*

Figure 2: Biomarker associations between subphenotypes and serology status

Comparison of the overall cohort and subphenotypes by Serology status. ***a,*** *Volcano plot of overall cohort.* ***b****, Volcano plot of subphenotype-1.* ***c****, Volcano plot of subphenotype-2.* ***d****, Volcano plot of subphenotype-3.* ***e-h****, Box, and violin plot of* ***(e)*** *IFN- λ1,* ***(f)*** *IL-6,* ***(g)*** *CCL20* a *chemokine increased during microbial insult and required for effective humoral responses[56],* ***(h)*** *IL-5 by overall and sub phenotypes by serology status. For volcano plots, upregulated proteins (higher in serology positive compared to serology negative) are colored red and defined as log2 fold change >0.3 and P ≤ 0.05. down regulated proteins (lower in serology negative compared serology positive) are colored as blue and defined as log2 fold change <-*

0.3 and P ≤ 0.05. For box and whisker plots the bottom border of box represents the 25th percentile; line bisecting the box represents median; upper border of the box is the 75th percentile. The whiskers represent 1.5 times the 75th (highest) and 25th (lowest) values.

Figure 3: Biomarker associations between subphenotypes and hospital mortality

Comparison of the overall cohort and subphenotypes by hospital mortality. ***a,*** *Volcano plot of overall cohort.* ***b****, Volcano plot of subphenotype-1.* ***c****, Volcano plot of subphenotype-2.* ***d****, Volcano plot of subphenotype-3.* ***e-h****, Box, and violin plot of* ***(e)*** *Angiopoietin-2,* ***(f)*** *CXCL10,* ***(g)*** *IL-6,* ***(h)*** *CCL4 by overall and sub phenotypes by mortality status. For volcano plots, upregulated proteins (higher in deceased patients compared to survivors) are colored red and defined as log2 fold change >0.3 and P ≤ 0.05. Down regulated proteins (lower in deceased patients compared to survivors) are colored as blue and defined as log2 fold change <-0.3 and P ≤ 0.05. For box and whisker plots the bottom border of box represents the 25th percentile; line bisecting the box represents median; upper border of the box is the 75th percentile. The whiskers represent 1.5 times the 75th (highest) and 25th (lowest) values.*

Figure 4: Treatment effect convalescent plasma compared to usual care for organ support free days by subphenotypes

Forest plot comparing organ support free days at day 21 (OSFD-21) of the overall cohort and by subphenotypes when treated with convalescent plasma, compared to usual care population. Median and inter-quartile range (IQR) for OFSD are displayed. Odds ratio was calculated using ordered logistic regression, and 95% confidence intervals are reported. Square dots represent odds ratio of respective row, and the black line denotes 95% confidence intervals. Odds ratio <1 favours control. The P value is reported based on test of heterogeneity estimated post ordered logistic regression. The odds ratio represents the average odds ratio for each possible cut points of the outcome variable. The proportional

odds assumption means that the odds ratios are about the same regardless of the cut-point of the ordinal outcome variable.

Table-1: Clinical characteristics of subphenotypes

Characteristic	Overall (n=1,239)	Phenotype-1 (n=870)	Phenotype-2 (n=128; 10.3%)	Phenotype-3 (n=241; 19.5%)
Allocation n (%)				
Convalescent plasma	737 (59.5%)	534 (61.4%)	67 (52.3%)	136 (56.4%)
Usual care	502 (40.5%)	336 (38.6%)	61 (47.7%)	105 (43.6%)
Age, median (IQR) y	61 (52, 70)	62 (53, 70)	58 (48, 65.5)	61 (52, 70)
Female n (%)	408 (32.9%)	286 (32.9%)	39 (30.5%)	83 (34.4%)
BMI (kg/BSA m^2)	30.9 (26.7, 36.3) [n=1,111]	30.5 (26.3, 36.0) [n=776]	33.4 (28.1, 37.1) [n=116]	30.9 (27.4, 36.1) [n=219]
Pre-existing conditions				
Diabetes	358 (28.9%)	247 (28.4%)	35 (27.3%)	76 (31.5%)
Respiratory disease	292 (23.6%)	204 (23.5%)	37 (28.9%)	51 (21.2%)
Severe CVS disease	103 (8.3%)	73 (8.4%) [n=848]	14 (10.9%) [n=124]	16 (6.6%) [n=236]
Immunosuppression treatment/disease	83 (6.7%) [n=1,237]	61 (7.0%)	6 (4.7%) [n=127]	16 (6.6%) [n=240]
SARS-CoV-2 type				
Wild Type	424 (34.2%)	301 (34.6%)	45 (35.2%)	78 (32.3%)
B.1.1.7	270 (21.8%)	183 (21.0%)	27 (21.1%)	60 (24.9%)

Inconclusive	391 (31.6%)	265 (30.5%)	39 (30.5%)	87 (36.1%)
Not available	154 (12.4%)	121 (13.9%)	17 (13.3%)	16 (6.6%)
SARS-CoV-2 viral load, median (IQR) (10^5 IU /ml)				
Wild Type	7.88 (0.62 - 96.28)	8.21 (0.63 – 115.68)	14.27 (2.35 – 88.0)	3.03 (0.34 – 47.28)
B.1.1.7	24.38 (1.39 – 248.65)	19.82 (1.32 – 185.69)	57.91 (0.38 – 420.74)	88.01 (2.60 – 307.7)
Inconclusive	0.01 (0.00001 – 0.047)	0.013 (0.0016 – 0.050)	0.0085 (0.00010 – 0.031)	0.01 (0.00010 – 0.05)
SARS-CoV-2 antibody n (%)				
Detected	846 (68.3%)	582 (66.9%)	95 (74.2%)	169 (70.1%)
Not detected	348 (28.1%	259 (29.8%)	29 (22.7%)	60 (24.9%)
Not available	45 (3.6%)	29 (3.3%)	4 (3.1%)	12 (5.0%)
SARS-CoV-2 antibody positive n (%)				
Wild Type	262 (64.8%)	184 (64.1%)	29 (67.4%)	49 (66.2%)
B.1.1.7	176 (66.7%)	115 (64.2%)	24 (88.9%)	37 (63.8%)
Inconclusive	318 (83.2%)	212 (80.9%)	32 (84.2%)	74 (90.2%)
Not available	90 (62.5%)	71 (62.8%)	10 (64.1%)	0 (60.0%)
APACHE II score, median (IQR)	13 (8, 19) [n=1,196]	13 (8, 19) [n=841]	11.5 (8, 17) [n=122]	13 (8, 18) [n=233]
Use and type of acute respiratory support, n (%)				
Non-invasive mechanical ventilation	562 (45.3%)	397 (45.6%)	61 (47.7%)	104 (43.2%)

Invasive mechanical ventilation	418 (33.7%)	290 (33.3%)	43 (33.6%)	85 (35.3%)
High-flow nasal cannula	243 (19.6%)	172 (19.8%)	23 (18.0%)	48 (19.9%)
None or supplemental oxygen only	16 (1.3%)			
COVID-19 therapy use				
Glucocorticoids	1,166 (94.1%)	820 (94.3%)	121 (94.5%)	225 (93.4%)
Remdesivir	442 (35.7%)	300 (34.5%)	48 (37.5%)	94 (39.0%)
Il-6 Receptor antagonists	41 (3.3%)			
Outcomes				
Overall				
Number of OSFD at D21* (median (IQR))	1 (-1, 21)	0 (-1, 16)	6 (-1, 17)	8 (-1, 17)
Hospital mortality				
Overall	444 (35.9%) [N=1214]	331 (38.7%) [n=855]	37 (30.1%) [N=123]	76 (32.2%) [n=236]
- Seropositive	260/827 (31.4%)	195/571 (34.2%)	21/91 (23.1%)	44/165 (26.7%)
- Seronegative	166/343 (48.4%)	123/255 (48.2%)	14/28 (50%)	29/60 (48.3%)

Author contributions

Drs Shankar-Hari, Dr Estcourt, Dr Rynne, Mr Fish had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Mr Fish and Dr Rynne contributed equally to this article.

Concept and design: Estcourt, Harvala, Menon, Roberts, Shankar-Hari.

Acquisition, analysis, or interpretation of data: Fish, Rynne, Jennings, Lamikara, Ratcliffe, Harvala, Estcourt, Menon, Roberts, Shankar-Hari.

Drafting of the manuscript: Fish, Rynne, Shankar-Hari.

Critical revision of the manuscript for important intellectual content: All authors.

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Obtained funding: Estcourt, Harvala, Menon, Roberts, Shankar-Hari.

Supervision: Shankar-Hari

Acknowledgements

We are grateful to the NIHR Clinical Research Network (UK) for their support of participant recruitment. We are grateful to the UK Blood Services (NHS Blood and Transplant, Northern Ireland Blood Transfusion Service, Scottish National Blood Transfusion Service and Welsh Blood Service) for the supply of convalescent plasma in the UK. The views expressed in this publication are those of the authors and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health and Social Care. This research was supported by the National Institute for Health and Care Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London and/or the NIHR Clinical Research Facility.

Conflicts of interests:

Dr Estcourt reported receiving grants from the National Institute for Health Research and European Union Horizon 2020. Dr Gordon reported receiving grants from the National Institute for Health Research and receiving personal fees from 30 Respiratory, GlaxoSmithKline, and Bristol Myers Squibb. DKM reports consultancy agreements, educational support, or research collaborations with NeuroTrauma Sciences LLC, GlaxoSmithKline Ltd, Calico Ltd, Lantmannen AB, Cortirio Ltd, and

Gryphon Inc; all outside the research in this manuscript. MSH reports receiving grant from the Chief Scientists Office, Scotland for time critical precision medicine in adult critically ill patients (TRAITS Programme) and highlights industry support for TRAITS research programme (https://www.ed.ac.uk/inflammation-research/clinical-trials/traits-ci-trial).

Funding/Support:

This study was supported by National Institute for Health (UKRIDHSC COVID-19 Rapid Response Rolling Call, "The use of convalescent plasma to treat hospitalized and critically ill patients with COVID-19 disease", Grant Reference Number COV19-RECPLAS). M.F. is supported by a National Institute of Academic Anesthesia BJA/RCoA fellowship (WKRO-2018-0047). JR is supported by the Marshall Scholarship and Clarendon Fund. ACG is funded by an NIHR Research Professorship (RP- 2015-06-18). DKM is supported by the NIHR through the Cambridge NIHR BRC, and the Addenbrooke's Charitable Trust. MS-H is funded by a clinician scientist fellowship 2016-16-011 from the National Institute for Health Research.

The REMAP-CAP platform trial has 4 regional nonprofit sponsors: Monash University, Melbourne, Australia (Australasian sponsor); Utrecht Medical Center, Utrecht, the Netherlands (European sponsor); St Michael's Hospital, Toronto, Ontario, Canada (Canadian sponsor); and the Global Coalition for Adaptive Research, San Francisco, California (US sponsor). REMAP-CAP trial was additionally funded by FP7-health-2013-innovation-1 (#602525) from the European Union Platform for European Preparedness Against Reemerging Epidemics grant, the Rapid European COVID-19 Emergency Research response (RECOVER) consortium by the European Union's Horizon 2020 research and innovation programme (#101003589), the UK National Institute for Health Research (NIHR) and the NIHR Imperial Biomedical Research Centre, Collection of UK plasma was funded by the DHSC through core funding under COVID-19 and EU SoHo Grants.

Role of the Funder/Sponsor:

The funders/ sponsors had no role in the design and conduct of the trial; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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The views expressed in this publication are those of the authors and not necessarily those of the National Health Service, the National Institute for Health Research, or the Department of Health and Social Care.

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