

Supplementary information

Longitudinal assessment of IFN-I activity and immune profile in critically ill COVID-19 patients with acute respiratory distress syndrome

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Supplemental Table 1. Immune response in critically ill COVID-19 patients. Immune parameters were measured 5 times (D0: within the first 48h, D3: between day3 and day 4, D7: between D7 and D9, D15: between D12 and D15, D20: between D20 and D25) after ICU admission in 64 COVID-19 patients. Results are presented as medians and interquartile ranges [Q1-Q3]. Numbers of available samples at each time-point are listed. ISG: Interferon stimulated genes. Ly: lymphocytes. Reference values are provided by routine clinical immunology laboratories at our institution.

| | D0 (n = 62) | D3 (n = 50) | D7 (n = 41) | D12 (n = 32) | D20 (n = 26) | Reference values |
|---|----------------------------|---------------------------|--------------------------|---------------------------|----------------------------|-------------------------|
| Ly count (cell/μL) | 653 [479 - 892] | 702 [499 - 919] | 750 [546 - 1014] | 1017 [634 - 1485] | 1236 [742 - 1709] | [1 000 – 2 800] |
| CD4+ T Ly count (cell/μL) | 298 [186 - 362] | 288 [189 - 405] | 324 [251 - 508] | 446 [266 - 701] | 550 [272 - 976] | [336 - 1 126] |
| CD8+ T Ly count (cell/μL) | 141 [84 - 201] | 142 [78 - 218] | 152 [88 - 272] | 209 [113 - 352] | 257 [151 - 424] | [125 - 780] |
| CD4 / CD8 Ly Ratio | 2.0 [1.3 – 3.0] | 1.9 [1.3 - 2.9] | 2.2 [1.4 - 3.5] | 2.2 [1.4 – 4.0] | 2.1 [1.1 - 3.0] | [1 - 4.9] |
| Monocyte HLA-DR (AB/C) | 11 125 [7 667 – 15 419] | 9 121 [6 194 – 12 765] | 7 347 [5 117 – 9 921] | 6 914 [3 987 – 12 155] | 10 838 [6 848 – 13 123] | [13 500 – 45 000] |
| IL-6 (pg/mL) | 108 [44 - 213] | 118 [45 - 217] | 68 [32 - 155] | 70 [38 - 105] | 29 [16 - 56] | < 7 pg/mL |
| IL-10 (pg/mL) | 20 [14 - 30] | 15 [8 - 28] | 13 [8 - 22] | 12 [7 - 17] | 8 [6 - 12] | < 8 pg/mL |
| TNF-α (pg/mL) | 18 [13 - 20] | 22 [15 - 30] | 24 [19 - 32] | 28 [17 - 40] | 20 [15 - 26] | < 10 pg/mL |
| IFN-γ (pg/mL) | 8 [5 - 16] | 3 [2 - 10] | 2 [1 - 5] | 2 [1 - 3] | 1 [1 - 3] | < 3 pg/mL |
| IFN-α (fg/mL) | 385 [67 – 2 407] | 35 [12 - 110] | 13 [6 - 33] | 10 [4 - 29] | 13 [8 - 20] | < 20 fg/mL |
| ISG score (Ratio) | 40 [12 - 68] | 8 [3 - 20] | 4 [2 - 11] | 1 [1 - 3] | 1 [1 - 4] | < 2.3 |

Supplemental Table 2. Immune response in critically ill COVID-19 patients with or without ARDS.

Immune parameters were measured 3 times (D0: within the first 48h, D3: between day3 and day 4, D7: between D7 and D9) during the first week after ICU admission in COVID-19 patients. Patients were separated in two groups based on presence (n = 40) or not (n = 24) of acute respiratory distress syndrome (ARDS) during the first 72h after admission according to Berlin definition. Medians and interquartile ranges [Q1-Q3] are shown. Numbers of available data are listed. Because of the high number of missing values for this set of biological parameters, no statistical analyses were performed.

| | No ARDS | | | ARDS | | |
|---|---------------------------------|-------------------------------|-------------------------------|--------------------------------|--------------------------------|-------------------------------|
| | D0 | D3 | D7 | D0 | D3 | D7 |
| Lactates (mM) | 1.2 [1 – 1.3] n = 12 | 1.5 [1.3 – 1.6] n = 3 | NA | 1.6 [1.3 – 2.2] n = 34 | 1.8 [1.5 – 2.3] n = 27 | 1.7 [1.5 – 2] n = 22 |
| Leucocytes (G/L) | 7.7 [4.4 – 8.8] n = 18 | 6.7 [5.8 – 8.3] n = 7 | 6.9 [5.9 – 7.7] n = 3 | 8.2 [6.8 – 9.6] n = 29 | 9.5 [8.1 – 13.6] n = 28 | 11.8 [9 – 13.7] n = 24 |
| Neutrophils (G/L) | 5.2 [3.1 – 7.3] n = 18 | 5.5 [3.9 – 5.9] n = 7 | 4.2 [3.8 – 5.9] n = 3 | 6.6 [4.4 – 8.3] n = 29 | 9.2 [6.8 – 11.7] n = 28 | 8.6 [6.8 – 11.5] n = 24 |
| Monocytes (G/L) | 0.35 [0.29 – 0.47] n = 17 | 0.6 [0.51 – 0.81] n = 7 | 0.8 [0.39 – 0.83] n = 3 | 0.33 [0.2 – 0.46] n = 29 | 0.5 [0.34 – 0.79] n = 28 | 0.9 [0.51 – 1.1] n = 23 |
| CRP (g/L) | 119 [67 – 191] n = 18 | 203 [114 – 295] n = 4 | 20 [9 – 32] n = 2 | 155 [103 – 262] n = 22 | 221 [198 – 333] n = 3 | 144 [51 – 181] n = 3 |
| Lymphocytes (cells/μL) | 769 [609– 1047] n = 22 | 941 [630 – 1292] n = 12 | 996 [804 – 1495] n = 6 | 615 [424 – 856] n = 39 | 559 [436 – 804] n = 37 | 702 [532 – 995] n = 35 |
| T CD4+ Lymphocytes (cells/μL) | 346 [256 – 431] n = 23 | 399 [267 – 643] n = 13 | 472 [313 – 920] n = 6 | 287 [140 – 342] n = 39 | 246 [176 – 365] n = 37 | 315 [234 – 496] n = 35 |
| T CD8+ Lymphocytes (cells/μL) | 178 [125 – 244] n = 23 | 211 [134 – 235] n = 13 | 217 [127 – 415] n = 6 | 120 [73 – 189] n = 38 | 133 [68 – 185] n = 36 | 148 [79 – 254] n = 34 |
| CD4/CD8 Ratio | 2 [1.4 – 2.4] n = 23 | 2 [1.5 – 2.7] n = 13 | 2.7 [1.7 – 4.8] n = 6 | 2.1 [1.2 – 3.3] n = 38 | 1.7 [1.3 – 3.1] n = 36 | 2.1 [1.4 – 3.6] n = 33 |
| B Lymphocytes (cells/μL) | 84 [78 – 120] n = 17 | 149 [107– 165] n = 5 | 165 [163 – 167] n = 2 | 94 [52 – 147] n = 32 | 84 [72 – 113] n = 15 | 57 [47 – 105] n = 6 |
| NK cells (cells/μL) | 84 [48 – 125] n = 17 | 69 [47 – 96] n = 5 | 85 [83 – 86] n = 2 | 96 [54 – 134] n = 32 | 59 [43 – 115] n = 15 | 19 [9.5 – 46.5] n = 5 |

Supplemental Table 3. Immune response in critically COVID-19 patients with ARDS according to status at D28. Immune parameters were measured 5 times (D0: within the first 48h, D3: between day3 and D4, D7: between D7 and D9, D15: between D12 and D15, D20: between D20 and D25) during the first month after ICU admission in COVID-19 patients with ARDS. Patients were stratified in two groups according to their status at D28: survivors (n = 26) or non-survivors (n = 14). Medians and interquartile ranges [Q1-Q3] are shown. Numbers of available data are listed. Non parametric Mann Whitney test was used to compare values between groups at the same time-point.* p < 0.05 and ** p < 0.005 versus corresponding value at the same time-point in survivors.

| | Survivors | | | | | Non-Survivors | | | | |
|--------------------------------------|------------------------------|------------------------------|-------------------------------|-------------------------------|--------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|
| | D0 | D3 | D7 | D15 | D20 | D0 | D3 | D7 | D15 | D20 |
| Lactates (mM) | 1.5 [1.1 – 2.1] n = 21 | 1.7 [1.4 – 2.2] n = 17 | 1.7 [1.5 – 2.0] n = 16 | 1.5 [1.3 – 1.7] n = 11 | 1.8 [0.9 – 3.1] n = 5 | 2.0 [1.6 – 2.5] n = 13 | 1.9 [1.7 – 3.7] n = 10 | 2.0 [1.5 – 2.2] n = 6 | 2.0 [1.4 – 2.3] n = 6 | 1.6 [1.4 – 1.7] n = 2 |
| Leucocytes (G/L) | 8 [7 – 11] n = 18 | 11 [8 – 16] n = 19 | 12 [8 – 14] n = 17 | 13 [10 – 17] n = 20 | 14 [10 – 20] n = 14 | 8 [6 – 9] n = 11 | 9 [7 – 12] n = 9 | 12 * [9 – 16] n = 7 | 11 [7 – 14] n = 5 | 12 [11 – 20] n = 3 |
| Neutrophils (G/L) | 6.8 [4.3 – 8.2] n = 18 | 9.2 [6.6 – 12] n = 19 | 8.7 [6.4 – 12] n = 17 | 10 [7.8 – 13.3] n = 20 | 11.2 [7.7 – 17] n = 14 | 6.6 [4.3 – 8.4] n = 11 | 7.7 [6.9 – 11.0] n = 9 | 8.1 [6.9 – 10.9] n = 7 | 8.1 [5.4 – 9.2] n = 5 | 9.5 [9.3 – 12.9] n = 3 |
| Monocytes (G/L) | 0.4 [0.2 – 0.5] n = 18 | 0.5 [0.4 – 0.8] n = 19 | 0.9 [0.5 – 1.2] n = 16 | 1 [0.6 – 1.5] n = 20 | 1 [0.6 – 1.1] n = 14 | 0.3 [0.2 – 0.5] n = 11 | 0.5 [0.2 – 0.8] n = 9 | 0.9 [0.7 – 1.1] n = 7 | 0.4 [0.2 – 1.1] n = 5 | 0.9 [0.6 – 1.7] n = 3 |
| CRP (g/L) | 168 [106 – 278] n = 15 | NA | NA | NA | NA | 120 [59 – 211] n = 6 | NA | NA | NA | NA |
| Lymphocytes (cells/μL) | 623 [430 – 898] n = 25 | 572 [405 – 852] n = 24 | 726 [482 – 1015] n = 24 | 988 [585 – 1510] n = 23 | 1141 [608 – 1737] n = 19 | 564 [375 – 849] n = 14 | 545 [503 – 790] n = 13 | 688 [532 – 980] n = 11 | 988 [732 – 1624] n = 8 | 1214 [730 – 1630] n = 6 |
| T CD4+ Lymphocytes (cells/μL) | 306 [153 – 356] n = 25 | 231 [167 – 344] n = 24 | 284 [189 – 485] n = 24 | 441 [237 – 627] n = 23 | 461 [203 – 730] n = 19 | 267 [117 – 309] n = 14 | 267 [176 – 427] n = 13 | 365 [302 – 503] n = 11 | 593 [344 – 888] n = 8 | 653 [449 – 1025] n = 6 |
| T CD8+ Lymphocytes (cells/μL) | 142 [67 – 212] n = 24 | 157 [58 – 224] n = 23 | 208 [84 – 287] n = 23 | 230 [111 – 370] n = 23 | 257 [156 – 427] n = 19 | 97 [75 – 143] n = 14 | 99 [69 – 159] n = 13 | 112 [65 – 144] n = 11 | 195 [95 – 221] n = 8 | 173 [115 – 410] n = 6 |
| B Lymphocytes (cells/μL) | 107 [69 – 154] n = 18 | 87 [68 – 108] n = 8 | 57 [51 – 118] n = 4 | 88 [47 – 102] n = 3 | NA | 80 [47 – 125] n = 14 | 80 [72 – 243] n = 7 | 66 [38 – 94] n = 2 | NA | NA |
| NK cells (cells/μL) | 100 [54 – 128] n = 18 | 61 [41 – 109] n = 8 | 16 [8 – 35] n = 4 | 40 [14 – 61] n = 3 | | 89 [51 – 160] n = 14 | 59 [43 – 271] n = 7 | NA | NA | NA |

Supplementary Table 4. Numbers of available values for each immune parameter at each time point. ARDS: acute respiratory distress syndrome. D: Day. mHLA-DR: monocytic expression of antigen-leukocyte DR. IL: interleukin. TNF: Tumor necrosis factor. IFN: interferon. ISG: interferon stimulated genes.

A- Numbers of values presented in Figure 2

| | No ARDS | | | ARDS | | |
|-----------------|---------|----|----|------|----|----|
| | D0 | D3 | D7 | D0 | D3 | D7 |
| mHLA-DR | 23 | 13 | 6 | 39 | 37 | 35 |
| T lymphocytes | 23 | 13 | 6 | 39 | 37 | 35 |
| IL-6 | 22 | 13 | 6 | 36 | 37 | 35 |
| IL-10 | 22 | 13 | 6 | 36 | 37 | 35 |
| TNF- α | 22 | 13 | 6 | 36 | 37 | 35 |
| IFN- γ | 22 | 13 | 6 | 36 | 37 | 35 |
| IFN- α 2 | 21 | 11 | 5 | 34 | 35 | 33 |
| ISG score | 21 | 11 | 5 | 32 | 34 | 34 |

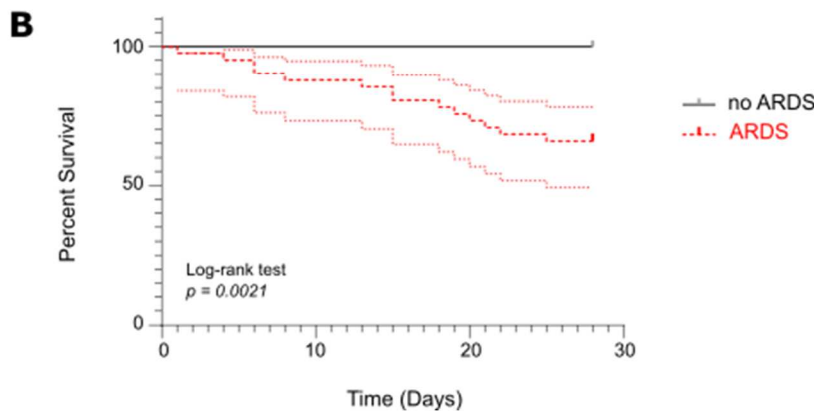
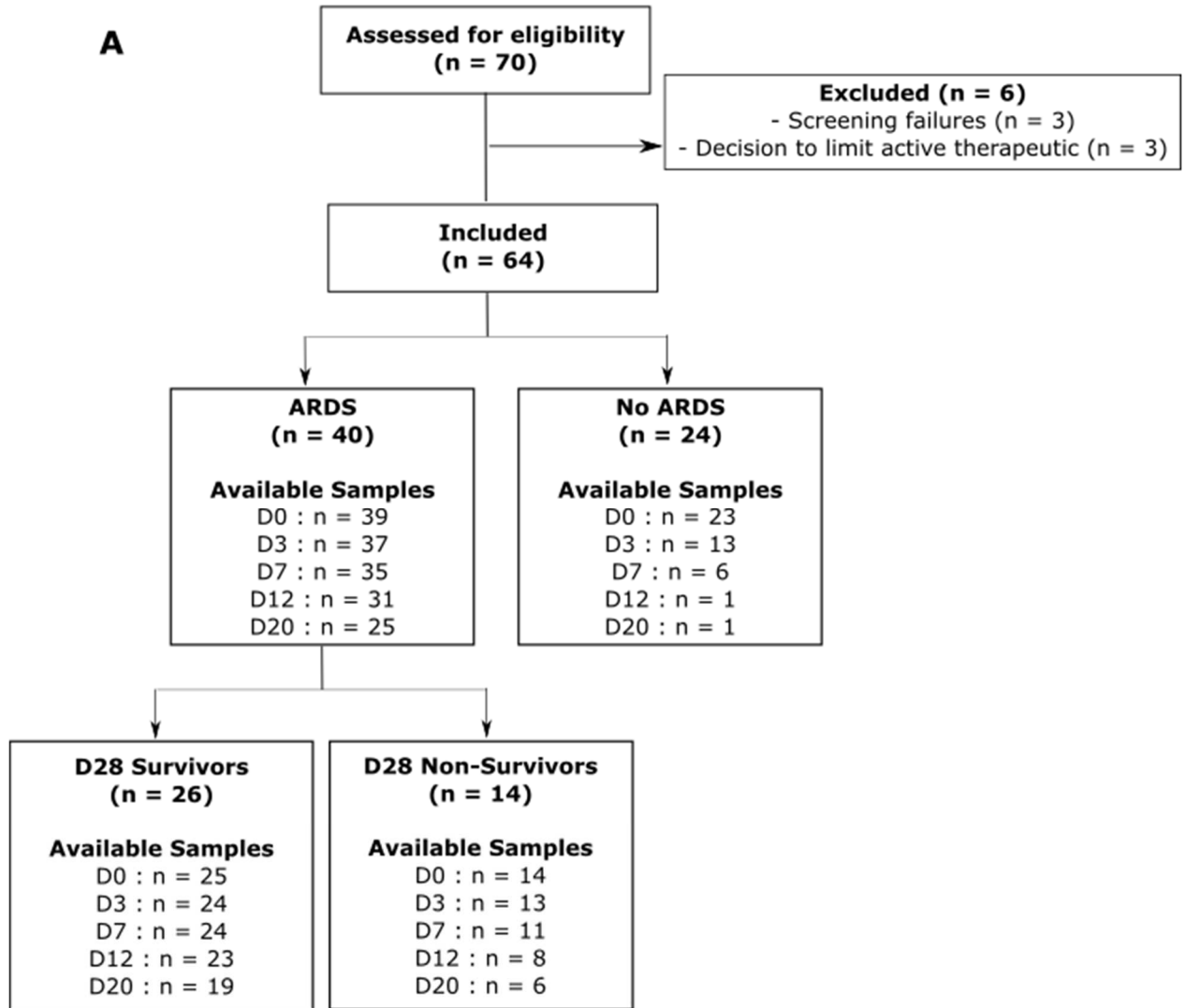
B- Numbers of values presented in Figure 3

| | Survivors | | | | | Non-survivors | | | | |
|-----------------|-----------|----|----|-----|-----|---------------|----|----|-----|-----|
| | D0 | D3 | D7 | D15 | D20 | D0 | D3 | D7 | D15 | D20 |
| mHLA-DR | 25 | 24 | 24 | 23 | 19 | 14 | 13 | 11 | 8 | 6 |
| T lymphocytes | 25 | 24 | 24 | 23 | 19 | 14 | 13 | 11 | 8 | 6 |
| CD4/CD8 ratio | 24 | 23 | 22 | 23 | 19 | 14 | 13 | 11 | 8 | 6 |
| IL-6 | 22 | 24 | 24 | 22 | 19 | 14 | 13 | 11 | 8 | 6 |
| IL-10 | 22 | 24 | 24 | 22 | 19 | 14 | 13 | 11 | 8 | 6 |
| TNF- α | 22 | 24 | 24 | 22 | 19 | 14 | 13 | 11 | 8 | 6 |
| IFN- γ | 22 | 24 | 24 | 22 | 19 | 14 | 13 | 11 | 8 | 6 |
| IFN- α 2 | 20 | 24 | 23 | 18 | 12 | 14 | 11 | 10 | 7 | 4 |
| ISG score | 20 | 22 | 23 | 22 | 19 | 12 | 12 | 10 | 7 | 6 |

Supplemental Figure 1. Flow Chart and Survival Curves.

A- Study flow diagram. Patients were split in two groups based on presence of acute respiratory distress syndrome (ARDS) according to Berlin definition during the first 72h after admission. Patients with ARDS were then analyzed according to the occurrence of deaths within 28 days after ICU admission. The numbers of available samples at each time-point in each subgroup of patients are shown. **B-** Kaplan-Meier survival curves over the first 28 days after admission are shown for patients with (dashed red line) or without ARDS (black line). 95 % confidence interval is shown for patients with ARDS. Log-rank test was used to compare survival between the two groups.

Supplemental Figure 1



Supplemental Figure 2. Impact of viral load at admission.

Semi-quantitative values of SARS-CoV-2 viral load in upper respiratory samples at ICU admission were retrieved from clinical data files for 40 patients. Patients were classified in 3 groups according to SARS-CoV-2 PCR Ct values: High viral load (High, n = 12), Medium viral load (Med, n =21), Low viral load (Low, n =7). Plasma interleukin-6 and SOFA score at admission are shown in each group. Data are presented as individual values and Tukey box plots. Pie charts represent the percentages of survivors (grey parts) and non-survivors (red parts) in each group.

Supplemental Figure 2

