# Distinct cellular immune profiles in the airways and blood of critically ill patients with COVID-19

Anno Saris[1], Tom D.Y. Reijnders[1], Esther J. Nossent[2,3], Alex R. Schuurman[1], Jan Verhoeff[4,5], Saskia D. van Asten[4,5,6], Hetty J. Bontkes[7], Siebe G. Blok[2], Janwillem Duitman[1], Harm Jan Bogaard[2,3], Leo Heunks[8], Rene Lutter[2,9], Tom van der Poll[1,10] *, Juan J. Garcia Vallejo[4,5] *, on behalf of the ArtDECO consortium and the Amsterdam UMC COVID study group.

## Supplemental information

### Supplemental methods

#### Subjects and study approval

The current study was part of the Amsterdam Study for DEep Phenotyping of COVID-19 disease (ArtDECO) 1 study, a cohort study of COVID-19 patients with persistent ARDS (mechanical ventilation > seven days). Per clinical protocol obtained left over biological samples were stored in the anonymized research Amsterdam UMC COVID-19 biobank (#2020-182). Informed consent for the use of samples and data was deferred until discharge from the ICU. In case of death, informed consent was requested from the patient's relatives. Study procedure was approved by the Review Committee Biobank of the Amsterdam UMC (2020-065). All patients from whom BALF mononuclear cells were available were included in the present study. The study is in accordance with the declaration of Helsinki and adheres to Dutch regulations. Healthy control BALF was collected in two explorative studies, the explorative RILCA and RILCO trials (Dutch ethical committee study numbers: NL48912.018.14 & NL53354.018.15). BALF controls (n=8) had no (history of) respiratory disease or comorbidities, 5/8 were male and were 38.8±18.1 years of age, non-allergic, non-smoking (or non-smoking for at least one year) and had a BMI of 25.8 kg/$m_2$ (range: 17.8-31.1). PBMC controls (n=10) were collected as part of the ELDER-BIOME project (clinicaltrials.gov identifier NCT02928367) and upon recruitment had no active respiratory disease, 9/10 were male and were 62.8±14.1 years of age with a median BMI of 28.0 kg/$m_2$ (range: 21.2-32.0). Both the RILCA and RILCO studies were approved by the internal IRB and participants provided written informed consent.

#### Isolation of BALF, plasma, peripheral blood mononuclear cells and BALF mononuclear cells

Prior to diagnostic bronchoalveolar lavage in COVID-19 patients, which was performed once weekly when patients did not show signs of clinical improvement, venous blood was drawn in EDTA and heparin tubes. EDTA blood was centrifuged 10 min at 1800g and supernatant plasma was collected and stored at -80°C. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood samples using standard Ficoll-Paque density gradient centrifugation (1000g, 20min, 21°C). During diagnostic bronchoscopy 2 x 20 ml 0,9 % NaCl at a (sub)segmental level, each aspirated immediately with low suction. From this, 10ml was used for microbiological diagnostic purposes and the remaining



Saris A, *et al. Thorax* 2021; 76:1010–1019. doi: 10.1136/thoraxjnl-2020-216256

(~3-20ml) was centrifuged (300g, 10min, 4°C). BALF supernatant was stored at -80° and cell pellet was resuspended in 2mM dithiotreitol (Sigma, Zwijndrecht, the Netherlands) to solubilize sputum and mucus. After 30 min at 4°C, cells were washed with PBS+1%BSA and mononuclear cells were isolated using Ficoll-Paque density gradient centrifugation (see supplemental table 2 for BALF cell composition prior to ficoll isolation). PBMCs and BALF mononuclear cells (BALFMCs) were cryopreserved in liquid nitrogen until further analysis. Healthy BALF was collected by instilling eight successive 20 ml aliquots of pre-warmed 0.9% NaCl instilled at a (sub)segmental level, each aspirated immediately with low suction, according to the recommendations of the NHLBI and the National Institute of Allergy and Infectious Diseases.[17] Fractions 3-8 were pooled, centrifuged for 10 minutes at 267g at 4°C and the supernatant was stored at -80°C till further analyses.

## Spectral flow cytometry

Cells were thawed and subsequently washed in IMDM+10%FCS+75U/ml DNAse (Sigma). To minimize day-to-day variation, all samples were thawed and stained on the same day. Next, 1 million cells (or as many as available in case of BALFMCs) were stained with live/dead stain, and subsequently monoclonal antibodies added sequentially: CCR7, γδ T cell receptor, all non-brilliant (ultra) violet (BV/BUV) or non-brilliant blue (BB) labelled markers and finally all BV, BUV and BB labelled antibodies. See supplemental table 1 for a list of all antibodies used for this staining. Cells were measured using a 5 laser Aurora system (Cytek Biosciences, Fremont, CA) and data analysed with SpectroFlo (Cytek Biosciences, Fremont, CA) and OMIQ (OMIQ Inc, Santa Clara, CA). Six of thirty-one BALF samples were excluded because too few viable CD45 + cells were measured (i.e. less than 2.000) and another five samples were excluded due to active prednisolone therapy as part of standard clinical care. PBMC samples were only included if a paired BALF sample was available. CD3+ and CD3- cells were analysed separately in each compartment and to cater for the different yield of viable CD45+ cells in cluster analyses, a maximum of 45.000 PBMC (both CD3+ or CD3-) and 10.000 CD3+ and 30.000 CD3- BALFMC were included from each sample. Manual gating strategy is depicted in supplemental figure 1 (PBMCs) and 2 (BALFMCs) and an overview of T cell subsets is presented in supplemental table 2.

## Measurements of soluble immunological mediators

Cytokines and chemokines were measured using Human Magnetic Luminex Assay according to the manufacturer's instructions (#LKTM014, R&D systems, MN, USA). Samples that were above the upper limit of quantification were set at the upper detection limit of the assay, while samples below detection limit were set at half of the of the lower detection limit. Anti-RBD and anti-NP IgG antibodies were measured in EDTA plasma samples at 100-1200 fold dilutions using ELISA as previously described.[11] Plates were coated with RBD or N protein and specific IgG antibodies were detected using anti-human IgG (MH16, Sanquin).



Saris A, *et al. Thorax* 2021; 76:1010–1019. doi: 10.1136/thoraxjnl-2020-216256

## Statistical analysis

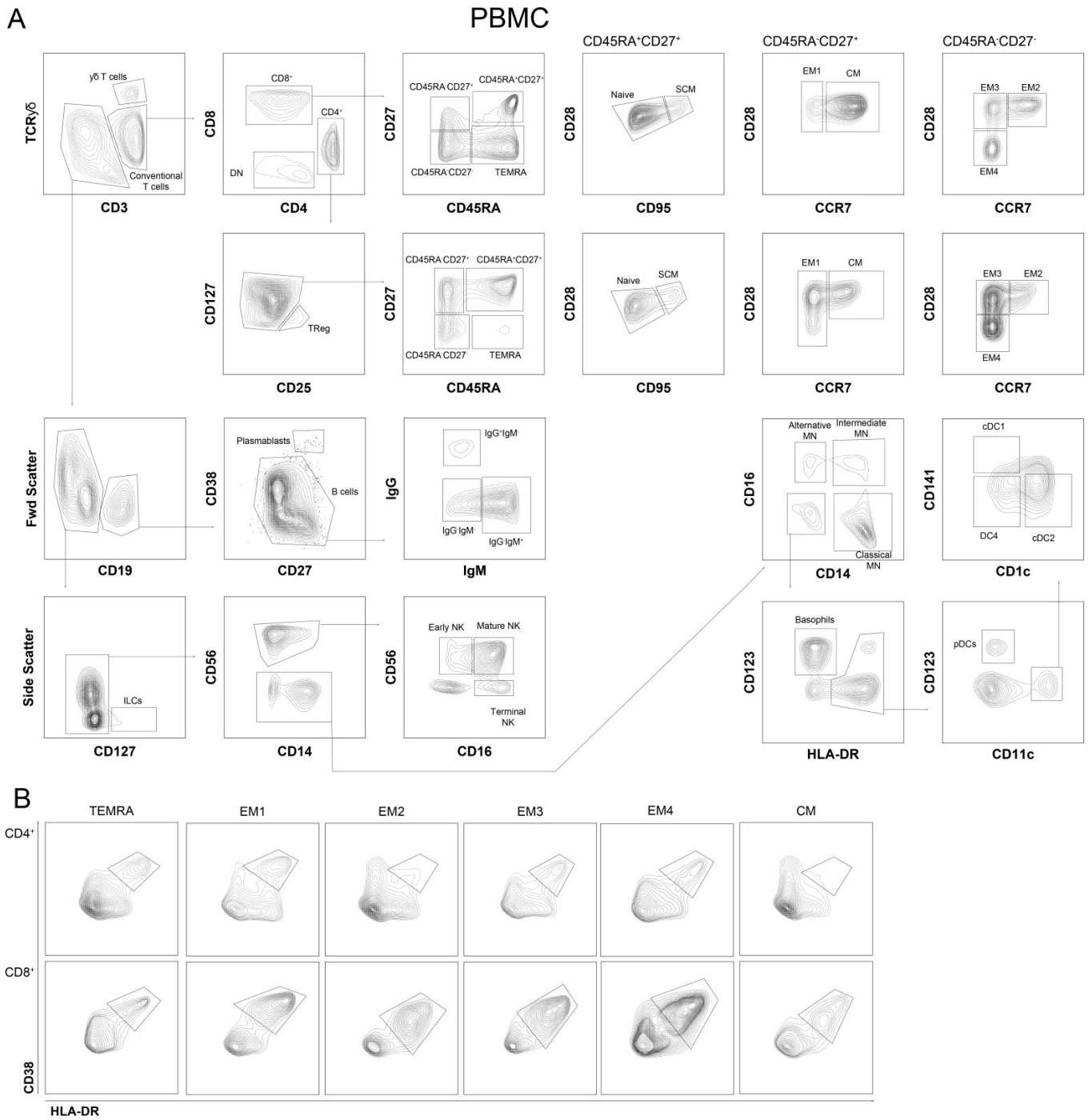
To investigate the differences between PBMC and BALF, effect of ICU stay and mortality on cell compositions and levels of inflammatory mediators, multiple analyses were performed wherein unsupervised clustering with OMIQ tSNE (OmiQ)) was performed. First, frequencies of immune cell subset were compared between PBMCs and BALFMCs, and soluble mediators in plasma and BALF. To aid comparability, from each patient only one sample was included in this analysis that was obtained around 14 days at the ICU and before prednisolone therapy (see supplemental figure 3). Statistical significance of observed differences were tested using Kruskal-Wallis (Figure 1+2: control vs patient PBMCs), Friedmans test (Figure 1+2: patient PBMC vs patient BALFMC), Mann Whitney U test (Figure 1+2: soluble mediators control vs patient) or Wilcoxon signed-rank test (Figure 1+2: soluble mediators patient plasma vs BALF). All these analysis were corrected for multiple testing using the Benjamini-Hochberg method. PBMC populations were associated with BALFMC populations using non-linear regression and spearman correlations without multiple-testing correction for hypothesis generation (Figure 3). Correlations are represented using hierarchical edge bundling plot generated using the circlize package in R as previously described.[18] Second, to investigate the effect of ICU stay on cell compositions and inflammatory marker levels, samples were stratified in ICU stay ≤14 days and >14 days (i.e. the last samples obtained before prednisolone therapy was initiated according to clinical protocol). For patients with more than one sample available in the indicated stratification, only one sample was included for the analysis (≤14 days (in case of multiple samples per patient: closest to 7 days at ICU) and >14 days (in case of multiple samples per patient: closest to 21 days at ICU); also see supplemental figure 3). Stratified patients characteristics are depicted in supplemental table 4. Statistical differences were tested using two-way ANOVA with multiple testing correction using Holm-Sidak (cell populations), or Mann-Whitney U test or Wilcoxon signed-rank test with multiple testing correction using the Benjamini-Hochberg method (soluble mediators). Third, the association of cell composition and soluble mediators with mortality during ICU stay was investigated in both peripheral blood and BALF (see supplemental table 5 for patient characteristics stratified on survival and supplemental figure 3 which samples have been included in the analysis). Statistical differences were tested using two-way ANOVA with multiple testing correction using Holm-Sidak (cell populations) or Mann-Whitney U test or Wilcoxon signed-rank test with multiple testing correction using the Benjamini-Hochberg method (soluble mediators). Statistical analysis was performed in the R statistical framework (Version 4.0.1, Vienna, Austria) or Graphad Prism v7.01 (GraphPad Software, San Diego, California, USA). Graphical presentation was performed using Graphpad Prism v7.01 (GraphPad Software), Adobe Illustrator CC v22.1 (Adobe, San Jose, California, USA), and R (Version 4.0.1).



Saris A, *et al. Thorax* 2021; 76:1010–1019. doi: 10.1136/thoraxjnl-2020-216256

## Supplemental Figures

**Supplemental figure 1**




Gating strategy of cell subsets in peripheral blood mononuclear cells (PBMCs; A) and gating of T cell activation using CD38 and HLA-DR expression (B).

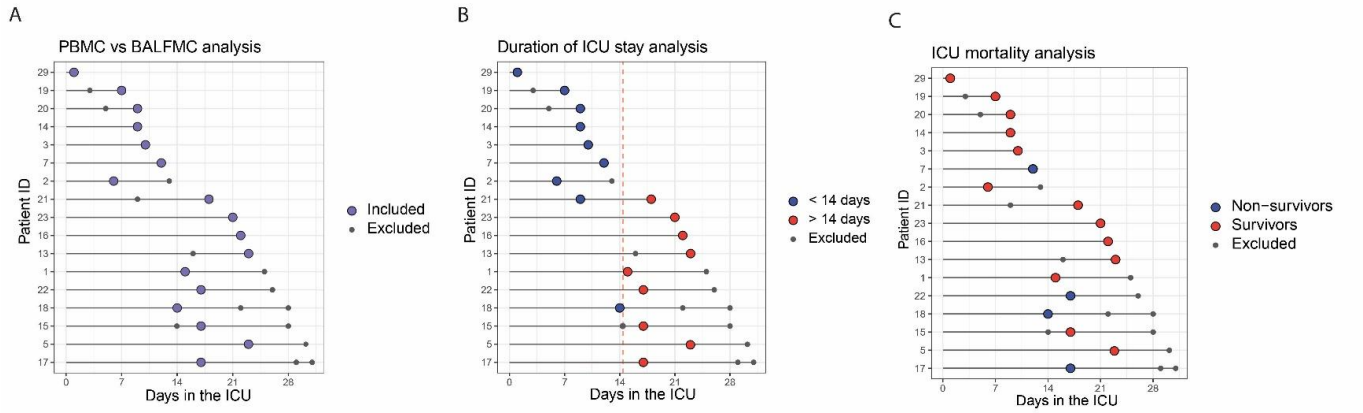
*Saris et al, thoraxjnl-2020-216256, supplemental information*

**Supplemental figure 2**


Gating strategy of cell subsets in BALF mononuclear cells (BALFMCs) (A) and of gating T cell activation using CD38 and HLA-DR expression (B).

*Saris et al, thoraxjnl-2020-216256, supplemental information*

**Supplemental figure 3**

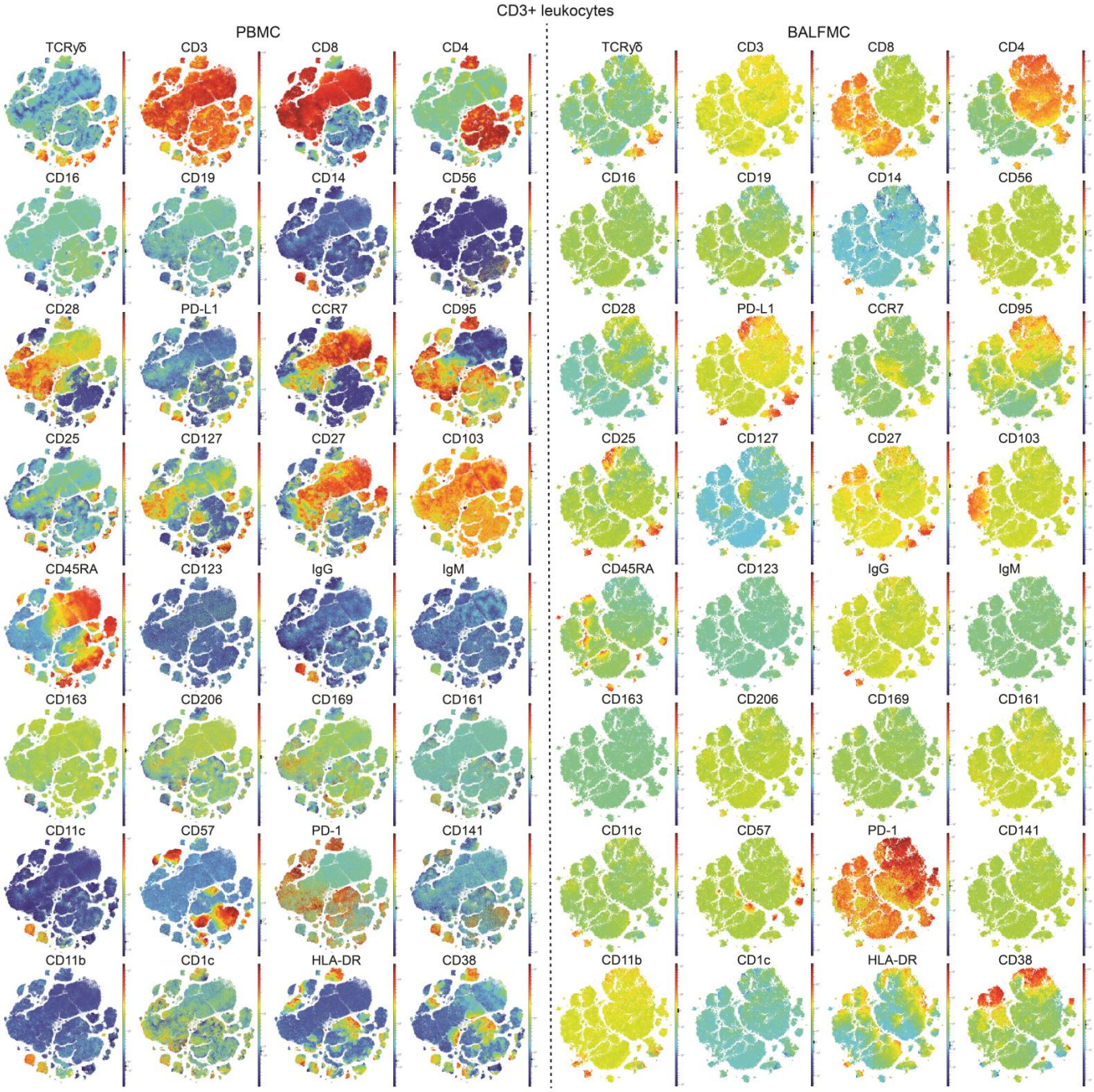



Swimmer plots displaying all patient samples that were collected of which 5 samples were excluded due to >2000 viable CD45+ cells and another 6 samples were excluded due to active prednisolone therapy. For each analysis samples that were included (red and blue circles) or excluded (small grey circles) are presented: comparing PBMCs with BALFMCs (A), duration of ICU stay (B) or ICU mortality (C).



Saris A, *et al. Thorax* 2021; 76:1010–1019. doi: 10.1136/thoraxjnl-2020-216256

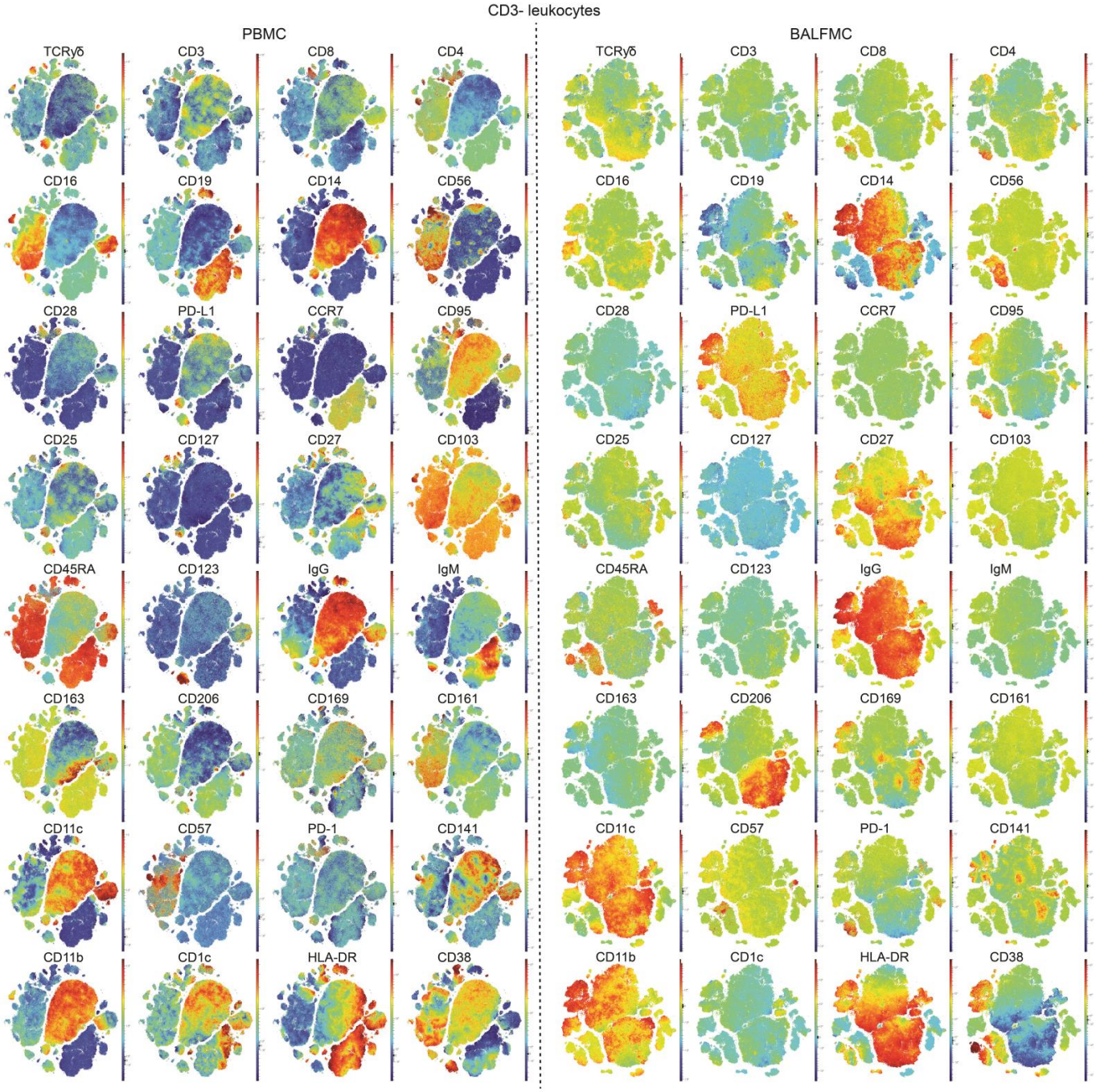
**Supplemental figure 4**




Expression patterns in viable CD45+CD3+ leukocytes of all markers that were measured. Omiq tSNE presentation of all markers individually in peripheral blood mononuclear cells (PBMCs) and BALF mononuclear cells (BALFMCs).

*Saris et al, thoraxjnl-2020-216256, supplemental information*

**Supplemental figure 5**




Expression patterns in viable CD45+CD3- leukocytes of all markers that were measured. Omiq tSNE presentation of all markers individually in peripheral blood mononuclear cells (PBMCs) and BALF mononuclear cells (BALFMCs).

*Saris et al, thoraxjnl-2020-216256, supplemental information*

## Supplemental tables

**Supplemental table1: overview of antibodies used in spectral flow cytometry panel**

| | Fluorochome | Catalog # | Vendor |
|---|---|---|---|
| Brilliant Stain Buffer Plus | N/A | 566385 | BD |
| LIVE/DEAD™ Fixable Blue Dead Cell Stain Kit, for UV excitation | N/A | L34962 | Thermo |
| **UV LASER** | | | |
| BUV395 Mouse Anti-Human CD45RA | BUV395 | 740315 | BD |
| BUV496 Mouse Anti-Human CD16 | BUV496 | 612944 | BD |
| BUV615 Mouse Anti-Human CD163 | BUV615 | 751355 | BD |
| BUV661 Mouse Anti-Human CD11c | BUV661 | 612967 | BD |
| BUV737 Mouse Anti-Human CD56 | BUV737 | 612766 | BD |
| BUV805 Mouse anti-Human CD169 | BUV805 | 748924 | BD |
| **VIOLET LASER** | | | |
| Brilliant Violet 421™ anti-human CD197 (CCR7) Antibody | BV421 | 353208 | Biolegend |
| CD123 Monoclonal Antibody (6H6), Super Bright 436 | Super Bright 436 | 62-1239-42 | Thermo |
| CD161 Monoclonal Antibody (HP-3G10), eFluor 450 | eFluor450 | 48-1619-42 | Thermo |
| BV480 Mouse Anti-Human CD103 | BV480 | 746742 | BD |
| Brilliant Violet 510™ anti-human CD3 Antibody | BV510 | 317332 | Biolegend |
| Brilliant Violet 570™ anti-human IgM Antibody | BV570 | 314517 | Biolegend |
| PacOrange Mouse Anti-Human CD8 | Pacific Orange | PO4958-5 | |
| BD Horizon™ BV605 Mouse Anti-Human IgG | BV605 | 563246 | BD |
| Brilliant Violet 650™ anti-human CD28 Antibody (clone CD28.2) | BV650 | 302946 | Biolegend |
| Brilliant Violet 785™ anti-human CD279 (PD-1) Antibody | BV785 | 329930 | Biolegend |
| **BLUE LASER** | | | |
| BD Horizon™ BB515 Mouse Anti-Human CD141 | BB515 | 566017 | BD |
| FITC anti-human CD57 Antibody | FITC | 359604 | Biolegend |
| Spark Blue™ 550 anti-human CD14 Antibody | SparkBlue550 | 367148 | Biolegend |
| PerCP anti-human CD45 Antibody | PerCP | 368506 | Biolegend |
| PerCP/Cyanine5.5 anti-human CD11b Antibody | PerCPCy5.5 | 301328 | Biolegend |
| TCR gamma/delta Monoclonal Antibody (B1.1), PerCP-eFluor 710 | PerCP-eF710 | 46-9959-42 | Thermo |
| **YELLOW/GREEN LASER** | | | |
| PE anti-human CD274 (B7-H1, PD-L1) Antibody | PE | 329706 | Biolegend |
| CD4 CF568 | CF568 | | Cytek |
| PE/Dazzle™ 594 anti-human 206 Antibody | PEDz594 | 321130 | Biolegend |
| PE/Cy5 anti-human CD95 (Fas) Antibody | PECy5 | 305610 | Biolegend |
| PE-Alexa Fluor 700 anti-human CD25 Antibody | PE-AF 700 | MHCD2524 | Thermo |
| **RED LASER** | | | |
| APC anti-human CD27 Antibody | APC | 356410 | Biolegend |
| Alexa Fluor® 647 anti-human CD1c Antibody | AF647 | 331510 | Biolegend |
| Spark NIR™ 685 anti-human CD19 Antibody | SparkNIR685 | 302270 | Biolegend |
| APC/Fire™ 750 anti-human HLA-DR Antibody | APCF750 | 307658 | Biolegend |
| APC-R700 Mouse Anti-Human CD127 | APC-R700 | 565185 | BD |
| CD38 APC-Fire810 | APC-Fire810 | Custom | Biolegend |



Saris A, *et al. Thorax* 2021; 76:1010–1019. doi: 10.1136/thoraxjnl-2020-216256

**Supplemental table 2: constitution of BALF cells before ficoll isolation**

| Date | Sample ID | Patient ID | Days at ICU | Living cells % | % CD3+ | % CD16+ | % CD56+ CD3+ | % CD19+ | % CD14++ | % CD66b+ | Unidentified | Notes on 'unidentified' |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15-4-2020 | 1781 | 18 | 14 | panel not yet operational | | | | | | | | |
| 16-4-2020 | 1919 | 15 | 13 | | | | | | | | | |
| 16-4-2020 | 1920 | 21 | 9 | | | | | | | | | |
| 17-4-2020 | 2012 | 14 | 9 | 97.8 | 8.1 | 4.2 | 0.6 | 1.9 | 17.9 | 62.5 | 4.9 | Partly macrophages? |
| 17-4-2020 | 2013 | 17 | 17 | 94.4 | 6.6 | 4.1 | 0.4 | 2.4 | 6.6 | 32.1 | 47.7 | Mostly debris |
| 17-4-2020 | 2014 | 2 | 6 | 38.1 | 0.2 | 5.3 | 0.1 | 1.9 | 5.5 | 84.9 | 2.1 | Mostly macrophages |
| 17-4-2020 | 2015 | 3 | 10 | 90.8 | 2.6 | 1.9 | 0.1 | 1.9 | 1.8 | 10.4 | 81.2 | Mostly debris |
| 17-4-2020 | 2022 | 1 | 15 | 94.0 | 1.0 | 1.3 | 0.1 | 1.1 | 16.9 | 63.6 | 16.0 | Mostly debris |
| 19-4-2020 | 2215 | 7 | 12 | 52.2 | 9.8 | 14.9 | 15.3 | 4.8 | 24.8 | 4.9 | 25.5 | Seem to be macrophages? |
| 19-4-2020 | 2216 | 20 | 9 | 96.9 | 12.6 | 4.0 | 0.8 | 1.7 | 22.0 | 25.9 | 33.0 | Partly macrophages |
| 19-4-2020 | 2234 | 19 | 7 | 81.5 | 24.3 | 4.1 | 2.0 | 2.4 | 20.1 | 13.4 | 33.6 | Debris and macrophages? |
| 20-4-2020 | 2326 | 22 | 17 | 98.8 | 0.0 | 3.0 | 0.0 | 2.3 | 7.6 | 65.8 | 21.4 | Seem to be macrophages? |
| 20-4-2020 | 2327 | 23 | 21 | 82.7 | 0.0 | 0.6 | 0.0 | 2.7 | 0.0 | 0.3 | 96.4 | MQs?? |
| 20-4-2020 | 2328 | 15 | 17 | 97.9 | 0.0 | 4.1 | 0.1 | 2.9 | 3.2 | 89.0 | 0.6 | - |
| 21-4-2020 | 2420 | 16 | 22 | 95.2 | 0.7 | 0.3 | 0.0 | 0.4 | 8.5 | 89.0 | 1.2 | |
| 23-4-2020 | 2668 | 13 | 23 | 96.8 | 0.7 | 1.2 | 0.1 | 1.2 | 8.7 | 78.6 | 9.4 | Partly macrophages |
| 23-4-2020 | 2678 | 5 | 30 | 99.3 | 0.3 | 0.2 | 0.0 | 0.6 | 4.6 | 84.7 | 9.6 | Partly macrophages |
| 25-4-2020 | 2901 | 21 | 18 | 96.0 | 4.8 | 2.3 | 0.3 | 1.2 | 10.9 | 77.9 | 2.7 | Partly macrophages |
| 29-4-2020 | 3266 | 17 | 29 | 96.0 | 3.1 | 13.1 | 4.5 | 6.5 | 15.5 | 3.0 | 54.4 | Seem to be macrophages? |
| 18-5-2020 | 4538 | 29 | 1 | 71.1 | 2.1 | 0.3 | 0.0 | 0.3 | 3.2 | 89.0 | 5.0 | |

**Supplemental table 3: percentages of T cell differentiation with phenotyping used during analysis**

| Tc subset | Phenotyping | PBMC | | BALF MC | |
|---|---|---|---|---|---|
| | | CD4 | CD8 | CD4 | CD8 |
| Naive | CD45RA+CD27+CD28+CD95- | 39.32% | 18.42% | 1.25% | 1.43% |
| EM1 | CD45RA-CD27+CD28+CCR7- | 4.34% | 9.19% | 0.49% | 0.79% |
| EM2 | CD45RA-CD27-CD28+CCR7+ | 6.44% | 1.86% | 24.52% | 5.18% |
| EM3 | CD45RA-CD27-CD28+CCR7- | 4.96% | 6.61% | 45.41% | 5.58% |
| EM4 | CD45RA-CD27-CD28-CCR7- | 4.75% | 9.71% | 12.88% | 39.01% |
| CM | CD45RA-CD27+CD28+CCR7+ | 24.75% | 6.75% | 0.98% | 0.19% |
| TEMRA | CD45RA-CD27-CD28+ | 4.33% | 42.04% | 2.53% | 16.62% |
| SCM | CD45RA+CD27+CD28+CD95+ | 6.71% | 5.42% | 0.08% | 0.13% |
| Treg | CD25++CD127- | 4.39% | | 8.29% | |
| Trm | CD103+CD28- | | | 3.57% | 31.07% |



Saris A, *et al. Thorax* 2021; 76:1010–1019. doi: 10.1136/thoraxjnl-2020-216256

**Supplemental table 4: patient characteristics stratified in samples collected <14 days and >14 days after ICU admission**

| Stratified by sampling time | Overall | <14 days | >14 days |
|---|---|---|---|
| n | 18* | 9 | 9 |
| **Demographics** | | | |
| Age | 62.56 (10.45) | 60.11 (12.03) | 65.00 (8.60) |
| Male sex | 17 (94.4) | 8 (88.9) | 9 (100.0) |
| Body Mass Index (BMI) | 28.98 (4.64) | 29.68 (5.67) | 28.29 (3.54) |
| **Comorbidities** | | | |
| Diabetes | 7 (38.9) | 4 (44.4) | 3 (33.3) |
| Chronic lung disease | 3 (16.7) | 2 (22.2) | 1 (11.1) |
| Cardiovascular disease | 6 (33.3) | 3 (33.3) | 3 (33.3) |
| Active malignancy | 1 (5.6) | 1 (11.1) | 0 (0.0) |
| No significant comorbidities | 5 (27.8) | 2 (22.2) | 3 (33.3) |
| **Biochemistry and blood counts** | | | |
| C-reactive protein (mg/L) | 197.50 [120.00, 333.50] | 198.00 [106.00, 344.00] | 197.00 [167.00, 332.00] |
| Hemoglobin (mmol/L) | 7.80 [6.27, 8.28] | 7.40 [6.80, 8.00] | 8.00 [6.10, 8.30] |
| Platelets (10^9/L) | 276.50 [215.00, 341.75] | 283.00 [253.00, 347.00] | 255.00 [180.00, 317.00] |
| Leukocytes (10^9/L) | 9.30 [8.40, 12.90] | 8.90 [8.20, 12.10] | 11.10 [8.60, 13.60] |
| Neutrophils (10^9/L) | 7.23 [6.02, 10.34] | 7.10 [5.95, 9.82] | 9.23 [6.22, 10.38] |
| Lymfocytes (10^9/L) | 0.88 [0.74, 1.47] | 0.87 [0.74, 1.51] | 0.88 [0.74, 1.36] |
| Monocytes (10^9/L) | 0.56 [0.36, 0.78] | 0.52 [0.35, 0.69] | 0.60 [0.51, 0.79] |
| **Disease severity** | | | |
| SOFA | 9.00 [7.00, 10.00] | 9.00 [4.00, 10.00] | 9.00 [3.00, 10.00] |
| **Outcome measures** | | | |
| Days in ICU | 30.00 [19.75, 39.75] | 20.00 [18.00, 38.00] | 36.00 [30.00, 40.50] |
| ICU mortality | 4 (27.2) | 2 (22.2) | 3 (33.3) |
| Overall mortality | 3 (22.2) | 2 (22.2) | 2 (22.2) |

*Results are reported as n (%), mean (standard deviation) or median [range]. ICU=Intensive care unit. *:one patient is included in both <14 days and >14 days after ICU admission.*



Saris A, *et al. Thorax* 2021; 76:1010–1019. doi: 10.1136/thoraxjnl-2020-216256

**Supplemental table 5: patient characteristics stratified in fatal and non-fatal COVID-19 cases**

| Stratified by ICU mortality | Overall | Alive | Dead |
|---|---|---|---|
| n | 17 | 13 | 4 |
| **Demographics** | | | |
| Age | 63.12 (10.39) | 62.15 (11.22) | 66.25 (7.41) |
| Male sex | 16 (94.1) | 12 (92.3) | 4 (100.0) |
| Body Mass Index (BMI) | 29.12 (4.85) | 30.01 (5.10) | 26.24 (2.61) |
| **Comorbidities** | | | |
| Diabetes | 5 (29.4) | 3 (23.1) | 2 (50.0) |
| Chronic lung disease | 4 (23.5) | | |
| Cardiovascular disease | 5 (29.4) | 3 (23.1) | 2 (50.0) |
| Active malignancy | 1 (5.9) | 0 (0.0) | 1 (25.0) |
| No significant comorbidities | 6 (35.3) | 4 (30.8) | 2 (50.0) |
| **Biochemistry and blood counts** | | | |
| C-reactive protein (mg/L) | 217.00 [167.00, 334.00] | 217.00 [188.00, 334.00] | 219.00 [105.00, 336.50] |
| Hemoglobin (mmol/L) | 7.29 (1.10) | 7.21 (0.94) | 7.55 (1.67) |
| Platelets (10^9/L) | 270.00 [215.00, 326.00] | 299.00 [255.00, 347.00] | 197.50 [178.75, 224.50] |
| Leukocytes (10^9/L) | 10.42 (3.35) | | |
| Neutrophils (10^9/L) | 7.23 [6.15, 10.25] | 6.92 [6.14, 10.18] | 9.72 [8.41, 10.25] |
| Lymfocytes (10^9/L) | 0.88 [0.74, 1.40] | 0.85 [0.74, 1.31] | 1.53 [0.69, 2.77] |
| Monocytes (10^9/L) | 0.56 [0.36, 0.75] | 0.44 [0.35, 0.72] | 0.68 [0.58, 0.81] |
| **Disease severity** | | | |
| SOFA | 9.00 [7.00, 10.00] | 9.00 [7.00, 10.00] | 9.00 [6.50, 11.25] |
| **Outcome measures** | | | |
| Days in ICU | 31.00 [19.50, 40.50] | 38.00 [19.50, 45.50] | 30.00 [25.50, 32.25] |
| ICU mortality | 4 (27.2) | 0 (0.0) | 4 (100) |
| Overall mortality | 3 (22.2) | 1 (7.7) | 4 (100) |

*Results are reported as n (%), mean (standard deviation) or median [range]. ICU=Intensive care unit.*



Saris A, *et al. Thorax* 2021; 76:1010–1019. doi: 10.1136/thoraxjnl-2020-216256