Characterization of the Inflammatory Response to Severe COVID-19 Illness

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ONLINE DATA SUPPLEMENT

Legends:

Figure E1. IL-1β:IL-10 ratio is increased in severe COVID-19 illness.

Plasma was obtained from $COVID_{stable}$ (n=20), $COVID_{ICU}$ (n=20) and CAP_{ICU} (n=15). IL-1β:IL-10 was significantly increased in $COVID_{ICU}$ (0.97+/-0.39) compared to $COVID_{stable}$ (0.27+/-0.12) and CAP_{ICU} (0.37+/-0.07), both $P<0.0001$).

Figure E2. Production of AAT by primary hepatocytes is increased in response to IL-6.

(A) AAT qRT-PCR results of primary hepatocytes (HepG2 cell line) following exposure to human IL-6 (50pg/ml) for 48 h. AAT gene expression was significantly increased in the presence of IL-6 at both time points (n=3, $P<0.05$). (B) The level of AAT secreted from HepG2 cells at 48 h following treatment with IL-6 was increased 1.5-fold (n=3, $P<0.05$).

Figure E3. Individual trends in circulating levels of AAT and IL-6 over time in the $COVID_{ICU}$ cohort.

(A) Circulating levels of AAT over time in ICU are displayed for the $COVID_{ICU}$ patients included in Fig. 3C. Patients who went on to good outcome are indicated in blue, while those who went on to poor outcome are in red. (B) Plasma IL-6 for the same individuals.

Figure E4. Individual IL-6:AAT ratio values over time in the $COVID_{ICU}$ cohort.

The IL-6:AAT ratio for each patient in the $COVID_{ICU}$ group included in Fig. 3C at each time point is depicted. Patients who went on to good outcome are indicated in blue, while those who went on to poor outcome are indicated in red. We also investigated whether outcome would

associate with a worsening or improving IL-6:AAT ratio, if at all, taking good outcome and poor outcome as ordinal variables, and the slope of the IL-6:AAT ratio as a continuous variable, For each patient who experienced a bad outcome, their IL-6:AAT ratio worsened – a negative slope of ratio over time – while each patient who had a good outcome had a positive slope. The R^2 value when Pearson's correlation was applied to the data set was 0.9.

Table E1. Protease inhibitor cocktail constituents

Protease inhibitor (1µl/ml)	Description
Tosyl-L-lysyl-chloromethane hydrochloride	Serine protease inhibitor
Pepstatin A	Inhibitor of aspartyl proteases
Pefabloc	Serine protease inhibitor
Ethylenediaminetetraacetic acid	Metallopeptidase inhibitor
Leupeptin	Inhibitor of cysteine, serine and threonine peptidases
Sodium fluoride	Phosphatase inhibitor
Sodium orthovanadate	Phosphatase inhibitor
Phenylmethylsulfonyl fluoride	Serine protease inhibitor

Table E2. Details of key resources used.

Reagent or resource	Supplier/source	Identifier
Antibodies		
PKM2	Cell Signaling Technology	#3198
phospho-PKM2 (Tyr105)	Cell Signaling Technology	#3827
HIF-1α	Cell Signaling Technology	#36169
β-actin	Cell Signaling Technology	#4267
Lamin B1	Cell Signaling Technology	#13435
Anti-rabbit IgG (HRP-linked)	Cell Signaling Technology	#7074
Anti-mouse IgG (HRP-linked)	Cell Signaling Technology	#7076
Critical Commercial Assays		
IL-1β ELISA (sensitivity: 1 pg/ml)	R&D	#DLB50
IL-6 ELISA (sensitivity: 0.7 pg/ml)	R&D	#D6050
IL-8 ELISA (sensitivity: 7.5 pg/ml)	R&D	#D8000C
IL-10 ELISA (sensitivity: 3.9 pg/ml)	R&D	#D1000B
sTNFR1 ELISA (sensitivity: 1.2 pg/ml)	R&D	#DRT100
Succinate assay	Abcam	#ab204718
Lactate assay	Abcam	#ab65330
Pyruvate assay	Abcam	#ab65342
Software and hardware		
Graphpad Prism 8.0	www.graphpad.com	sales@graphpad.com
SpectraMax M3 plate reader	Molecular Devices	N/A
Chemi Doc MP System	Bio-Rad	#17001402
Image Lab	Bio-Rad	https://www.selectscience.net/products/image-lab-software-(170-9690)/?prodID=115956
Hydrasys electrophoresis platform	Sebia	#PN1200
Other		
Nuclear extract kit	Active Motif	#40010
Hydragel 18 A1AT Isofocusing kit	Sebia	#PN4357

Table E3. Clinical features of the COVID$_{ICU}$ cohort at time of ICU admission

Parameter	Value
Temperature >38^{o}C	8 (66)
Heart rate >100 beats per minute	7 (58)
Respiratory rate >20 breaths per minute	12 (100)
SaO_2 <80% or requiring FiO_2 ≥60%	11 (92)
PaO_2	7.15 +/- 1.41
Acute confusion	3 (25)
Mean arterial pressure	86.25 +/- 13.16
qSOFA score	1.33 +/- 0.65
PaO_2:FiO_2 on arrival to ICU	157 +/- 67

Data presented as absolute number (%) or mean +/- SD; FiO_2: fraction of inspired oxygen; PaO_2: partial pressure of arterial oxygen in kPa; mean arterial pressure and PaO_2:FiO_2 in mmHg; qSOFA: quick sequential organ failure assessment.

Table E4. Clinical characteristics of the HC cohort

Number	15
Age in years	39.24 +/- 13.16
Male/female	9/6
Smoking history	
Current	0 (0)
Former	2 (13)
Never	13 (87)
Vaping history	
Current	0 (0)
Former	0 (0)
Never	15 (100)

Table E5. Clinical characteristics of the CAP_{ICU} cohort

Number	15
Age in years	59.66 +/- 17.82
Male/female	9/6
Airway microbiology	
Streptococcus pneumoniae	8 (53)
Haemophilus influenzae	3 (20)
Staphylococcus aureus	3 (20)
Pseudomonas aeruginosa	1 (7)
Positive respiratory viral swab	5 (33)
Comorbidities	
Hypertension	7 (47)
Coronary artery disease	7 (47)
Diabetes mellitus	2 (13)
Obesity	6 (40)
Chronic lung disease	6 (40)
Chronic kidney disease	6 (40)
Smoking history	
Current	5 (33)
Former	7 (47)
Never	3 (20)
Vaping history	
Current	1 (7)
Former	0 (0)
Never	14 (93)

Data presented as absolute number (%) or mean +/- SD; Some patients had more than one organism identified, therefore % totals for respiratory pathogens exceed 100%; Of those with S. aureus, two had recent influenza.

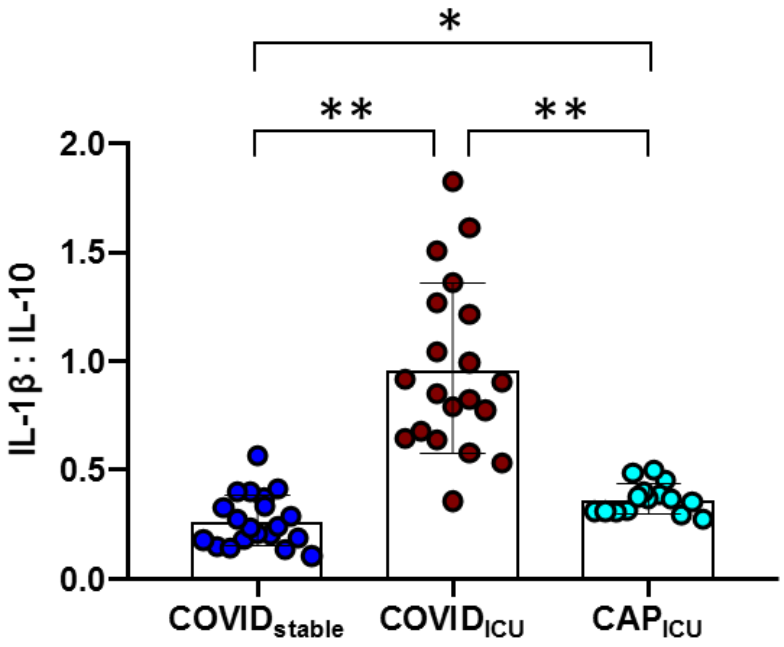

Figure E1.

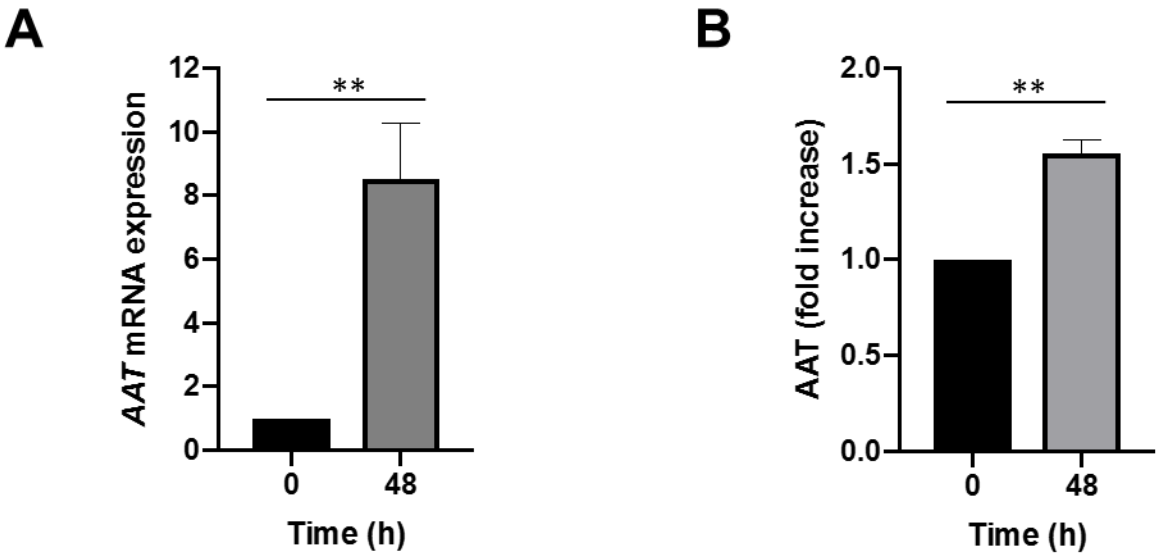
A
AAT mRNA expression
12
10
8
6
4
2
0
**
0
48
Time (h)
B
AAT (fold increase)
2.0
1.5
1.0
0.5
0.0
**
0
48
Time (h)

Figure E2.

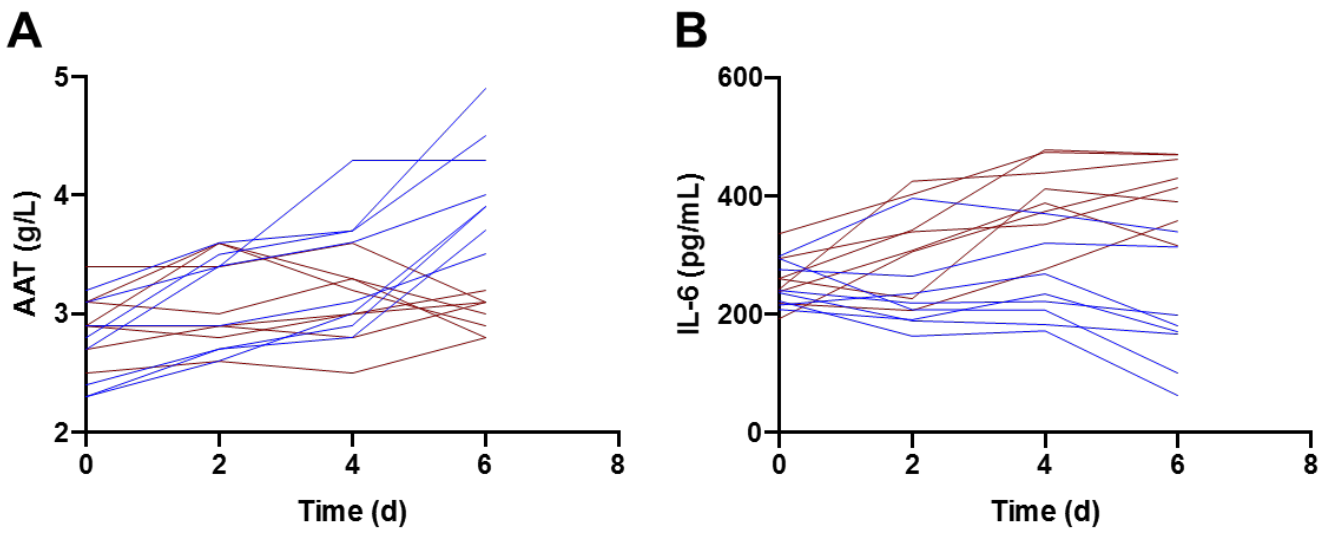

Figure E3.

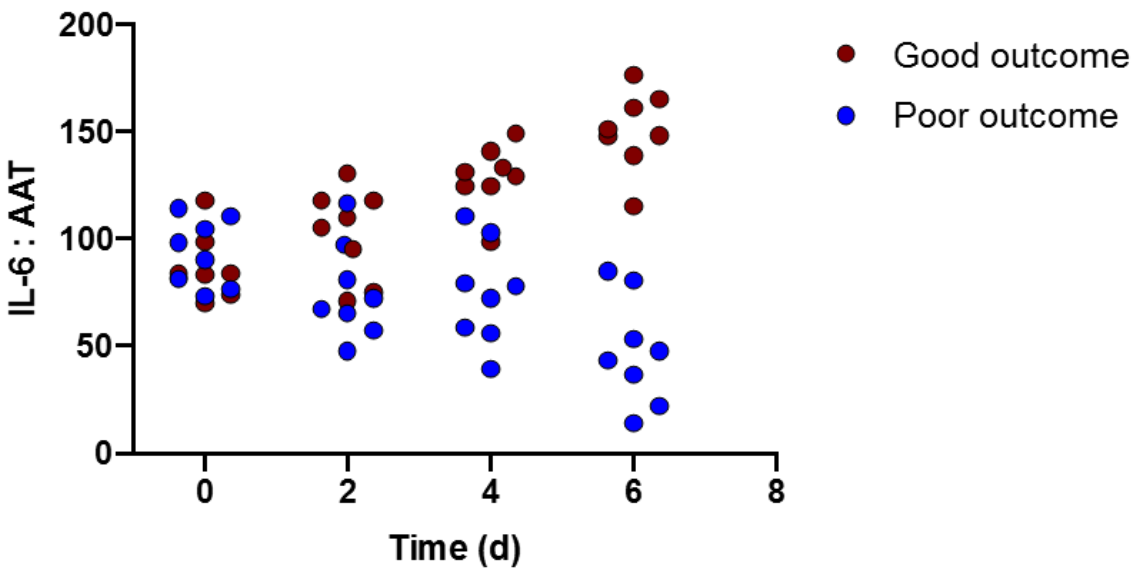
200
150
100
50
0
IL-6 : AAT
0
2
4
6
8
Time (d)
Good outcome
Poor outcome

Figure E4.