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## **Adaptive lymphocyte profile analysis discriminates mild and severe forms of COVID-19 after solid organ transplantation**

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[Running title : lymphocyte profile after transplant during COVID-19](#)

Authorship:

ADB, ET, NK, SF and OM designed the study

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ADB, YL, FA, JI, FV and ET analyzed the data and wrote the paper

NK, SF, OM, and ADB followed the patients

NK, SF and OM reviewed the paper

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## Abbreviations

SOT, Solid Organ Transplantation; COVID-19, Coronavirus Disease; mTOR, mammalian Target of Rapamycin; NK, Natural Killer cells; Tregs, regulatory T cells.

## Abstract

Solid organ transplant recipients are at high risk for the development of severe forms of COVID-19. However, the role of immunosuppression in the morbidity and mortality of the immune phenotype during COVID-19 in transplant recipients remains unknown. In this retrospective study, we compared peripheral blood T and B cell functional and surface markers, as well as serum antibody development during 29 cases of mild (World Health Organization 9-point Ordinal Scale (WOS) of 3-4) and 22 cases of severe COVID-19 (WOS 5-8) in solid organ transplant (72% kidney transplant) recipients hospitalized in our center. Patients who developed severe forms of COVID-19 presented significantly lower CD3<sup>+</sup> (median 344/mm<sup>3</sup> (inter quartile range 197; 564) vs. 643/mm<sup>3</sup> (397; 1251), and CD8<sup>+</sup> T cell counts (124/mm<sup>3</sup> (76; 229) vs. 240/mm<sup>3</sup> (119; 435)). However, activated CD4<sup>+</sup> T cells were significantly more frequent in severe forms (2.9% (1.37; 5.72) vs. 1.4% (0.68; 2.35)), counterbalanced by a significantly higher proportion of Tregs (3.9% (2.35; 5.87) vs 2.7% (1.9; 3.45)). A marked decrease in the proportion of NK cells was noted only in severe forms. In the B cell compartment, transitional B cells were significantly lower in severe forms (1.2% (0.7; 4.2) vs. 3.6% (2.1; 6.2)). Nonetheless, a majority of transplant recipients developed antibodies against SARS-CoV-2 (77% and 83% in mild and severe forms respectively). Thus, our data revealed immunological differences between mild and severe forms of COVID 19 in solid organ transplant recipients, similar to previous reports in the immunocompetent population.

## Keywords

Organ Transplantation, COVID-19, Lymphopenia, Exhaustion, regulatory T cells, NK-cells

## Introduction

Solid Organ Transplant (SOT) recipients are a high-risk population for the development of severe forms of COVID-19, with an in-hospital mortality rate reported ranging from 20% to 30%<sup>1,2</sup>. Although comorbidities associated with severe SARS-CoV-2 infection<sup>2,3</sup> such as cardiovascular diseases, obesity, hypertension, and diabetes are frequent in this population, factors that influence a substantial proportion of severe disease are not well understood. For instance, it is unknown whether the immune response changes that are observed in the general population are also present during COVID-19 in SOT patients<sup>2</sup>.

A significant immune dysregulation correlated with COVID-19 severity, with an increase in the level of pro-inflammatory cytokines<sup>4</sup> and impaired interferon type-I response to elevated interleukin-6<sup>5</sup>, dysregulation of innate immune cells (HLA class II downregulation on monocytes<sup>6</sup> or dysregulation of the mTOR pathway in dendritic cells<sup>7</sup>), and acquired immune cell changes (lymphopenia<sup>8</sup> and T cell exhaustion<sup>9</sup>). Song et al<sup>10</sup> reported a sharp difference between mild and severe cases of COVID-19 in 41 immunocompetent patients, with a major CD3+, CD4+ and CD8+ and NK cell lymphopenia, excessive T-cell activation, a higher expression of T-cell inhibitory molecules, and a higher expression of cytotoxic molecules in CD8+ T cells in severe cases compared to mild cases. Zheng et al<sup>9</sup> reported an overexpression of the inhibitory molecule NKG2A in CD8+T cells and NK cells in patients with severe forms, suggesting a state of functional exhaustion in cytotoxic lymphocytes in severe forms of COVID-19, in 55 immunocompetent patients with COVID-19.

Standard immunosuppression after solid organ transplantation may have variable consequences on lymphocyte homeostasis and functions. Therefore, we retrospectively

examined the impact of SARS-Cov2 infection on innate and adaptive lymphocytes in immunocompromised SOT recipients with moderate or severe COVID-19.

## **Patients and Method**

### Patients

This retrospective study was conducted in the Department of Nephrology of Toulouse (registration number RnIPH 2021 sou-16 s, Supporting information 1).

From March to November 2020, 69 SOT recipients who were hospitalized in our department for a COVID infection proven by the detection of SARS-CoV-2 by PCR in nasopharyngeal swab. Fifty-one of them were included in this retrospective cohort. The eighteen remaining patients, that presented a mild form, were excluded from the analysis because of lack of immunological data.

COVID-19 severity was assessed at admission and then each day, in accordance with the World Health Organization (WHO) 9-point Ordinal Scale (WOS) for clinical improvement consisting of the following categories: 0) Uninfected - no evidence of infection; 1) Ambulatory - no limitation of activities; 2) Ambulatory - limitation of activities; 3) Hospitalized, mild - no oxygen therapy; 4) Hospitalized, mild - oxygen by mask or nasal cannula; 5) Hospitalized, severe - non-invasive ventilation or high flow oxygen; 6) Hospitalized, severe - intubation and mechanical ventilation; 7) Hospitalized, severe - ventilation + additional organ support; 8) Death. Patients were then divided into two categories, according to the worst score obtained during the follow-up: Mild (WOS 3, 4) and Severe (WOS 5, 6, 7, 8).



## Immunological analysis

The first immunological analysis was performed during the first five days post admission to our department. Serial analyses were then performed, if possible, during hospitalization, each week until the discharge.

All staining was performed on fresh (<24h) whole blood samples drawn by venipuncture in EDTA coated tubes. Membrane immunostaining was performed as follows: 100µl of blood was incubated with the appropriate amount of antibodies for 15 mins at room temperature, followed by red cell lysis with either FACS Lyse buffer (BD Biosciences) or Versalyse (Beckman Coulter). After washing with Cellwash™ (BD Biosciences), the cell pellet was re-suspended in the same buffer before rapid analysis by flow cytometry. Intracellular staining was performed as follows: 100µl of blood was fixed and permeabilized with the Perfix-nc kit (Beckman Coulter), according to the manufacturer's instructions. Fixed/permeabilized blood was then incubated with the appropriate amount of mAbs for 1h, washed and processed for flow cytometry analysis. Lymphocyte subsets were enumerated by addition in the appropriate stained samples of 100µl of Flow-Count fluorospheres (Beckman Coulter), as per the manufacturer's instructions.

Data was acquired using a Navios flow cytometer (Beckman Coulter), and data analysis was carried out with the Kaluza analysis software (Beckman Coulter). The gating strategy is presented in the Supporting Information 2.

## Virological analysis

Positivity for SARS-CoV-2 was diagnosed through nasopharyngeal swab samples using a home-brew real-time PCR (RT-PCR) or a TMA assay on the Panther instrument (Hologic®).

The total antibody against SARS-CoV-2 in serum samples were tested using enzyme linked immunosorbent assay (ELISA) kit supplied by Beijing (Wantai Biological Pharmacy Enterprise Co., Ltd., China), according to the manufacturer's instructions. Briefly, the ELISA for total antibodies detection (IgG/IgM/IgA) was developed based on double-antigens sandwich immunoassay, using mammalian cell expressed recombinant antigens contained the receptor binding domain (RBD) of the spike protein of SARS-CoV-2 as the immobilized and HRP conjugated antigen. Samples were considered as positive if the S/Co was  $> 1$ .

## Statistical analyses

Results were expressed as median with interquartile ranges, unless stated otherwise. Continuous variables between groups were compared by unpaired T test and categorical variables by two-sided chi-square or two-sided Fisher's exact test, when necessary. Unpaired parametric or non-parametric tests were chosen according to the Gaussian-based data analysis. Spearman coefficient was used for correlation analyses and Pearson coefficient was established for linear regression analyses. All statistical analyses were performed with GraphpadPrism® 8.4.2 software (GraphPad Software Inc., San Diego, CA, USA). A p value  $< 0.05$  was considered statistically significant.

## Results

### 1. Patient's characteristics

Fifty-one Solid Organ Transplant (SOT) patients were included in the analysis (29 (57%) mild forms and 22 (43%) severe forms) (Table 1). A large majority of patients were kidney transplant recipients (21/29 (72%) and 19/22 (86%) in mild and severe forms respectively ( $p=0.31$ )). Three kidney transplant patients with a mild form, and one kidney recipient with a severe form received a re-transplantation. A large majority of patients received a triple, tacrolimus-based therapy. Immunosuppression management was protocolized for all patients as follows: mycophenolic Acid (MPA), mammalian target of rapamycin (mTOR) inhibitors and costimulation signal blockers were immediately stopped at diagnosis of COVID19, whereas tacrolimus was maintained (or introduced) with a trough target of 3-5 ng/ml. It was the first year post transplantation for twelve patients (41%) with a mild form and eight (36%) with a severe form (4/12 and 2/8 respectively received anti-T lymphocyte globulin ( $p > 0.99$ ), 0/12 and 1/8 received a course of anti-CD20 monoclonal antibodies ( $p=0.40$ )); among them 8 mild cases and 7 severe cases were in the first 3 months post transplantation (3/8 and 2/7 respectively received anti-T lymphocyte globulin ( $p=0.99$ ), 0/8 and 1/7 respectively received anti-CD20 monoclonal antibodies ( $p=0.47$ )). At admission, eGFR was lower in patients who later developed a severe form compared to mild forms. Among the 22 SOT recipients with severe COVID-19, 17 (77%) required mechanical ventilation. One patient in each group had a thromboembolism. Four patients with a severe form died from COVID-19 (18.2%), compared to none among the mild forms ( $p=0.03$ ).

## 2. Mild vs Severe forms of COVID 19 in solid organ transplant recipients

We first compared patients affected by mild versus severe COVID-19 using data collected at the closest time before the worst clinical situation (Table 1). The time between the first symptom of COVID-19 and sample analysis was comparable in both groups (3 days (1; 5) and 6 days (0; 16) days respectively in mild and severe forms,  $p= 0.16$ ). The lymphocyte count was lower in patients presenting a severe form ( $526/\text{mm}^3$  (278; 782) in severe forms, vs.  $815/\text{mm}^3$  (560; 1506) in mild forms,  $p= 0.04$ ), (figure 1A). The NK cell count was comparable in both groups ( $100/\text{mm}^3$  (63; 135) in severe forms vs.  $49/\text{mm}^3$  (23; 156) in mild forms,  $p= 0.16$ ). CD3+ T cells were lower in severe forms ( $344/\text{mm}^3$  (197; 564) vs.  $643/\text{mm}^3$  (397; 1251),  $p= 0.04$ ), as well as the number of CD8+ T cells ( $124/\text{mm}^3$  (76; 229) vs.  $240/\text{mm}^3$  (119; 435),  $p= 0.05$ ). Naive and memory T cell subsets (both in the CD4+ and the CD8+ compartments) were similar between both groups (Figure 1B). However, the proportion of Tregs (3.9% (2.35; 5.87) vs 2.7% (1.9; 3.45),  $p= 0.02$ ), and CD4+ DR+/CD38+ T cells (2.9% (1.37; 5.72) vs. 1.4% (0.68; 2.35),  $p= 0.005$ ) was higher in severe forms compared to mild forms. We also analyzed markers associated with exhaustion (PD-1, TIGIT, CD39), effector differentiation (CD57), and cytotoxic functions (perforin, granzyme B). We did not find any statistical difference for these markers between mild and severe COVID-19. Nonetheless, in severe forms a positive correlation was found between Tregs frequencies and the percentage of CD4 and CD8 T cells expressing TIGIT, CD39+ (for CD4+) and PD-1 (for CD8+). Further a negative correlation was observed between CD4+TIGIT+ and CD4+

perforin/granzyme B + frequencies (Figure 1C). The proportion of unconventional  $\gamma\delta$  T cells was comparable in both groups (2.5% T cells (1.25; 7.00) in mild forms, vs. 3.5% (2.35; 5.70) in severe forms),  $p= 0.31$ ). Similar results were obtained when kidney transplant patients alone were analyzed (Supporting information 3). Therefore, severe forms presented a more important lymphopenia, with an intense activation of adaptive immunity, associated with suggestive signs of exhaustion.

B cell numbers did not differ between the two groups (Figure 1A); However, the proportion of CD24<sup>high</sup>CD38<sup>high</sup> transitional B cells was lower in severe forms than in mild forms (1.2% (0.7; 4.2) vs. 3.6% (2.1; 6.2),  $p= 0.03$ ) (Figure 1D). We also observed in severe forms a positive correlation between the proportion of memory CD27+ B cells and the proportion of CD4+ EM cells (Figure 1E), suggestive of a co-regulation of these two subsets. Similar results were obtained when kidney transplant patients alone were analyzed (Supporting information 3). The results remained unchanged when we had excluded two patients from the mild form group and one patient from the severe form group in whom samples were obtained after the administration of dexamethasone (n=2) or tocilizumab (n=1) (data not shown).

### **3. The kinetics of expression of the different T cell compartments during COVID-19**

Serial blood tests were carried out for seventeen patients (9 mild and 8 severe forms, supporting information 4). During hospitalization, a negative correlation was observed in the number of NK cells and the duration of severe forms (Spearman  $r= -0.36$ ,  $p= 0.05$ ), whereas a weak positive correlation was observed in mild forms (Spearman  $r=0.27$ ,  $p= 0.02$ ), (Figure 2A).

Similar results were obtained when kidney transplant patients alone were analyzed (Supporting information 3).

We then compared the differential expression of T and B cell markers in patients who had had a mild form whether or not it developed into a severe form during hospitalization (Figure 2B). A decrease in the proportion of NK cells was observed only in severe forms during the first week (20.0% (10.7; 33.0) on the first analysis vs 7.2% (4.4; 13.8) during the first week,  $p= 0.08$ ). An increase in CD3+ T cells was observed in both groups, but later in the severe forms. However, CD4+ T cell distribution was stable in both groups. Tregs and the different CD4+ memory subsets presented a similar evolution in both groups (Supporting information 5). Nonetheless, the proportion of activated CD4+, as well as CD4+ T cells expressing perforin and granzyme B, PD1+ CD4+ and CD39+ CD4+ exhaustion markers tended to be at a higher level in severe forms compared to mild forms. CD8+ T cell counts tended to be lower over time in severe forms compared to mild forms. The change in B cell compartment was similar in both groups, except for CD21<sup>low</sup> memory B cells, which were more elevated in severe forms at admission ( $p= 0.03$ ), and remained at a higher level when the disease worsened.

#### **4. SARS-CoV-2 antibody detection**

Among the 44 (26 mild, 18 severe) patients screened for anti-SARS-CoV-2 antibodies (time of screening 28 (18.5; 58) and 21 (15; 46) days post diagnosis ( $p=0.46$ )): 20 /26 (76.9%) with a mild form and 15/18 (83.3%) with a severe form developed a positive serology ( $p= 0.72$ ). Fourteen of the fifteen patients that presented a COVID-19 during the first three months post transplantation seroconverted. Among them, four patients had receive a T cell depleting agent and one patient had receive both T and B cell depleting agents for ABO

incompatible kidney transplantation. The latter was the sole patient who did not seroconvert. Among the 20 patients that presented a COVID-19 during the first-year post transplantation, three of them did not seroconvert. The use of a depleting agent was not associated with no seroconversion (4/6 patients that had receive a T and or B cell depleting agent have seroconvert, vs. 13/14 patients without depleting agents,  $p= 0.20$ ).

Patients with a negative serology presented higher level of CD4+PD1+ ( $p= 0.06$ ) or CD8+PD1+ ( $p=0.04$ ) comparing with patients that seroconverted (Supporting information 6).

## Discussion

During 2020, the pandemic caused by severe acute respiratory syndrome coronavirus 2 had a marked impact on solid organ transplant recipients. While SARS-CoV-2 infection is frequently asymptomatic in the general population, severe forms seem to be higher by 13 to 50% in solid organ transplant recipients<sup>11</sup>. The course of infection in the general population is now well documented. A comprehensive understanding of immune responses in COVID-19 transplant recipients is fundamental to defining the best management for these patients.

The first step in immune response is driven by the secretion of pro-inflammatory cytokines by innate immune cells to inhibit viral replication, recruit other immune cells to the infection site and stimulate adaptive immune response<sup>12</sup>. A dysregulated innate immune response, mainly with a type I interferon response driven by genetic susceptibilities such as inborn errors of Toll-like receptor-3, IRF-7 dependent type I interferon, or neutralizing antibodies to interferon, was previously associated with severe forms of COVID 19<sup>5,13-15</sup>. Moreover, T and B cell responses are implicated in preventing SARS-CoV-2 viral clearance, and are detectable as early as the first week after the onset of COVID-19 symptoms<sup>16</sup>. As previously demonstrated in the immunocompetent population<sup>1,4,5,17</sup>, we found that severe forms of COVID-19 in SOT recipients were associated with lower CD3+ T cells. There are several hypotheses to explain this phenomenon, including a direct cytopathic effect on infected immune cells, pulmonary recruitment of lymphocytes from blood, or T cell apoptosis during infection<sup>18,19</sup>. Memory CD8+ T cells can recognize the MHC-class I molecules of cells infected by viruses, leading to their elimination and subsequent clearance of many viruses including SARS-CoV-2<sup>20</sup>. Lymphopenia could be directly associated with a



higher level of viral load or delayed viral clearance, leading to a cytokine storm and destructive tissue inflammation<sup>18</sup>. Lymphopenia is frequent in SOT recipients, and could at least partially explain the high proportion of severe forms of COVID-19 in this population. A recent study<sup>21</sup> suggested a delayed SARS-CoV-2 specific T cell response in kidney transplant recipient, which may also participate in the development of severe forms in this population. In addition, we found a higher level of regulatory T cells in severe forms compared to mild forms. Interestingly, we observed in severe forms only a correlation between the percentage of Tregs and the expression of exhaustion-related markers TIGIT, CD39 in CD4+ T cells and PD1+ and CD39+ in CD8+ T cells. Conflicting results were observed concerning the frequency of Tregs and outcomes in general population<sup>22-24</sup>. Exhaustion was suspected to be a mechanism to maintain immune cell homeostasis<sup>25</sup>, and to participate in the progression of the disease severity<sup>20,26</sup>. The correlation between the percentage of Tregs and the expression of exhaustion markers in severe forms of COVID-19 in our patients might be seen as a reflection of the intense and prolonged activation of the immune system. However, further studies are required to better understand the role of Tregs in acute infections, and relations with CD4+ and CD8+ T inhibitory receptors expression in immunocompromised recipients.

As previously shown in immunocompetent population<sup>20,27,28</sup>, our data also suggest, a marked difference in the change in NK cell count during infection in mild and severe cases. The exact reasons for these kinetics could involve lung sequestration during pneumonia and an apoptosis mechanism directly due to SARS-CoV-2<sup>29</sup>. In any event, the decrease in circulating NK cells during infection could facilitate viral spread<sup>29</sup>.

We observed changes in the B cell compartment during infection that were similar to those described in non-transplant, immunocompetent patients<sup>30</sup>. First, we observed a sharp difference in CD24<sup>high</sup>CD38<sup>high</sup> transitional B cells between mild and severe patients. A higher percentage of transitional B cells during mild COVID 19 infection compared to severe cases was previously reported in the general population<sup>30</sup>. Infectious diseases could promote the expansion of transitional B cells which play a direct protective role by differentiation into antibody secreting cells<sup>31</sup>. We also observed in severe forms a positive correlation between the proportion of memory B cells and CD4+ EM T cells, suggesting a strong activation of T and B cells during COVID-19. Nonetheless, although most patients seroconverted during the course of the infection, a significant proportion did not (23% and 17% of moderate and severe COVID-19, respectively). These numbers are higher than those described in the general population. Two out of nine seronegative patients had been treated with anti-T lymphocyte globulin and/or anti-CD20 therapies and displayed no detectable circulating B cells. Recently Burack and colleagues<sup>32</sup> reported similar results in a cohort of 70 solid organ transplant recipients, in which only 51% of patients developed antibodies after COVID-19. Interestingly, time post transplantation (OR=1.26,  $p= 0.002$ ) and the use of more than 2 immunosuppressive agents (OR=0.26,  $p= 0.03$ ) were significantly associated with the seroconversion<sup>32</sup>.

Based on these different observations, one could hypothesize that delayed and weak specific T cell and neutralizing humoral response to SARS-Cov-2 caused by immunosuppression lead to virus immune neutralization escape<sup>33</sup>, prevent rapid clearance of the virus leading to severe disease forms. Early reports investigating the response to vaccines tend to demonstrated also a weak and delayed response to vaccination in solid organ transplant recipients<sup>34,35</sup>.

The expression of different T and B cell markers differed between patients that presented mild forms and those initially mild who developed severe forms. Patients who developed severe forms presented since the diagnosis a lower CD3+, CD8+ T cell, a higher expression of PD1 or CD39 in CD4+ T cells, comparing with mild forms that did not developed severe forms. Further studies including a larger number of patients are needed to identify the optimal biomarker that predicts disease severity.

Interestingly, as previously demonstrated in general population, we observed a higher proportion of male recipients and lower kidney function in severe forms of COVID-19<sup>36</sup>. Acute kidney injury occurs frequently among patients with COVID-19 and is associated with poor prognosis<sup>37-39</sup>. Kidney susceptibility to SARS-CoV-2 infection is in part related to the expression of angiotensin-converting enzyme 2 receptor which is used as a port of the viral entry into targeted cells<sup>40</sup>. Case series of naïve kidney biopsies identified acute tubular necrosis as the main histological finding<sup>41,42</sup>, but some patients with genetical predisposition (i.e apolipoprotein L1 G1 risk allele homozygosity) could develop collapsing focal segmental glomerulosclerosis<sup>43,44</sup>. Renal dysfunction could participate in dysregulation of inflammation (e.g, CRP and IL-6 levels), nitrogen, carbon (glucose and free fatty acid) metabolism, fueling viral proliferation<sup>45</sup>.

Our study has various limitations. First, anti-rejection treatment used in our patients can have a dramatic impact on lymphocyte numbers and phenotype. Unfortunately, these parameters are not accessible; therefore, the influence that the immune status of our patients had on the course of COVID-19 and the dynamics of immune cells that we studied cannot be inferred. Specifically, due to the relative low number of patients recently transplanted that received a T or B cell depleting agent, we were not able to draw robust

conclusions about the role of induction on the outcome. Further studies investigating the impact of depleting agents and the durability of antibodies in patients having received depleting agents is required. Second, some patients with mild forms were not included in our study due to the absence of immunological samples. However, our large cohort of SOT recipients was well defined, with clear clinical differences between mild and severe patients, comparable to previously published data<sup>46</sup>. Third, since all patients received the same immunosuppressive treatment, with only the maintenance of a low dose of tacrolimus (and mTOR inhibitors, antimetabolites, or second signal inhibitor withdrawal), we were unable to investigate the best strategy to reduce severe forms of COVID-19. However, the uniform immunosuppressive regimen allowed us to exclude biases in the interpretation of immunological analysis. Future large studies concerning the management of immunosuppressive therapies during COVID 19 are required to address this issue, especially concerning the role of the most lymphopenia-inducing treatments, such as T cell depleting agents. Similarly, it should be noted that in our study, treatments against SARS-CoV-2 such as antibiotics, convalescent patient plasma, or immunomodulatory agents (e.g. anti-IL-6 receptor blockers) changed over time. However, since these treatments were proposed only for patients who experienced a worsening clinical status associated with COVID-19 therapies, this did not influence the immunological results.

In summary, our data revealed sharp differences between mild and severe forms of COVID 19 infections that are similar to what is observed in the general population. CD3 and CD8 lymphopenia was highly associated with severe COVID 19 infections in solid organ transplant recipients. Prospective studies to investigate the effect of immunosuppression management are urgently needed.

## References

1. Akalin E, Azzi Y, Bartash R, et al. Covid-19 and Kidney Transplantation. *N Engl J Med*. April 2020;NEJMc2011117. doi:10.1056/NEJMc2011117
2. Nair V, Jandovitz N, Hirsch JS, et al. COVID-19 in kidney transplant recipients. *Am J Transplant*. 2020;20(7):1819-1825. doi:10.1111/ajt.15967
3. Wu C, Chen X, Cai Y, et al. Risk Factors Associated with Acute Respiratory Distress Syndrome and Death in Patients with Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med*. 2020;180(7):934-943. doi:10.1001/jamainternmed.2020.0994
4. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5
5. Hadjadj J, Yatim N, Barnabei L, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science (80- )*. 2020;369(6504):718-724. doi:10.1126/science.abc6027
6. Wilk AJ, Rustagi A, Zhao NQ, et al. A single-cell atlas of the peripheral immune response in patients with severe COVID-19. *Nat Med*. 2020;26(7):1070-1076. doi:10.1038/s41591-020-0944-y
7. Arunachalam PS, Wimmers F, Mok CKP, et al. Systems biological assessment of immunity to mild versus severe COVID-19 infection in humans. *Science (80- )*. 2020;369(6508):1210-1220. doi:10.1126/SCIENCE.ABC6261
8. Cao X. COVID-19: immunopathology and its implications for therapy. *Nat Rev Immunol*. 2020;20(5):269-270. doi:10.1038/s41577-020-0308-3
9. Zheng M, Gao Y, Wang G, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cell Mol Immunol*. 2020;17(5):533-535. doi:10.1038/s41423-020-0402-2
10. Song JW, Zhang C, Fan X, et al. Immunological and inflammatory profiles in mild and severe cases of COVID-19. *Nat Commun*. 2020;11(1). doi:10.1038/s41467-020-17240-2
11. Azzi Y, Bartash R, Scalea J, Loarte-Campos P, Akalin E. COVID-19 and Solid Organ Transplantation: A Review Article. *Transplantation*. 2021;105(1):37-55. doi:10.1097/TP.0000000000003523
12. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol*. 2020;20(6):363-374. doi:10.1038/s41577-020-0311-8
13. Zhang Q, Bastard P, Liu Z, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science (80- )*. 2020;370(6515). doi:10.1126/SCIENCE.ABD4570
14. Bastard P, Rosen LB, Zhang Q, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science*. 2020;370(6515). doi:10.1126/science.abd4585
15. Van Der Made CI, Simons A, Schuurs-Hoeijmakers J, et al. Presence of Genetic Variants among Young Men with Severe COVID-19. *JAMA - J Am Med Assoc*. 2020;324(7):663-673. doi:10.1001/jama.2020.13719
16. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell*. 2020;181(7):1489-1501.e15. doi:10.1016/j.cell.2020.05.015

17. Guan W, Ni Z, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. 2020;382(18):1708-1720. doi:10.1056/nejmoa2002032
18. Azkur AK, Akdis M, Azkur D, et al. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. *Allergy Eur J Allergy Clin Immunol*. 2020;75(7):1564-1581. doi:10.1111/all.14364
19. Cizmecioglu A, Cizmecioglu HA, Goktepe MH, et al. Apoptosis-Induced T Cell Lymphopenia Is Related to COVID-19 Severity. *J Med Virol*. December 2020:jmv.26742. doi:10.1002/jmv.26742
20. Zheng HY, Zhang M, Yang CX, et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. *Cell Mol Immunol*. 2020;17(5):541-543. doi:10.1038/s41423-020-0401-3
21. Favà A, Donadeu L, Sabé N, et al. SARS-CoV-2-specific serological and functional T-cell Immune responses during acute and early COVID-19 convalescence in Solid Organ Transplant patients. *Am J Transplant*. March 2021:ajt.16570. doi:10.1111/ajt.16570
22. Sadeghi A, Tahmasebi S, Mahmood A, et al. Th17 and Treg cells function in SARS-CoV2 patients compared with healthy controls. *J Cell Physiol*. 2021;236(4):2829-2839. doi:10.1002/jcp.30047
23. Galván-Peña S, Leon J, Chowdhary K, et al. Profound Treg perturbations correlate with COVID-19 severity. *bioRxiv*. December 2020. doi:10.1101/2020.12.11.416180
24. Meckiff BJ, Ramírez-Suástegui C, Fajardo V, et al. Imbalance of Regulatory and Cytotoxic SARS-CoV-2-Reactive CD4+ T Cells in COVID-19. *Cell*. 2020;183(5):1340-1353.e16. doi:10.1016/j.cell.2020.10.001
25. Wang W, Su B, Pang L, et al. High-dimensional immune profiling by mass cytometry revealed immunosuppression and dysfunction of immunity in COVID-19 patients. *Cell Mol Immunol*. 2020;17(6):650-652. doi:10.1038/s41423-020-0447-2
26. Zheng M, Gao Y, Wang G, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cell Mol Immunol*. 2020;17(5):533-535. doi:10.1038/s41423-020-0402-2
27. Wen W, Su W, Tang H, et al. Immune cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing. *Cell Discov*. 2020;6(1). doi:10.1038/s41421-020-0168-9
28. Giamarellos-Bourboulis EJ, Netea MG, Rovina N, et al. Complex Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure. *Cell Host Microbe*. 2020;27(6):992-1000.e3. doi:10.1016/j.chom.2020.04.009
29. Masselli E, Vaccarezza M, Carubbi C, et al. NK cells: A double edge sword against SARS-CoV-2. *Adv Biol Regul*. 2020;77. doi:10.1016/j.jbior.2020.100737
30. Sosa-Hernández VA, Torres-Ruiz J, Cervantes-Díaz R, et al. B Cell Subsets as Severity-Associated Signatures in COVID-19 Patients. 2020;11:1. doi:10.3389/fimmu.2020.611004
31. Oliviero B, Varchetta S, Mele D, et al. Expansion of atypical memory B cells is a prominent feature of COVID-19. *Cell Mol Immunol*. 2020;17(10):1101-1103. doi:10.1038/s41423-020-00542-2
32. Burack D, Pereira MR, Tsapepas DS, et al. Prevalence and Predictors of SARS-CoV-2 Antibodies among Solid Organ Transplant Recipients with Confirmed Infection. *Am J Transplant*. February 2021. doi:10.1111/ajt.16541
33. Clark SA, Clark LE, Pan J, et al. SARS-CoV-2 evolution in an immunocompromised host reveals

- shared neutralization escape mechanisms. *Cell*. March 2021. doi:10.1016/j.cell.2021.03.027
34. Benotmane I, Gautier-Vargas G, Cognard N, et al. Weak anti–Severe Acute Respiratory Syndrome Coronavirus 2 antibody response after the first injection of an mRNA Coronavirus Disease 2019 vaccine in kidney transplant recipients. *Kidney Int*. March 2021. doi:10.1016/j.kint.2021.03.014
  35. Chavarot N, Ouedrani A, Marion O, et al. Poor Anti-SARS-CoV-2 Humoral and T-cell Responses After 2 Injections of mRNA Vaccine in Kidney Transplant Recipients Treated with Belatacept. *Transplantation*. April 2021. doi:10.1097/TP.0000000000003784
  36. Gao Y dong, Ding M, Dong X, et al. Risk factors for severe and critically ill COVID-19 patients: A review. *Allergy Eur J Allergy Clin Immunol*. 2021;76(2):428-455. doi:10.1111/all.14657
  37. Cheng Y, Luo R, Wang K, et al. Kidney disease is associated with in-hospital death of patients with COVID-19. *Kidney Int*. 2020;97(5):829-838. doi:10.1016/j.kint.2020.03.005
  38. Hirsch JS, Ng JH, Ross DW, et al. Acute kidney injury in patients hospitalized with COVID-19. *Kidney Int*. 2020;98(1):209-218. doi:10.1016/j.kint.2020.05.006
  39. Ng JH, Hirsch JS, Wanchoo R, et al. Outcomes of patients with end-stage kidney disease hospitalized with COVID-19. *Kidney Int*. 2020;98(6):1530-1539. doi:10.1016/j.kint.2020.07.030
  40. Shang J, Wan Y, Luo C, et al. Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci U S A*. 2020;117(21). doi:10.1073/pnas.2003138117
  41. Su H, Yang M, Wan C, et al. Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. *Kidney Int*. April 2020. doi:10.1016/j.kint.2020.04.003
  42. Sharma P, Uppal NN, Wanchoo R, et al. COVID-19–Associated Kidney Injury: A Case Series of Kidney Biopsy Findings. *J Am Soc Nephrol*. 2020;31(9):1948-1958. doi:10.1681/ASN.2020050699
  43. Kissling S, Rotman S, Gerber C, et al. Collapsing glomerulopathy in a COVID-19 patient. *Kidney Int*. 2020;98(1):228-231. doi:10.1016/j.kint.2020.04.006
  44. Wu H, Larsen CP, Hernandez-Arroyo CF, et al. AKI and collapsing glomerulopathy associated with covid-19 and apol1 high-risk genotype. *J Am Soc Nephrol*. 2020;31(8):1688-1695. doi:10.1681/ASN.2020050558
  45. Thomas T, Stefanoni D, Reisz JA, et al. C L I N I C A L M E D I C I N E COVID-19 infection alters kynurenine and fatty acid metabolism, correlating with IL-6 levels and renal status. 2020. doi:10.1172/jci.insight.140327
  46. Cravedi P, Mothi SS, Azzi Y, et al. COVID-19 and kidney transplantation: Results from the TANGO International Transplant Consortium. *Am J Transplant*. 2020;20(11):3140-3148. doi:10.1111/ajt.16185

Tables

1. Main patient characteristics

Variable	Mild forms (n=29)	Severe forms (n=22)	p-value
<b>Medical past</b>			
<b>Medical history of</b>			
- Chronic respiratory insufficiency, yes (%)	8 (27.6)	7 (31.8)	0.74
- Cardiovascular events, yes (%)	10 (34.5)	11 (50)	0.26
- Hypertension, yes (%)	21 (72.4)	19 (86.3)	0.23
- Cancer, yes (%)	4 (13.8)	2 (9)	0.61
- Diabetes mellitus, yes (%)	8 (27.6)	11 (50)	0.10
- Smoking, yes (%)	5 (17.2)	4 (18.1)	0.89
Dialysis at admission, yes (%)	1 (3.4)	1 (4.5)	0.85
<b>Transplanted organ</b>			
- Kidney, n (%)	21 (72.4)	19 (86.5)	0.28
- Liver, n (%)	4 (13.84)	1(4.5)	
- Combined Kidney-Pancreas, n (%)	0	1 (4.5)	
- Heart, n (%)	4 (13.8)	1 (4.5)	
<b>Parameters at admission</b>			
Recipient age, mean (± SD)	55 ± 11	56 ± 15	0.63
Recipient gender, male (%)	17 (57)	19 (86)	0.06
Body Mass Index (kg/m <sup>2</sup> ), mean (SD)	27.3 ± 5.0	28.8 ± 7.2	0.70
IS at admission, n (%)			0.64
Tac / MMF/ S	23	17	
Tac / mTORi ± S	4	2	
Co-stimulation inhibitors* /MMF/S	1	2	
mTORi/S	0	1	
Time between transplantation – Sars-CoV-2 diagnosis, months (median, IQR <sub>25-75</sub> )	49.4 (2; 108)	26.7 (0.9; 77)	0.39
Time between first symptom of COVID-19 – hospitalization (days) median, (IQR <sub>25-75</sub> )	4 (2; 6)	3 (1; 5)	0.40
Time between SARS-CoV-2 diagnosis – first immunological analysis (days) median, (IQR <sub>25-75</sub> )	3 (1; 5)	6 (0; 16)	0.18
CT-scan evidence of COVID-19 pneumonia n (%)	22 (75.9)	19 (86)	0.48
Severity of CT-scan lesions (%)			0.31
<25	12 (41.4)	4 (18)	
25-50	8 (27.6)	12 (54.5)	
>50	2 (6.9)	3 (13.6)	
Oxygen requirement, yes n (n%)	6 (20.7)	9 (40.9)	0.13
SaO <sub>2</sub> (%)	97.8 ± 1.6	96.6 ± 3.4	0.22
<b>Biologic parameters mean (± SD)</b>			
- Serum creatinine (µmol/L)	125 ± 52	163 ± 58	0.008
- CKD-EPI eGFR (mL/min/1.73m <sup>2</sup> )	52.2 ± 23.5	35.8 ± 14.9	0.0007
- Ferritin (µg/L)	802 ± 1018	786 ± 508	0.15
- Troponin (µg/L)	23.6 ± 24	60.7 ± 76	0.08
- C-Reactive Protein (mg/L)	61 ± 81	95 ± 81	0.02
- Serum albumin (g/L)	31.1 ± 6	31.5 ± 10	0.72
- Platelets (G/mm <sup>3</sup> )	195 ± 79	194 ± 99	0.37



- Serum Interleukin 1 $\beta$ (pg/mL), median, (IQR <sub>25-75</sub> )	0.6 (0.3-1.0)	0.7 (0.5; 0.9)	0.51
- Serum Interleukin 6 (pg/mL), median, (IQR <sub>25-75</sub> )	10.0 (7.3- 53.9)	14.7 (4.3; 55.9)	0.99
- Serum TNF $\alpha$ (pg/mL), median, (IQR <sub>25-75</sub> )	18.7 (12.9; 26.4)	16.2 (11.0; 28.0)	0.99
- Serum IL8 (pg/mL), median, (IQR <sub>25-75</sub> )	10.8 (8.35; 13.5)	8.6 (4.0; 11.2)	0.22
Hospitalization follow-up			
Treatments n (%)			
Azithromycin	9 (31)	6 (27)	0.77
Third generation cephalosporin	23 (79)	21 (95)	0.12
Hydroxychloroquine	4 (14)	0	0.12
Dexamethasone	8 (28)	17 (77)	0.0006
Time (days) between Dexamethasone – sample analysis*	-1.5 (-3.5 ; 0)	-2.5 (-12 ; 0)	0.23
IL6-R blockers	0	4 (18)	0.03
Time (days) between IL6-R blockers- sample analysis**	-	-1 (-7 ; 4)	-
Convalescent plasma therapy	1 (3)	2 (9)	0.57
Outcomes, n (%)			0.05
Oxygen therapy	7 (24)	22 (100)	
Non-invasive ventilation / High flow oxygen	0	5 (23)	
Invasive ventilation	0	17 (77)	

Abbreviations: IS, Immunosuppression; Tac, tacrolimus; MMF, mycophenolate mofetil; S; Steroids; mTORi, inhibitors of mammalian target of rapamycin; eGFR, Estimated Glomerular Filtration Rate.

Costimulation inhibitors were represented by anti-CD40 monoclonal antibodies in mild (n=1) and severe case (n=1), and belatacept in one case with severe disease.

\*Blood sample analyses were performed before dexamethasone for 6/8 mild forms and 16/17 severe forms.

\*\* Blood sample analyses were performed before IL6-R blockers therapy in 3 / 4 severe forms.

## Figures legend

Figure 1 A-E. Comparison of NK, conventional and regulatory T, and B cell compartment in mild and severe forms of COVID-19

Severe forms were analyzed using the immunological sample taken at the closest time before the worse clinical situation.

A: Total lymphocyte count, CD3+, CD4+, CD8+ T cell count, proportion of NK cells,  $\delta\gamma$  T cells, and CD19+ B cells.

B: T cell compartment: CD4+ memory T cell compartment, Tregs, activation senescence and exhaustion markers, and functional markers. CD8+ memory T cell compartment, activation senescence and exhaustion markers, and functional markers.

C: Correlation between the percentage of Tregs and CD4+ TIGIT+ or CD4+ CD39+ cells, and CD8+ PD1+ or CD8+ CD39+, and CD4+TIGIT+ and CD4+perforin+/granzyme B + in mild and severe forms.

The linear regression analysis was assessed using a Pearson correlation.

D: B cell compartment: naive, transitional, activated, memory B cells, and plasmablasts.

Data are represented as median with interquartile ranges.

E: Correlation between the percentage of CD27+ memory B cells and CD4+ EM T cells in mild and severe forms.

The linear regression analysis was assessed using a Pearson correlation.

\*:  $p < 0.05$

Figure 2 A-B: Comparison of NK and conventional, T, and B cell compartment in an initially mild case that became severe after the first blood test, and those that remained mild

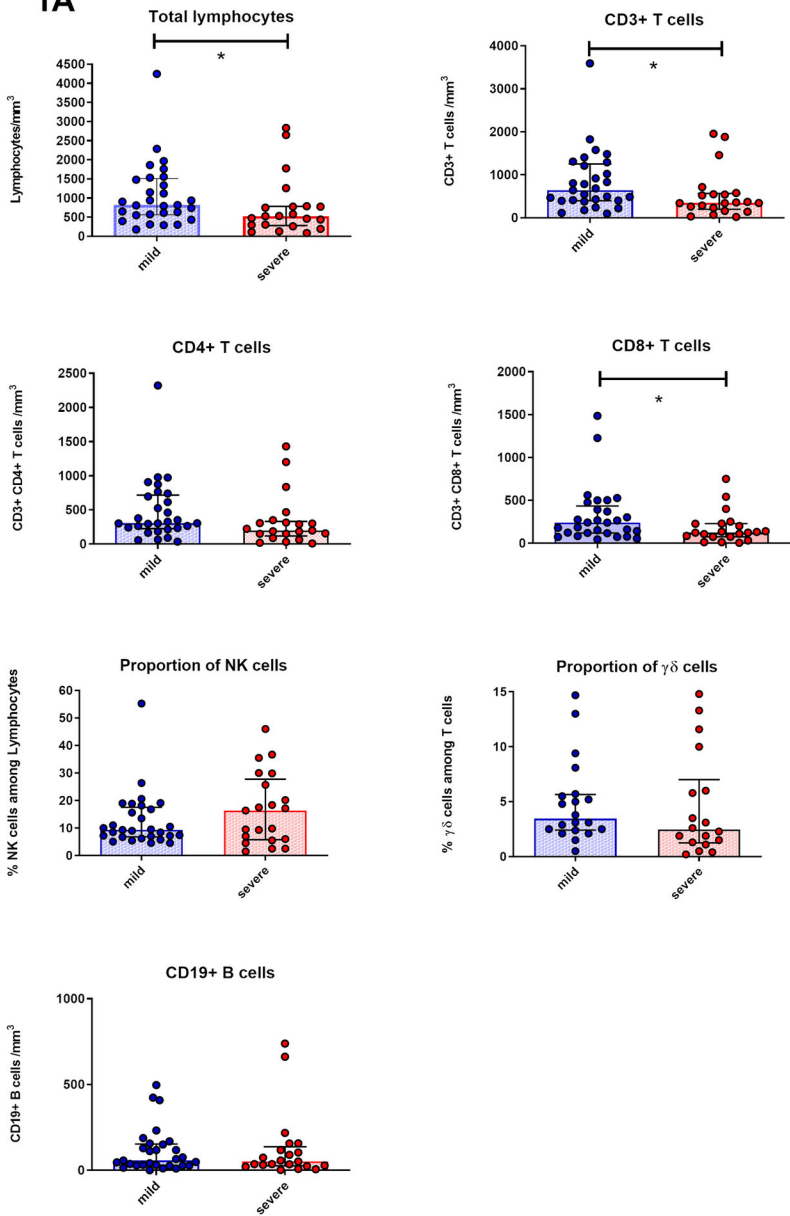
A. NK cell count over time in mild and severe forms.

B. CD3+, activated CD4+, PD1+ and CD39+ CD4+ T cells, Perforin+ / Granzyme B + CD4+ T cells, CD8+ T cells, and memory CD19+ CD21<sup>low</sup> B cells.

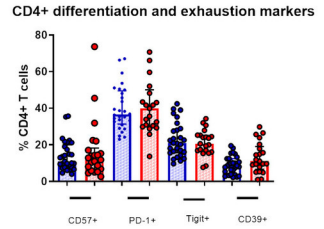
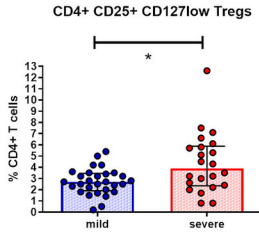
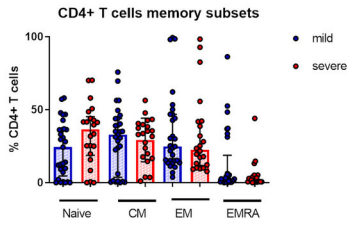
Data are represented as mean with standard error of the mean.

\*:  $p < 0.05$

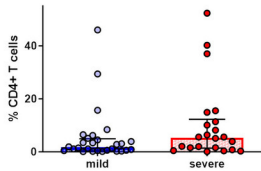
1A



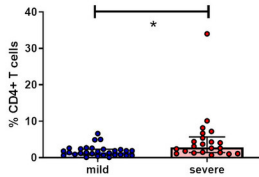
# 1B



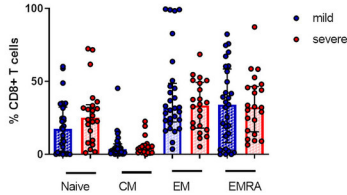
### CD4+ Perforin and Granzyme expression



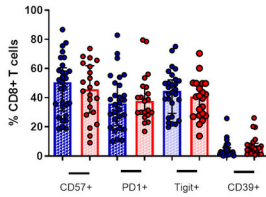
### CD4+ 38/DR+ activation markers



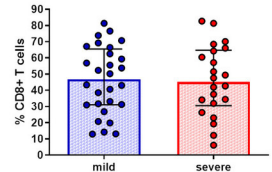
### CD8+ T cell memory subsets



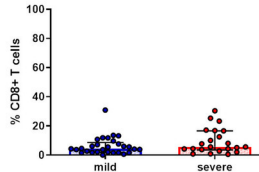
### CD8+ differentiation and exhaustion markers



### CD8+ Perforin and Granzyme expression

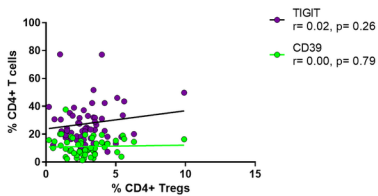


### CD8+ DR/38+ activation markers

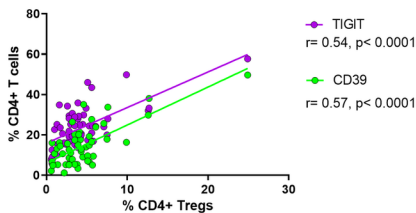


1C

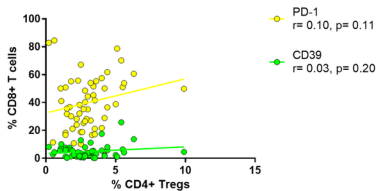
% CD4+ TIGIT+ and CD4+ CD39+ / % Tregs  
in mild forms



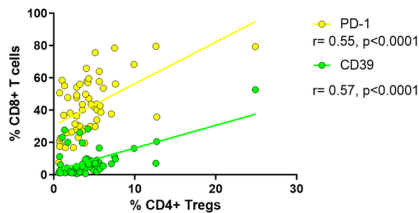
% CD4+ TIGIT+ or CD4+ CD39+ / % Tregs  
in severe forms



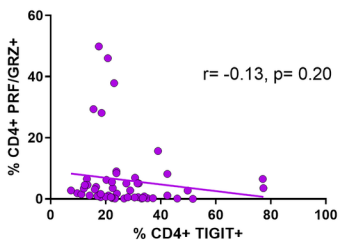
% CD8+ PD1+ or CD8+ CD39+ / % Tregs  
in mild forms



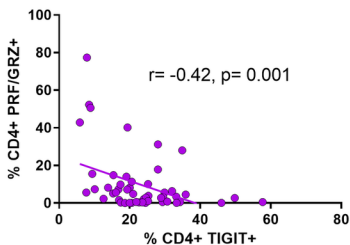
% CD8+ PD1+ or CD8+ CD39+ / % Tregs  
in severe forms



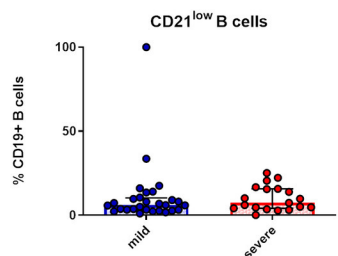
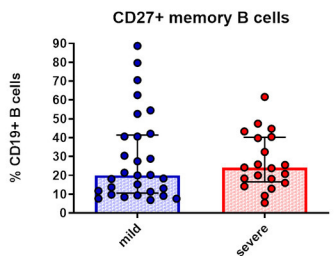
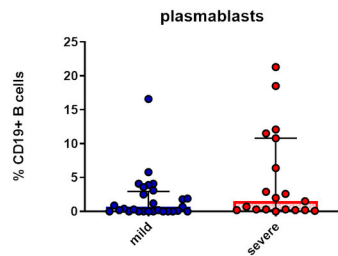
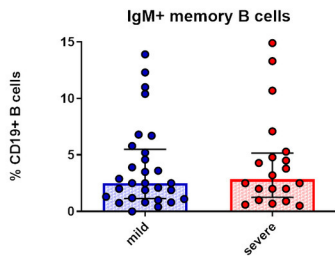
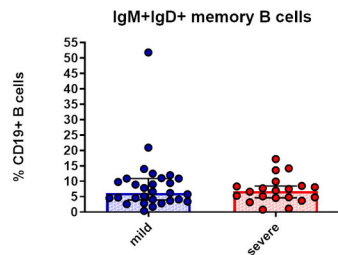
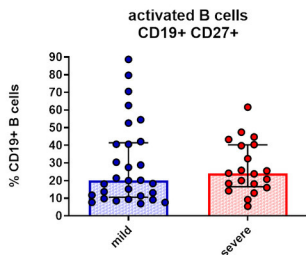
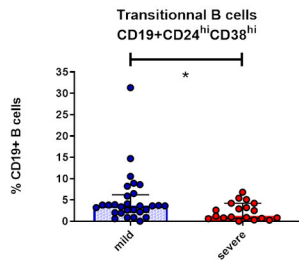
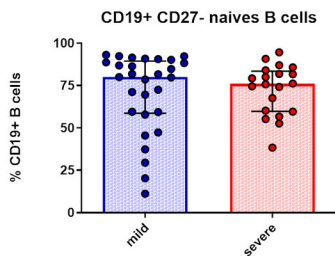
% CD4+ PRF/GZM+ / CD4+ TIGIT+  
in mild forms



% CD4+ PRF/GZM+ / CD4+ TIGIT+  
in severe forms

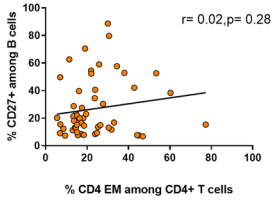


## 1D

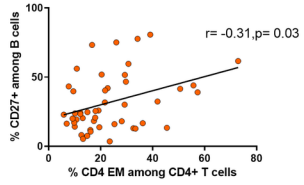


# 1E

**% CD27+ / % CD4 EM  
in mild forms**

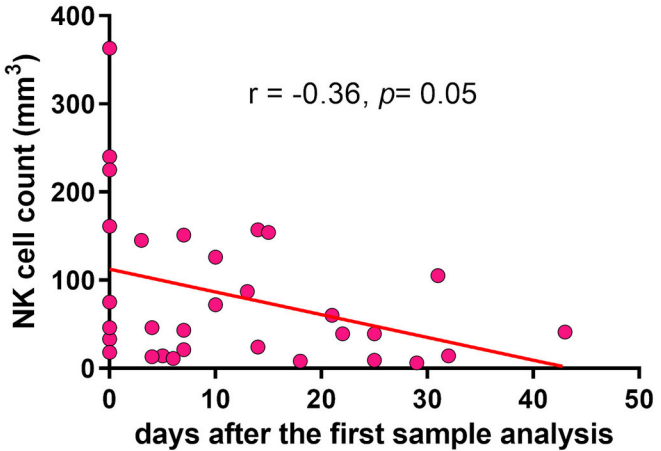


**% CD27+ / % CD4 EM  
in severe forms**

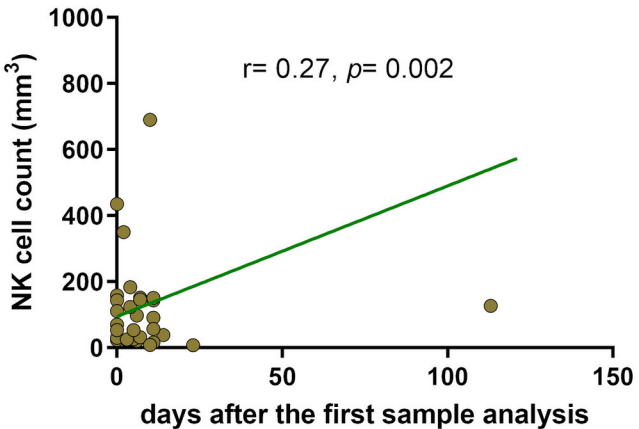


**A**

### NK cells over time in severe forms



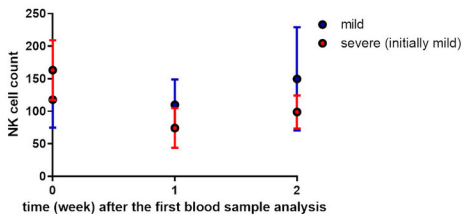
### NK cells over time in mild forms



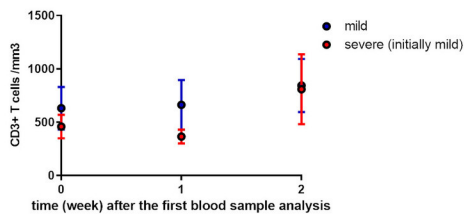


# 2B

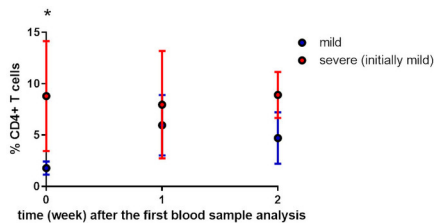
### NK cells over time



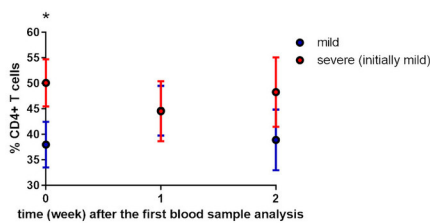
### CD3+ T cells



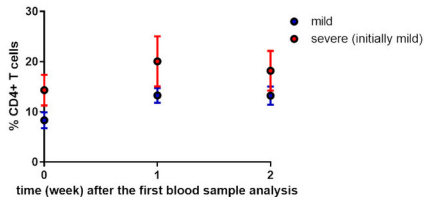
### activated DR+/CD38+ CD4+ T cells



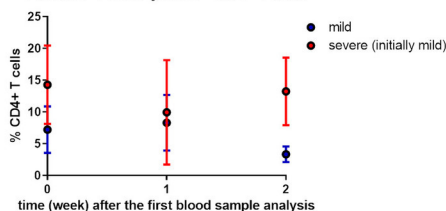
### PD1+ CD4+ T cells over time



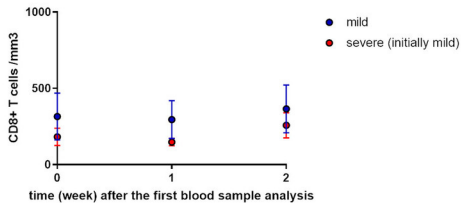
### CD39+ CD4+ T cells



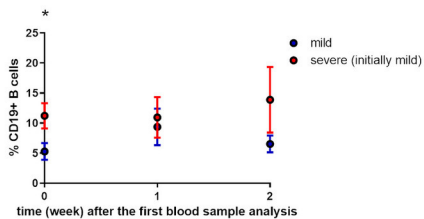
### Perforin+ / Granzyme B + CD4+ T cells



### CD8+ T cells



### CD21 low memory B cells

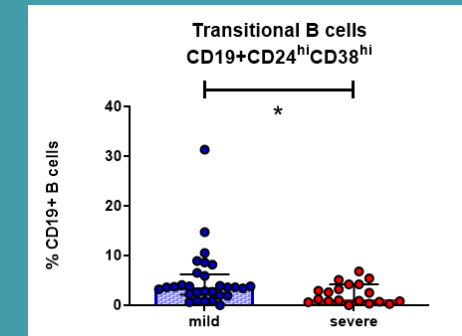
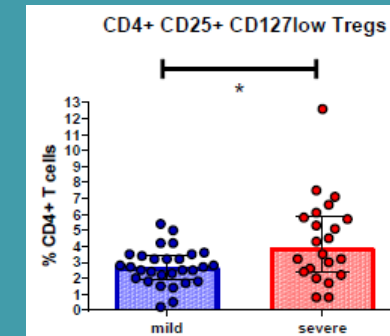
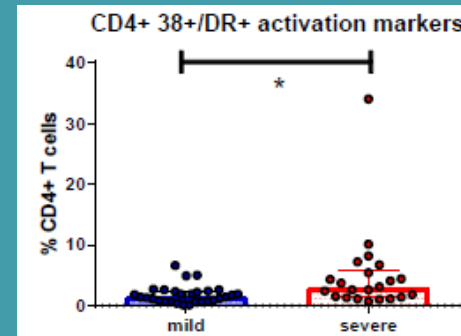


# Adaptive lymphocyte profile analysis discriminates mild and severe forms of COVID-19 after solid organ transplantation

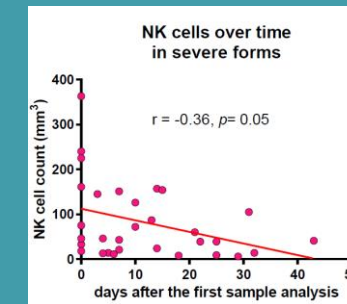
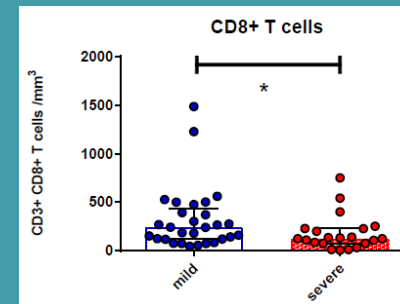
## Patients and Method

- 51 solid organ transplant patients hospitalized from 03/20 to 11/20 with COVID-19 (29 mild and 22 severe)  
Including 40 kidney, 5 liver, 5 heart, 1 combined pancreas-kidney, transplant recipients
- Immunological analyses:  
CD3/CD4/CD8 (conventional, Tregs, Tex) cells  
B cells/NK cells  
serological status
- Comparison before the worst clinical situation, and in kinetics

## Outcomes: severe forms presented



Higher activated CD4+ T cells & Tregs, lower transitional B cells  
Lower rate of CD8+ T cells, NK cells



## CONCLUSION

Immunological differences between mild and severe forms of COVID 19 are observed in solid organ transplant recipients