Nature and dimensions of the systemic hyper-inflammation and its attenuation by convalescent plasma in severe COVID-19

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Summary: In-depth characterization of the systemic hyper-inflammation in severe COVID-19 revealed the dominant cytokines and identified MCP3 to be a key correlate for clinical improvement. An anti-inflammatory role of convalescent plasma therapy independent of its neutralizing antibody content was revealed, which together with neutralizing antibodies contributed to rapid reductions in hypoxia.

ABSTRACT

Novel coronavirus SARS-CoV2, causing coronavirus disease 2019 or COVID-19, led to significant morbidity and mortality. While most suffer from mild symptoms, some patients progress to a severe disease with acute respiratory distress syndrome (ARDS) and an associated systemic hyper-inflammation. First to characterize key cytokines and their dynamics in this hyper-inflammatory condition, we assessed abundance and correlative expression of a panel of forty eight cytokines in patients progressing to ARDS, as compared to patients with mild disease. Then in an ongoing randomized control trial of convalescent plasma therapy (CPT), we analyzed rapid effects of CPT on the systemic cytokine dynamics, as a correlate for the level of hypoxia experienced by the patients. We identified an anti-inflammatory role of CPT independent of its neutralizing antibody content. Neutralizing antibodies as well as reductions in circulating interleukin-6 and interferon gamma induced protein 10, contributed to marked rapid reductions in hypoxia in response to CPT.

Keywords:

SARS-CoV-2; COVID-19; Cytokine; Convalescent plasma; hyper-inflammation; Hypoxia; Acute respiratory distress syndrome

The pandemic caused by the novel coronavirus SARS-CoV2 led to significant morbidity and mortality worldwide. The disease caused by SARS-CoV2, the coronavirus disease 2019 or Covid-19, has a spectrum of symptoms spread over two distinct phases in the symptomatic individuals. The variable symptomatology in the first milder phase is usually followed by recovery [1]. In a fraction of infected individuals this milder phase progresses to a more severe disease characterized by gradually worsening hypoxia and in some to acute respiratory distress syndrome (ARDS), leading to fatal outcomes in a number of them [1, 2]. A hyper-immune activation response is associated with the severe symptoms, characterized by a systemic deluge of inflammatory cytokines [3, 4, 5].

Different therapeutic approaches are being explored, either by repurposing specific anti-viral agents, viz. remdesivir (6), or by using corticosteroids to affect immunomodulation [7]. In addition, convalescent plasma therapy (CPT) emerged as a widely tried strategy against COVID-19, being explored in a number of clinical trials worldwide [8. 9, 10]. CPT is an age-old strategy for passive immunization, with primary intention to supplement non-recovering patients with antibodies against specific pathogens [9, 11]. Several previous pandemics of respiratory infections saw successful therapeutic use of CPT, starting from the 1918 Spanish flu to the more recent SARS and Ebola epidemic (12-15).

In the present study, first we aimed at an in-depth characterization of the nature and dimensions of the cytokines involved in the systemic hyper-inflammation encountered in COVID-19 patients with ARDS, as compared to mild disease, and then, in a randomized control clinical trial on COVID-19 CPT, explored effects of convalescent plasma (CP), if any, on mitigation of this systemic immune hyperactivation phenotype. We revealed a multi-dimensional nature of the systemic hyper-inflammation in severe COVID-19. Moreover, we identified an anti-inflammatory effect of CPT in terms of attenuation of the systemic cytokine deluge, which contributed to rapid mitigation of hypoxia, in addition to the neutralizing antibody content of CP.

METHODS

Ethical approval

The randomized control trial (RCT) on CPT and all associated studies and the human sampling needed for them were done with informed consent from the patients as per ethical approval from the Institutional Review Boards of CSIR-Indian Institute of Chemical Biology, Kolkata, India (IICB/IRB/2020/3P), Institutional Review Boards of Medical College Hospital, Kolkata (MC/KOL/IEC/NON-SPON/710/04/2020), India and Infectious Disease & Beleghata General Hospital

(ID & BG Hospital), Kolkata, India (IDBGH/Ethics/2429). The RCT was approved by Central Drugs Standard Control Organisation (CDSCO) under Ministry of Health & Family Welfare, Govt. of India (approval no. CT/BP/09/2020) and registered with Clinical Trial Registry of India (CTRI No. CTRI/2020/05/025209).

Plasma cytokine analysis

Plasma was isolated from peripheral blood of patients collected in EDTA vials and cytokine levels (pg/ml) were measured using the Bio-Plex Pro Human Cytokine Screening Panel 48-Plex Assay (Bio-Rad, Cat No. 12007283) which quantitates 48 cytokines (FGF basic, Eotaxin, G-CSF, GM-CSF, IFN-γ, IL-1β, IL-1ra, IL-1α, IL-2Rα, IL-3, IL-12 (p40), IL-16, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, GRO-α, HGF, IFN-α2, LIF, MCP-3, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, IP-10, MCP-1, MIG, β-NGF, SCF, SCGF-β, SDF-1α, MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF-α, VEGF, CTACK, MIF, TRAIL, IL-18, M-CSF and TNF-β). The patient plasma samples were diluted (1:3) in sample diluent and the assay was performed using manufacturer's protocol. The plate was run and analyzed using Bio-Plex® 200 System (Bio-Rad).

RNA Isolation and RT-PCR for SARS-CoV2

Nasopharyngeal swabs were collected from all patients on the day of enrolment and directly put in TRIzol. RNA from these TRIzol samples were extracted using chloroform-isopropanol method followed by quantitation using NanoDrop (Thermo Fisher Scientific). qRT-PCR for SARS-CoV-2 detection was performed using the STANDARD M nCoV Real-Time Detection kit (SD Biosensor), approved by Indian Council of Medical Research (ICMR), India. A cycle threshold (Ct-values) cut-offs mean value for both RdRp and E gene was considered for interpreting the results. A CY5 labeled Internal Control was used. Average CT values for two SARS-CoV2 targets (RdRp and E) were used for the study as a surrogate for viral load.

SARS-CoV-2 Surrogate Virus Neutralization Assay

Neutralizing antibodies (nAb) against SARS-CoV-2 in CP samples from peripheral blood of convalescent donors were detected using GenScript SARS-CoV-2 Surrogate Virus Neutralization kit (Cat no-L00847). The kit consists of recombinant SARS-CoV-2 Spike protein receptor binding domain fragment conjugated with horseradish peroxidase (HRP-RBD) & human ACE2 receptor protein (hACE2). Assay was performed according to manufacturer's protocol. Plasma samples were diluted at a ratio of 1:10. Since presence of SARS-CoV-2 nAb in the CP samples will result in inhibition of interaction between HRP-RBD and plate-bound human ACE2 protein, and subsequent development of color, assay results are interpreted as inhibition rate of assay reaction. Inhibition rate is calculated

as follows: Inhibition = {1- (O.D value of sample/O.D value of negative control)} × 100 [Inhibition values ≥20% signify positive detection of nAb]. In case of recipient patients the amount of nAb received was calculated considering the amount (ml) of CP transfused to them.

ELISA for anti SARS-CoV-2 IgG

Levels of Immunoglobulin G (IgG) specific for SARS-CoV-2 in the CP were detected using EUROIMMUN Anti-SARS-CoV-2 (IgG) Elisa kit (Cat No- EI 2606-9601 G). Assay was performed according to manufacturer's protocol. The assay wells are pre-coated with recombinant S1 domain of the SARS-CoV-2 spike protein. CP samples were diluted with provided sample dilution buffer at a ratio of 1:101 (vol/vol). Presence of anti SARS-CoV-2 IgG antibodies in the CP was measured using the following formula: Ratio = Extinction of the control or CP samples/Extinction of calibrator (Ratio ≥ 1.1 is interpreted as positive). In case of recipient patients the amount of IgG received was calculated considering the amount (ml) of CP transfused to them.

Standard-of-care

At the clinical trial site (ID & BG Hospital, Kolkata, India) standard-of-care (SOC) in all patients with evidence for ARDS were: O2 therapy as per requirement in all patients, dexamethasone or equivalent corticosteroid in all patients, for patients with D-dimer <1000 Fibrinogen Equivalent Units (FEU) prophylactic anticoagulation and for patients with D-dimer >1000 ng/ml FEU therapeutic anticoagulation using either low molecular weight heparin or unfractionated heparin, appropriate broad-spectrum antibiotic therapy based on clinical and microbiological assessment, blood sugar was maintained below 200mg/dl using appropriate anti-diabetic therapy, appropriate anti-hypertensive agents were used to maintain systolic blood pressure 100-140 mm of Hg, diastolic blood pressure at 70-90 mm of Hg and mean arterial pressure >65 mm of Hg. Awake proning for 6-8 hours/day was attempted in all patients with ARDS. O2 therapy was designed to maintain SpO2 >95% using different devices with different efficiencies in O2 supplementation (as denoted by FiO2), viz. nasal cannula, face mask, face mask with reservoir, and in patients unable to maintain SpO2 above 90% with face mask with reservoir, high flow nasal cannula (HFNC) or in some cases mechanical ventilation (MV). For S/F ratio kinetics, a value of 89.99 was used for data-points where either HFNC or MV was in use. Of note S/F ratio kinetics was analyzed only in patients having data for at least one intervening time-point in addition to day 1 and day 5.

Convalescent plasma therapy

CP was collected from convalescent donors (recovered from RT-PCR positive SARS-CoV-2 infection at least 28 days prior to donation) by apheresis at the Department of Blood Transfusion and Immunohematology, Medical College Hospital, Kolkata, India. All donors were tested for their anti-Spike IgG content in addition to routine screening tests to exclude major blood borne pathogens before apheresis. We randomized the ARDS patients into either standard-of-care (SOC) group as controls or added two consecutive doses of ABO-matched 200ml CP on two consecutive days to their standard care (CPT group), the first transfusion being on the day of recruitment (Day 1).

Co-occurrence analyses

Co-occurrence among each pair of cytokines in patient plasma was calculated using Pearson correlation (r). Absolute values of the cytokines were used for the calculation of correlation network and threshold was set to $r>0.5$, $p<0.01$ for the complete set of cytokines from mild (n=13) and ARDS (n=33) conditions, and to $r>0.3$, $p<0.05$ for the significantly different cytokines between mild and ARDS and between SOC (n=16) and CPT (n=17) sets at T2. All calculations were done using 'Hmisc' R package and converted to a network file using 'igraph'. Visualisation of the network was performed using Cytoscape. Each cytokine was color coded and node size was set in proportion to the median fold change as compared to the same cytokine in the mild datasets.

Statistical analyses

Differential abundance of cytokines was evaluated by Mann-Whitney U Test and Wilcoxon Matched Pairs Test for categorical and ordinal data, respectively. To test the monotonous relationship between CT value and plasma cytokine, spearman's rank correlation was used, as was done between nAb content and IgG antibody content for CP. In order to explain the $SFR_{5D}AUC$ as a function of a combination of cytokines and nAb, linear regression was applied on the ranks of each of the parameters and was implemented using R. The statistical significance was defined as a p value <0.05 (*) and p value <0.01 (**) with two-sided tests, unless otherwise mentioned. Statistical tests were also performed in Statistica 64 (StatSoft).

RESULTS

Subject recruitment and sampling

We recruited patients suffering from COVID-19 at the ID & BG Hospital, Kolkata, India, who either had mild COVID-19 disease (WHO Clinical Progression Score 1-4; N=13, 12 males and 1 female, aged 41.1 ± 11.2 years, enrolled after 2.5 ± 2 days after hospitalization) or more severe disease showing evidence for ARDS with PaO2/FiO2 ratio between 100-300 (WHO Clinical Progression Score 5-6;

N=33, 26 males and 7 females, aged 60.03 ± 11.5 years, enrolled after 4 ± 2.8 days after hospitalization). We randomized the recruited ARDS patients into either standard-of-care (SOC) group or added two doses of 200ml CP on two consecutive days to their standard care (CPT group). First transfusion of ABO-matched convalescent plasma was done on the day of enrolment (day 1), followed by another transfusion on the next day (day 2). Patient plasma samples were taken on day 1 before CP transfusion (time-point 1 or T1) and again on day 3 or 4 post-transfusion (time-point 2 or T2) to assess the rapid effects of CP transfusion on the systemic cytokine milieu.

Nature of systemic hyper-inflammation and its relationship with viral load in severe COVID-19

First, we analyzed a panel of 48 cytokines in plasma at T1 for deeper characterization of the systemic hyper-inflammation through comparison between patients having milder disease and patients with evidence of ARDS. We identified a panel of 14 molecules that were significantly higher in ARDS patients (Figure 1A, Supplemental figure 1). In addition, we found a significant decrease in plasma abundance of TNF-related apoptosis-inducing ligand (TRAIL) in ARDS.

The viral load (estimated by average cycle threshold values from real time PCR for two viral genes from nasopharyngeal swabs, collected concomitant to plasma sampling) was significantly higher (lower cycle threshold values) in patients with milder disease, perhaps due to earlier sampling in their disease course (Figure 1B). Plasma abundance of a number of cytokines in mild disease was negatively correlated with concomitant viral load, presumably representing the cytokine component of an efficient protective immunity in the milder phase (Figure 1C). Interestingly, in patients with ARDS there was rather a significant positive correlation of viral load with a few specific cytokines (Figure 1C). Increasing age of the patients was significantly correlated with higher plasma abundance of a number of cytokines, though except IFNγ none of them were significantly associated with ARDS (Figure 1D).

Severe COVID-19 is characterized by correlated induction of multiple cytokines

Next to gather some insight on the dimensions of the systemic hyper-inflammation, we constructed the correlative networks (Pearson correlation, with a cut-off of $R > 0.5$, $p < 0.01$) among individual members of the whole panel of cytokines and compared between mild and ARDS patients (Supplemental figure 2A,B). We also separately constructed similar correlative networks (Pearson correlation, with a cut-off of $R > 0.5$, $p < 0.05$) among the cytokines that were found to be significantly dysregulated in ARDS (Figure 2). Interestingly, in ARDS we found more robust assimilation of

correlative networks among the cytokines with greater number of edges compared to mild disease (156 edges in mild vs 356 edges in ARDS among the whole panel of 48 cytokines and 23 edges in mild disease vs 52 edges in ARDS among the cytokines significantly dysregulated in ARDS). In this analysis most notable was a five member cytokine module, comprising of IL-6, monocytes chemotactic protein 3 (MCP3), MIP1α, IL-1RA and interferon gamma induced protein 10 (IP10), showing robust correlative upregulation. On the other hand, a notable omission from this correlative network, despite a significantly higher abundance in ARDS, was that of the mesenchymal cell-derived pleiotropic cytokine hepatocyte growth factor (HGF).

Rapid attenuation of systemic cytokine levels in response to therapy

We then analyzed the aforementioned cytokine panel in all ARDS patients at T2 and explored if the T1 to T2 change was different between SOC and CPT groups. Intriguingly, on analyzing the panel of 15 cytokines depicted in Figure 1A, we found a notable effect of CPT, as compared to SOC, in reducing the levels of a number of them, viz. IL-6, IP10 and macrophage colony stimulating factor (MCSF) (Figure 3A,B; Supplemental table 1). On the other hand, none of the major cytokines driving the hyper-inflammatory state was found to be significantly reduced at T2 in patients receiving SOC (Supplemental table 1). This anti-inflammatory effect was dependent on neither the anti-SARS-CoV-2 spike Immunoglobulin G (IgG) content of the transfused CP nor its nAb content (Figure 3A). Of note here, we found strong correlation between anti-spike IgG content of CP and its nAb content, as measured in an in vitro assay [16] ($R=0.8564$, $P<0.0001$; Figure 3C).

This anti-inflammatory effect of CP was also apparent when we compared the correlative networks at T2 of both the whole panel of cytokines (Supplemental figure 2 C,D) as well as among the 15 cytokines found to be significantly dysregulated in ARDS (Figure 4) and compared them between the SOC and CPT groups. Residual correlative edges among the cytokines at T2 were significantly less in number in case of CPT in contrast to SOC group (334 edges in SOC group vs 135 edges in CPT group among the whole panel of 48 cytokines and 47 edges in SOC group vs 33 edges in CPT group among cytokines significantly dysregulated in ARDS), representing an attenuation of the systemic hyper-inflammation in response to CPT and a trend toward a cytokine milieu quite similar to one found in the early milder phase.

Effect of convalescent plasma on rapid mitigation of hypoxia

To explore if the effect of therapy on the systemic hyper-inflammation was correlated with concomitant clinical status of the ARDS patients, we decided to assess their requirement for oxygen supplementation (as measured by fraction of inspired oxygen or FiO2) for maintaining O2 saturation of circulating hemoglobin at a physiological level (O2 saturation as measured by pulse oximetry or SpO2). This offered a quantifiable, comparable and relevant clinical parameter across all ARDS patients. Thus the short-term clinical outcome in the ARDS patients were assessed by the kinetics of SpO2/FiO2 ratio (S/F ratio) for 5 days following enrolment (Figure 5A). This data was then processed to represent clinical improvement over 5 days with respect to day 1 by calculating the area under curve (AUC) for the S/F Ratio curve ($SFR_{5D}AUC$) (Figure 5B). CPT was found to affect faster mitigation of hypoxia, as compared to patients receiving SOC only (Figures 5A, B). We noted gradual abrogation of this differential response on reductions in hypoxia third day onwards after 2^{nd} dose of CPT, indicating that a sustained benefit may require additional transfusions of CPT in some patients.

Reduction in the chemokine MCP3 at T2, as compared to T1 was significantly associated with mitigation of hypoxia, irrespective of whether patients received CPT or not, thus identifying a major pathogenic molecule underlying COVID-19 ARDS (Figure 5C). In patients receiving CPT, as expected, improvements in S/F ratio was significantly correlated with nAb content of CP they were transfused with. Interestingly, in a linear regression analysis we identified that IL-6 and IP10, the two major ARDS-associated cytokines that were affected by the anti-inflammatory effect of CPT as described in Figure 3A, also played a major role in this rapid mitigation of hypoxia in combination with nAb content (Figure 5D; Supplemental table 2).

DISCUSSION

In this study we have been able to chart the nature of the characteristic immune hyper-activation associated with diseases severity in COVID-19 patients, to a considerable resolution, by looking at a great number of cytokines at two timepoints. A panel of 14 cytokines dominated the systemic deluge, in terms of statistically significant increase in their plasma abundance compared to mild disease. Plasma abundance of a single cytokine, TRAIL, was found to be significantly reduced in severe COVID-19 patients. TRAIL is known to be expressed in cytotoxic T cells and NK cells and involved in killing of virus-infected host cells [17]. Decrease in circulating TRAIL may signify obviation of infected host cell-directed cytotoxicity at this later phase of the disease.

In patients with ARDS there was rather a significant positive correlation of viral load with a few specific cytokines. Among them IL-8 and G-CSF presumably represent the usual neutrophil recruitment response triggered by residual virus-infected cells. Notably most of the major cytokine components of the systemic hyper-inflammation, viz. IL-6, IFNγ, IL-1RA, MIP1α, seemed not to correlate any way with the concomitant viral load in patients with ARDS. But notable exception here were MCP3 and IP10, which may represent pathogenic links between host-virus interactions in the earlier phase of the disease and the hyper-immune activation that ensue later in a fraction of patients and warrant further mechanistic studies. Although the number of patients recruited in the study was small the statistical robustness makes the findings notable.

As discussed earlier, despite a significantly higher abundance in ARDS, the mesenchymal cell-derived pleiotropic cytokine HGF was interestingly not part of the correlative network of cytokines at any time-point. Systemic abundance of HGF perhaps represents an anti-inflammatory as well as tissue regeneration response at the face of the systemic inflammatory assault, as shown earlier [18]. Although a proinflammatory role of HGF, targeting neutrophils through c-MET receptor signaling, has also been described recently and thus may be of interest to explore in the context of severe COVID-19 [19].

On analyzing the kinetics of the cytokine components of the systemic hyper-inflammation in both the SOC and CPT groups after enrolment we noted reduced abundance of a number of major cytokines preferentially in patients receiving CPT. Moreover, this cytokine attenuation effect was not found to be correlated with the anti SARS-CoV2 spike IgG content or the nAb content of the transfused CP. Of note, beyond providing specific pathogen-neutralizing antibodies, CP has the potential to drive other biological effects, for example immunomodulation as well as endothelial stabilization [20, 21]. This may result from other constituents of CP, viz. non-specific immunoglobulins acting through the Fc receptors, anti-inflammatory components of the complement system, coagulation factors, in addition to other anti-inflammatory proteins and cytokines [20].

A number of clinical trials, with varying study designs and scopes, are ongoing in different parts of the world that are evaluating therapeutic efficacy of CPT in COVID-19. Therapeutic use of CPT was not found to have any considerable safety issue in a large study in USA [8]. But there have been contradictory reports in terms of measurable clinical benefits offered by CPT. A number of RCTs

could not register any clinical benefit of CPT in severe COVID-19 patients [22, 23]. On the other hand in several case series early into the pandemic as well as in a few RCTs therapeutic benefits were recorded [24-26]. In view of the variable reports it is imperative to explore if there are patient subgroups who may or may not benefit from CPT.

We report here that beneficial effect of CPT perhaps mechanistically goes beyond just passive immunization of the recipients and thus should further be explored mechanistically to identify other anti-inflammatory factors in CP. We envisage that this anti-inflammatory effect of CPT may affect mitigation of other longer term systemic effects of the hyper-inflammation encountered in severe COVID-19, full appreciation of which awaits end-point analyses in our trial as well as further meta-analyses of data from other clinical trials on CPT accomplished or ongoing elsewhere.

Thus, this study characterizes the nature and dimensions of the systemic hyper-inflammation in patients suffering from acute respiratory distress syndrome, which could identify a number of hitherto unappreciated features of the disease pathogenesis in severe COVID-19. Moreover, we report here an anti-inflammatory effect of COVID-19 convalescent plasma, independent of, but acting in synergy with, its neutralizing antibody content, which may prove to be a composite predictor of response to convalescent plasma therapy in COVID-19 and should be explored while analyzing the clinical outcomes of trials ongoing throughout the world.

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Author contributions. D.G. conceptualized the study. D.G. and Y.R. designed the study protocol. P.Ba. and R.D. did the plasma cytokine analysis. J.S. performed serological studies. S.P. and A.L. contributed to the analysis and computation. Y.R. and S.R.P. recruited patients, maintained clinical data and supervised clinical management. R.R., R.M., K.C., S.B., A.M., M.M.R., B.S.S., D.R., R.C., B.S. contributed to patient management. J.S.V., S.S. and R.P. did the RT-PCR for SARS-CoV2. S.C. contributed in immunological studies. S.P. and P.Bh. recruited convalescent donors, D.B., C.M. and P.Bh. performed donor screening, apheresis and biobanking of convalescent plasma. D.G. and S.P. interpreted data, D.G. wrote the manuscript. All authors approved the manuscript.

Conflicts of interest. The authors declare no competing interests.

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Figure legends

Figure 1. Nature of the systemic hyper-inflammation in severe Covid-19 disease. A. Heatmap representing normalized plasma abundance of 15 major cytokines compared between patients with mild disease (n=13) or ARDS (n=33) at enrolment, made with Morpheus. Rows are clustered using one minus Pearson correlation distance and average linkage. **B.** Comparison between the CT values of RT-PCR for SARS-CoV2 target genes from the nasopharyngeal swabs from patients with mild disease and patients progressing to ARDS. **C.** Extent of correlation between the plasma levels of cytokines and concomitant viral loads as measured by average cycle threshold values for real time PCR of two SARS-CoV2 target genes, compared between patients with mild disease and ARDS. Spearman correlation values are arranged in ascending order and significance are marked by either * ($P<0.05$) or ** ($P<0.01$). **D.** Plasma level of cytokines having significant ($P<0.05$) correlation with age of patients with ARDS. The Spearman R value is given for each of the colour coded cytokine with age.

Figure 2. Correlative dynamics of the plasma cytokine abundance in severe COVID-19. Correlation network of major cytokines at T1 for both mild disease group (n=13) and ARDS group (n=33) are shown. Pearson correlations with $R> 0.3$ and $P<0.05$ are considered.

Figure 3. Attenuation of hyper-inflammation and mitigation of hypoxia in response to convalescent plasma. A. Heatmap representing log2 fold change between T1 and T2 in the plasma abundance of major cytokines associated with ARDS compared between SOC (n=16) and CPT (n=17) groups. Heatmap is made with Morpheus and rows are clustered using one minus Pearson correlation distance and average linkage. Anti-Spike IgG content and nAb content of the convalescent plasma transfused are represented for the respective patients in the CPT group. **B.** Reduction in plasma levels of IL-6, IP10 and M-CSF from T1 to T2, compared between SOC and CPT groups. The percentage difference of median value at T2 time point in compare to T1 was also calculated for three of the cytokines. **C.** Correlation between the anti SARS-CoV2 spike IgG content of convalescent plasma transfused to patients and their neutralization efficacy in the in vitro assay.

Figure 4. Rapid effect of therapy on the correlative dynamics of cytokine strom in severe COVID-19. Correlation network of major cytokines at T2 compared between SOC (n=16) and CPT (n=17) groups ar e shown. Pearson correlations with R> 0.3 and P<0.05 are considered.

Figure 5. Role of convalescent plasma on rapid mitigation of hypoxia in severe COVID-19. A. Kinetics of SpO2/FiO2 ratio in patients over five days post-enrolment, compared between SOC and CPT groups. Comparison of day means was performed by Welch's t-test and significant differences are marked by * (P<0.05). The plasma transfusion days are also marked by arrows. **B.** Violin plot represents the comparison of $SFR_{5D}AUC$ for the patients between SOC and CPT groups. **C.** Correlation of log2 fold change between T1 and T2 for plasma level of MCP3 and $SFR_{5D}AUC$. The Spearman correlations values for SOC and CPT groups are given with their significance level. **D.** $SFR_{5D}AUC$ relationships with nAb content of transfused plasma, log2 fold change of plasma levels of IL-6 and IP10 in the CPT group. The plot is generated by plotly package in R.

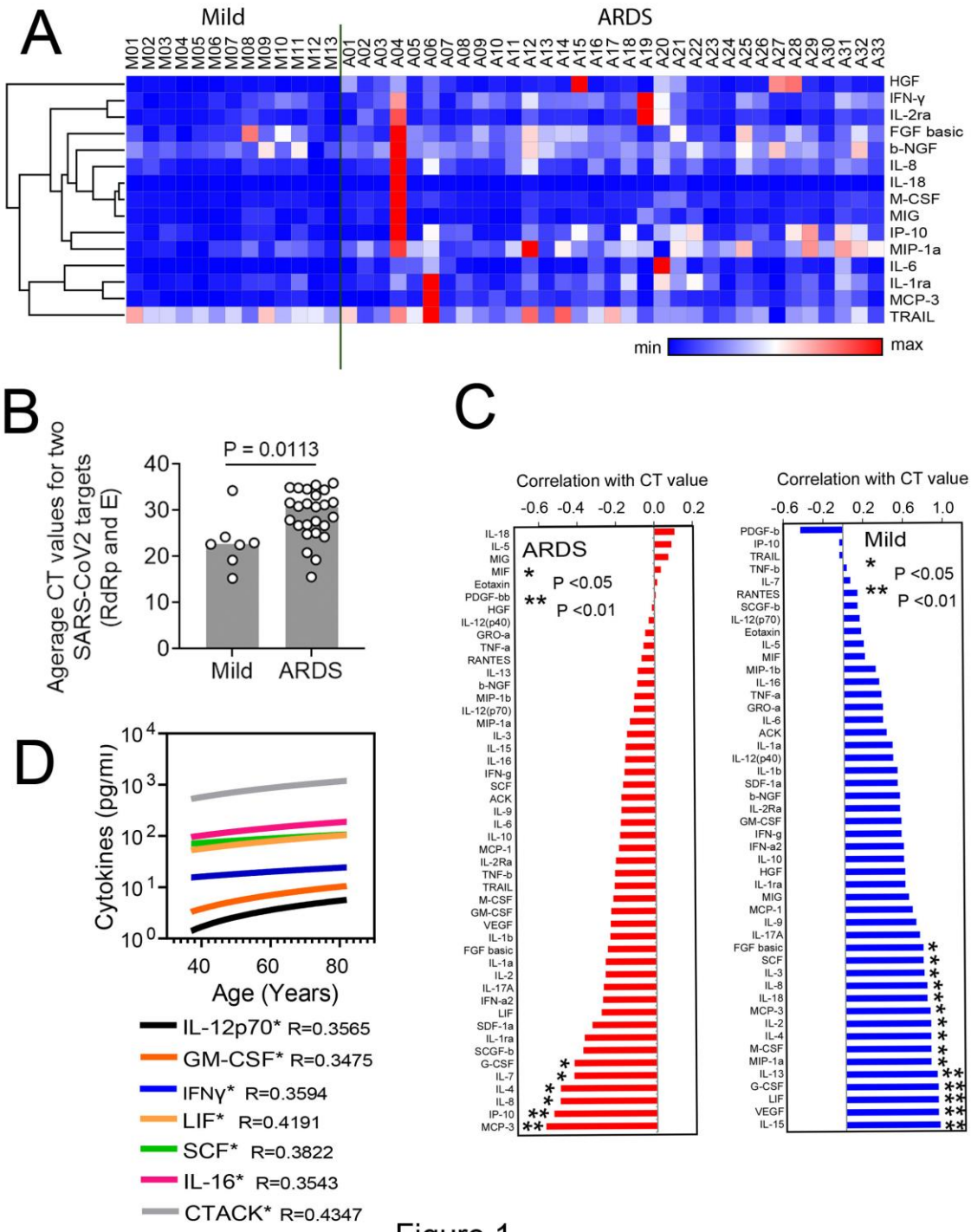
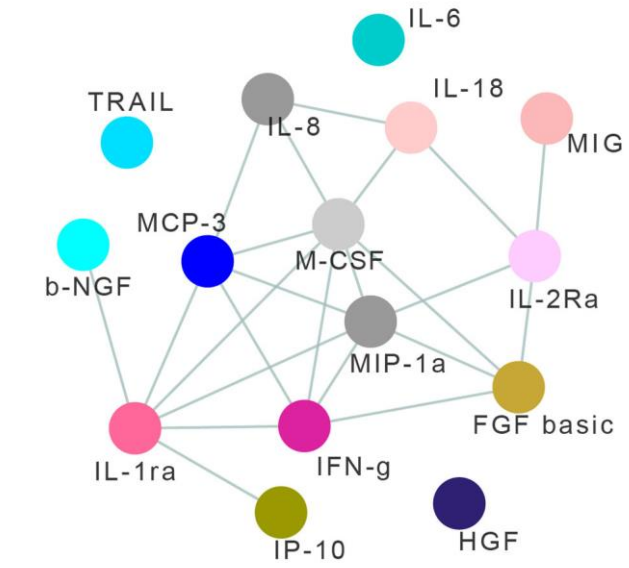
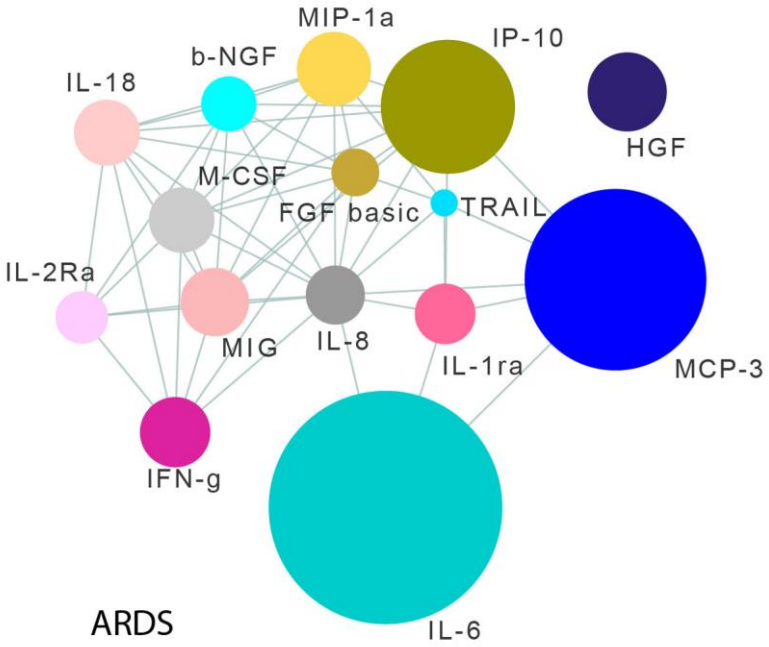

A
Mild
ARDS
M01 M02 M03 M04 M05 M06 M07 M08 M09 M10 M11 M12 M13
A01 A02 A03 A04 A05 A06 A07 A08 A09 A10 A11 A12 A13 A14 A15 A16 A17 A18 A19 A20 A21 A22 A23 A24 A25 A26 A27 A28 A29 A30 A31 A32 A33
HGF
IFN-γ
IL-2ra
FGF basic
b-NGF
IL-8
IL-18
M-CSF
MIG
IP-10
MIP-1a
IL-6
IL-1ra
MCP-3
TRAIL
min
max
B
Agerage CT values for two SARS-CoV2 targets (RdRp and E)
P = 0.0113
40
30
20
10
0
Mild
ARDS
C
Correlation with CT value
-0.6 -0.4 -0.2 0.0 0.2
ARDS
* P <0.05
** P <0.01
IL-18
IL-5
MIG
MIF
Eotaxin
PDGF-bb
HGF
IL-12(p40)
GRO-a
TNF-a
RANTES
IL-13
b-NGF
MIP-1b
IL-12(p70)
MIP-1a
IL-3
IL-15
IL-16
IFN-g
SCF
ACK
IL-9
IL-6
IL-10
MCP-1
IL-2Ra
TNF-b
TRAIL
M-CSF
GM-CSF
VEGF
IL-1b
FGF basic
IL-1a
IL-2
IL-17A
IFN-a2
LIF
SDF-1a
IL-1ra
SCGF-b
G-CSF
IL-7
IL-4
IL-8
IP-10
MCP-3
Correlation with CT value
-0.6 -0.2 0.2 0.6 1.0
Mild
* P <0.05
** P <0.01
PDGF-b
IP-10
TRAIL
TNF-b
IL-7
RANTES
SCGF-b
IL-12(p70)
Eotaxin
IL-5
MIF
MIP-1b
IL-16
TNF-a
GRO-a
IL-6
ACK
IL-1a
IL-12(p40)
IL-1b
SDF-1a
b-NGF
IL-2Ra
GM-CSF
IFN-g
IFN-a2
IL-10
HGF
IL-1ra
MIG
MCP-1
IL-9
IL-17A
FGF basic
SCF
IL-3
IL-8
IL-18
MCP-3
IL-2
IL-4
M-CSF
MIP-1a
IL-13
G-CSF
LIF
VEGF
IL-15
D
Cytokines (pg/ml)
10^4
10^3
10^2
10^1
10^0
40 60 80
Age (Years)
IL-12p70* R=0.3565
GM-CSF* R=0.3475
IFNγ* R=0.3594
LIF* R=0.4191
SCF* R=0.3822
IL-16* R=0.3543
CTACK* R=0.4347

Figure 1



Mild



ARDS

Figure 2

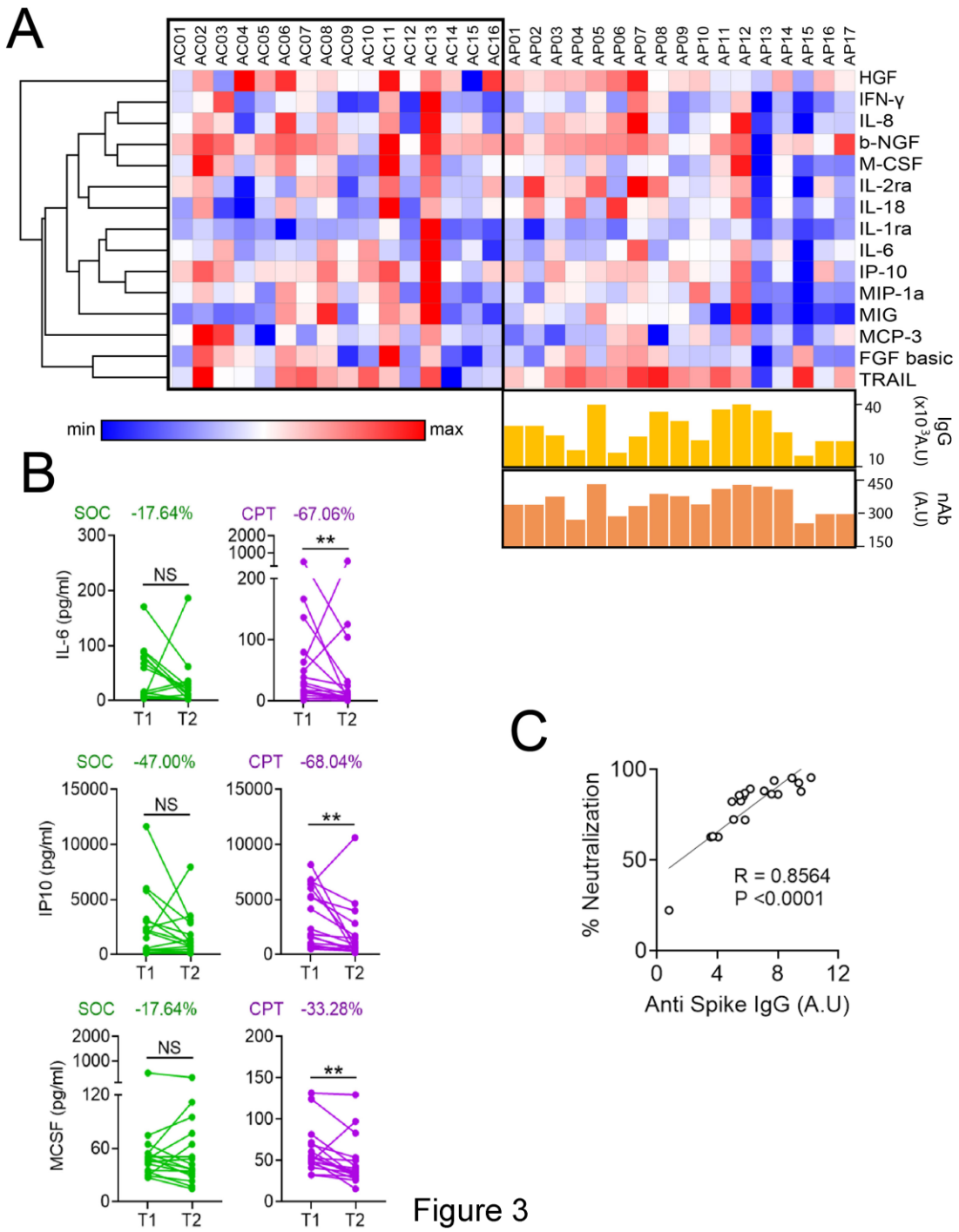

A
AC01 AC02 AC03 AC04 AC05 AC06 AC07 AC08 AC09 AC10 AC11 AC12 AC13 AC14 AC15 AC16
AP01 AP02 AP03 AP04 AP05 AP06 AP07 AP08 AP09 AP10 AP11 AP12 AP13 AP14 AP15 AP16 AP17
HGF
IFN-γ
IL-8
b-NGF
M-CSF
IL-2ra
IL-18
IL-1ra
IL-6
IP-10
MIP-1a
MIG
MCP-3
FGF basic
TRAIL
min
max
IgG (x10³A.U)
nAb (A.U)
B
SOC -17.64%
CPT -67.06%
IL-6 (pg/ml)
NS
**
SOC -47.00%
CPT -68.04%
IP10 (pg/ml)
SOC -17.64%
CPT -33.28%
MCSF (pg/ml)
T1
T2
C
% Neutralization
R = 0.8564
P <0.0001
Anti Spike IgG (A.U)

Figure 3

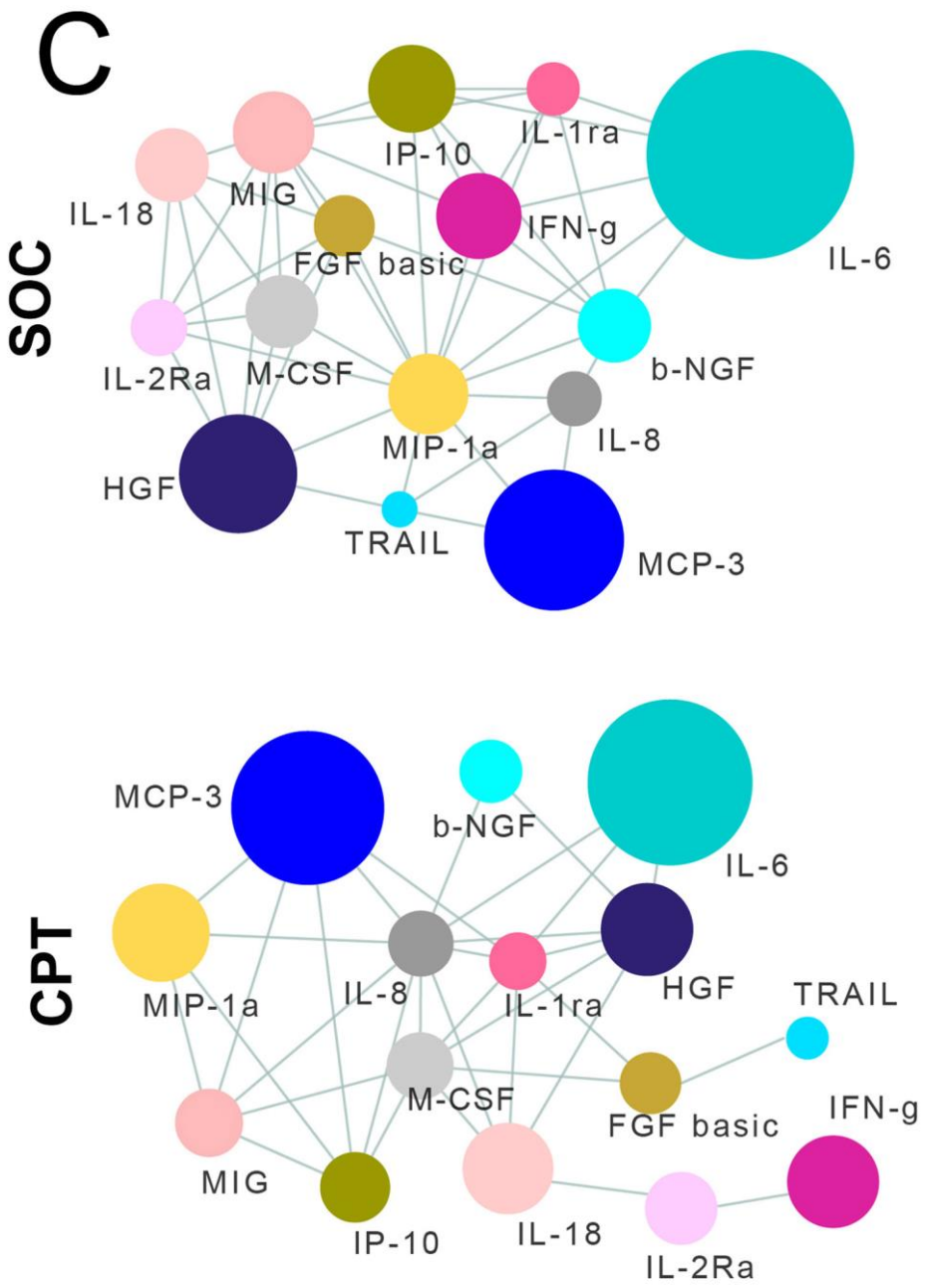

C
SOC
IP-10
IL-1ra
IL-18
MIG
IFN-g
FGF basic
IL-6
IL-2Ra
M-CSF
b-NGF
MIP-1a
IL-8
HGF
TRAIL
MCP-3
CPT
MCP-3
b-NGF
IL-6
MIP-1a
IL-8
IL-1ra
HGF
TRAIL
M-CSF
FGF basic
IFN-g
MIG
IP-10
IL-18
IL-2Ra

Figure 4

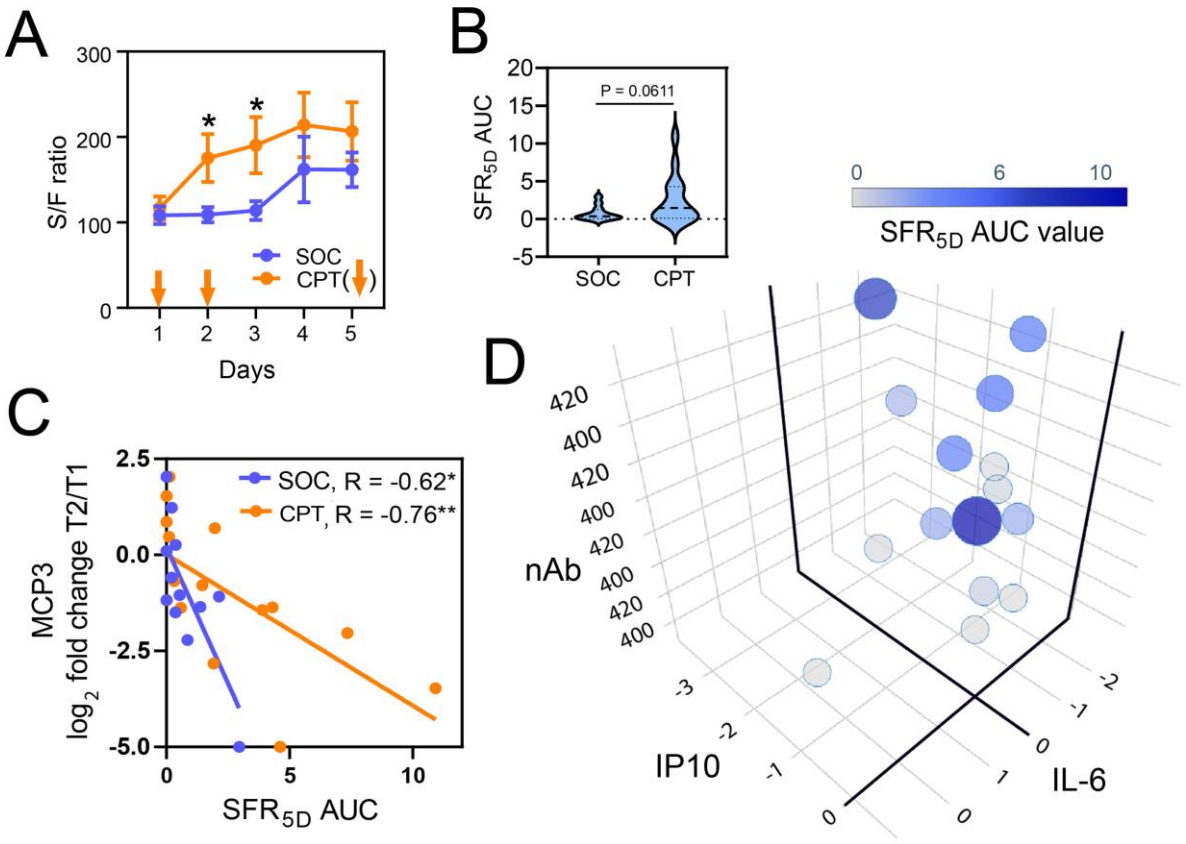

A
300
200
100
0
S/F ratio
*
*
SOC
CPT(↓)
1
2
3
4
5
Days
B
20
15
10
5
0
-5
SFR5D AUC
P = 0.0611
SOC
CPT
0
6
10
SFR5D AUC value
C
2.5
0.0
-2.5
-5.0
MCP3
log2 fold change T2/T1
SOC, R = -0.62*
CPT, R = -0.76**
0
5
10
SFR5D AUC
D
nAb
420
400
IP10
IL-6
-3
-2
-1
0
1
2

Figure 5