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

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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 $CD3^+$ (median 344/mm^3 (inter quartile range 197; 564) vs. 643/mm^3 (397; 1251)) and $CD8^+$ T cell counts (124/mm^3 (76; 229) vs. 240/mm^3 (119; 435)). However, activated $CD4^+$ 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.

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Solid organ transplant (SOT) recipients are a high-risk population for the development of severe forms of coronavirus disease 2019 (COVID-19), with an in-hospital mortality rate reported ranging from 20% to 30%.[1,2] Although comorbidities associated with severe infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2),[2,3] 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 patients with SOT.[2]

A significant immune dysregulation correlated with COVID-19 severity, with an increase in the level of pro-inflammatory cytokines[4] and impaired interferon type I response to elevated interleukin-6,[5] dysregulation of innate immune cells (human leukocyte antigen class II downregulation on monocytes[6] or dysregulation of the mammalian target of rapamycin pathway in dendritic cells[7]), and acquired immune cell changes (lymphopenia[8] and T-cell exhaustion[9]). Song *et al.*[10] reported a sharp difference between mild and severe cases of COVID-19 in 41 immunocompetent patients, with a major CD3+, CD4+, CD8+, and natural killer (NK) cell lymphopenia, excessive T-cell activation, higher expression of T-cell inhibitory molecules, and higher expression of cytotoxic molecules in CD8+ T cells in severe cases than in mild cases. Zheng *et al.*[9] reported overexpression of the inhibitory molecule NK Group member 2A (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 effect of SARS-CoV-2 infection on innate and adaptive lymphocytes in immunocompromised SOT recipients with moderate or severe COVID-19.

METHODS

Patients

This retrospective study was conducted in the Department of Nephrology and Organ Transplantation, CHU de Toulouse (registration number RnIPH 2021 sou-16 s, Supplementary Supporting Information S1).

From March to November 2020, 69 SOT recipients were hospitalized in our department for a COVID-19 infection proven by the detection of SARS-CoV-2 by polymerase chain reaction in nasopharyngeal swab samples. Fifty-one of them were included in this retrospective cohort. The 18 remaining patients, who presented a mild form, were excluded from the analysis because of the lack of immunological data.

COVID-19 severity was assessed at admission and then each day in accordance with the World Health Organization 9-point ordinal scale 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 – noninvasive ventilation or high flow oxygen; (6) hospitalized, severe – intubation and mechanical ventilation; (7) hospitalized, severe – ventilation plus additional organ support; and (8) death. Patients were then divided into 2 categories according to the worst score obtained during follow-up: mild (World Health Organization 9-point ordinal scale of 3 and 4) and severe (World Health Organization 9-point ordinal scale of 5–8).

Immunological analysis

The first immunological analysis was performed during the first 5 days postadmission to our department. Serial analyses were then performed, if possible, during hospitalization, each week until the discharge.

All staining was performed on fresh (<24 hour) whole blood samples drawn by venipuncture in ethylenediamine tetraacetic acid (EDTA)–coated tubes. Membrane immunostaining was performed as follows: 100 μl of blood was incubated with the appropriate amount of antibodies for 15 minutes 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 resuspended in the same buffer before rapid analysis by flow cytometry. Intracellular staining was performed as follows: 100 μl 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 monoclonal antibodies for 1 hour, washed, and processed for flow cytometry analysis. Lymphocyte subsets were enumerated by addition in the appropriate stained samples of 100 μl of Flow-Count Fluorospheres (Beckman Coulter) as per the manufacturer's instructions.

Data were acquired using a Navios flow cytometer (Beckman Coulter), and data analysis was performed with the Kaluza analysis software (Beckman Coulter). The gating strategy is presented in Supplementary Supporting Information S2.

Virological analysis

Positivity for SARS-CoV-2 was diagnosed through nasopharyngeal swab samples by using a home-brew real-time polymerase chain reaction or a transcription-mediated amplification (TMA) assay on the Panther instrument (Hologic).

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

Statistical analyses

The results were expressed as median with interquartile range unless stated otherwise. Continuous variables were compared between groups by using an unpaired *t* test and categorical variables by using the 2-sided χ^2 or 2-sided Fisher exact test, when necessary. Unpaired parametric or nonparametric 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 using GraphPad Prism version 8.4.2 (GraphPad Software Inc.). A *P* value of <0.05 was considered statistically significant.

RESULTS

Patient characteristics

Fifty-one patients with SOT 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 of 29 [72%] and 19 of 22 [86%] in mild and severe forms, respectively; $P = 0.31$). Three kidney transplant patients with a mild form and 1 kidney transplant recipient with a severe form received retransplant. A large majority of patients received triple tacrolimus-based therapy. Immunosuppression management was protocolized for all patients as follows: mycophenolic acid, mammalian target of rapamycin inhibitors, and costimulation signal blockers were immediately discontinued at the time of diagnosis of COVID-19, whereas tacrolimus was maintained (or introduced) with a trough target of 3 to 5 ng/ml. It was the first year post-transplantation for 12 patients (41%) with a mild form and 8 (36%) with a severe form (4 of 12 and 2 of 8, respectively, received anti–T-lymphocyte globulin; $P > 0.99$ and 0 of 12 and 1 of 8, respectively, 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 of transplantation (3 of 8 and 2 of 7, respectively, received anti–T-lymphocyte globulin; $P = 0.99$ and 0 of 8 and 1 of 7, respectively, received anti-CD20 monoclonal antibodies; $P = 0.47$). At admission, the estimated glomerular filtration rate was lower in patients who later developed a severe form than in

Table 1 | Main patient characteristics

Variable	Mild forms (n = 29)	Severe forms (n = 22)	P value
Medical past			
Medical history of			
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
Transplanted organ			
Kidney	21 (72.4)	19 (86.5)	0.28
Liver	4 (13.84)	1(4.5)	
Combined kidney and pancreas	0 (0)	1 (4.5)	
Heart	4 (13.8)	1 (4.5)	
Parameters at admission			
Recipient age, yr	55 ± 11	56 ± 15	0.63
Recipient sex, male	17 (57)	19 (86)	0.06
Body mass index, kg/m^2	27.3 ± 5.0	28.8 ± 7.2	0.70
IS at admission			0.64
Tac/MMF/S	23	17	
Tac/mTORi ± S	4	2	
Costimulation inhibitors[a]/MMF/S	1	2	
mTORi/S	0	1	
Time between transplantation and SARS-CoV-2 diagnosis, mo	49.4 (2 to 108)	26.7 (0.9 to 77)	0.39
Time between the first symptom of COVID-19 and hospitalization, d	4 (2 to 6)	3 (1 to 5)	0.40
Time between SARS-CoV-2 diagnosis and the first immunological analysis, d	3 (1 to 5)	6 (0 to 16)	0.18
CT scan evidence of COVID-19 pneumonia	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	6 (20.7)	9 (40.9)	0.13
SaO_2, %	97.8 ± 1.6	96.6 ± 3.4	0.22
Biological parameters			
Serum creatinine, μmol/l	125 ± 52	163 ± 58	0.008
CKD-EPI eGFR, ml/min per 1.73 m^2	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^3	195 ± 79	194 ± 99	0.37
Serum interleukin-1β, pg/ml	0.6 (0.3 to 1.0)	0.7 (0.5 to 0.9)	0.51
Serum interleukin-6, pg/ml	10.0 (7.3 to 53.9)	14.7 (4.3 to 55.9)	0.99
Serum TNF-α, pg/ml	18.7 (12.9 to 26.4)	16.2 (11.0 to 28.0)	0.99
Serum interleukin-8, pg/ml	10.8 (8.35 to 13.5)	8.6 (4.0 to 11.2)	0.22
Hospitalization follow-up			
Treatments			
Azithromycin	9 (31)	6 (27)	0.77
Third-generation cephalosporin	23 (79)	21 (95)	0.12
Hydroxychloroquine	4 (14)	0 (0)	0.12
Dexamethasone	8 (28)	17 (77)	0.0006
Time between dexamethasone and sample analysis, d[b]	−1.5 (−3.5 to 0)	−2.5 (−12 to 0)	0.23
IL6-R blockers	0 (0)	4 (18)	0.03
Time between IL6-R blockers and sample analysis, d[c]	—	−1 (−7 to 4)	–
Convalescent plasma therapy	1 (3)	2 (9)	0.57
Outcomes			0.05
Oxygen therapy	7 (24)	22 (100)	
Noninvasive ventilation/high flow oxygen	0 (0)	5 (23)	
Invasive ventilation	0 (0)	17 (77)	

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; COVID-19, coronavirus disease 2019; CT, computed tomography; eGFR, estimated glomerular filtration rate; IL6-R, interleukin-6 receptor; IS, immunosuppression; MMF, mycophenolate mofetil; mTORi, mammalian target of rapamycin inhibitor; S; steroid; SaO_2, arterial saturation of oxygen; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Tac, tacrolimus; TNF-α, tumor necrosis factor-α.
[a]Costimulation inhibitors were represented by anti-CD40 monoclonal antibodies in mild (n = 1) and severe (n = 1) cases and belatacept in 1 case with severe disease.
[b]Blood sample analyses were performed before dexamethasone for 6 of 8 mild forms and 16 of 17 severe forms.
[c]Blood sample analyses were performed before IL6-R blocker therapy in 3 of 4 severe forms.
Data are expressed as mean ± SD, median (interquartile range), or n (%).

those who developed mild forms. Of 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 of COVID-19 (18.2%) as compared with none among those with mild forms ($P = 0.03$).

Mild versus severe forms of COVID-19 in SOT recipients

We first compared patients affected by mild versus severe COVID-19 by 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 [1–5] and 6 [0–16] days in mild and severe forms, respectively; $P = 0.16$). The lymphocyte count was lower in patients presenting a severe form (526/mm^3 [278–782/mm^3] in severe forms vs. 815/mm^3 [560–1506/mm^3] in mild forms; $P = 0.04$) (Figure 1a). The NK cell count was comparable in both groups (100/mm^3 [63–135/mm^3] in severe forms vs. 49/mm^3 [23–156/mm^3] in mild forms; $P = 0.16$). CD3+ T cells were lower in severe forms (344/mm^3 [197–564/mm^3] vs. 643/mm^3 [397–1251/mm^3]; $P = 0.04$), as well as the number of CD8+ T cells (124/mm^3 [76–229/mm^3] vs. 240/mm^3 [119–435/mm^3]; $P = 0.05$). Naive and memory T-cell subsets (both in the CD4+ and CD8+ compartments) were similar in both groups (Figure 1b). However, the proportion of regulatory T cells (Tregs) (3.9% [2.35%–5.87%] vs. 2.7% (1.9%–3.45%]; $P = 0.02$) and that of CD4+DR+/CD38+ T cells (2.9% [1.37%–5.72%] vs. 1.4% [0.68%–2.35%]; $P = 0.005$) were higher in severe forms than in mild forms. We also analyzed markers associated with exhaustion (programmed death receptor-1 [PD-1], T-cell immunoreceptor with Ig and ITIM domains [TIGIT], and CD39), effector differentiation (CD57), and cytotoxic functions (perforin and 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 Treg 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 γδ T cells was comparable in both groups (2.5% of 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 (Supplementary Supporting Information S3). 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 2 groups (Figure 1a). However, the proportion of CD24^{high}CD38^{high} 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+ effector memory (EM) cells (Figure 1e), suggestive of coregulation of these 2 subsets. Similar results were obtained when kidney transplant patients alone were analyzed (Supplementary Supporting Information S3). The results remained unchanged when we had excluded 2 patients from the mild form group and 1 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).

Kinetics of expression of the different T-cell compartments during COVID-19

Serial blood tests were conducted for 17 patients (9 mild and 8 severe forms) (Supplementary Supporting Information S4). 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 (Supplementary Supporting Information S3).

We then compared differential expression of T- and B-cell markers in patients who had had a mild form irrespective of whether it developed into a severe form during hospitalization (Figure 2b). A decrease in the proportion of NK cells was observed in only severe forms during the first week (20.0% [10.7%–33.0%] during 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 (Supplementary Supporting Information S5). Nonetheless, the proportion of activated CD4+, as well as CD4+ T cells expressing perforin and granzyme B, PD-1+CD4+, and CD39+CD4+ exhaustion markers tended to be at a higher level in severe forms than in mild forms. CD8+ T-cell counts tended to be lower over time in severe forms than in mild forms. The change in B-cell compartment was similar in both groups, except for CD21^{low} 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.

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 postdiagnosis; $P = 0.46$), 20 of 26 (76.9%) with a mild form and 15 of 18 (83.3%) with a severe form had a positive serology ($P = 0.72$). Fourteen of 15 patients who presented COVID-19 during the first 3 months post-transplantation seroconverted. Among them, 4 patients had received a T-cell depleting agent and 1 patient had received both T- and B-cell depleting agents for ABO-incompatible kidney transplantation. The latter was the sole patient who did not seroconvert. Of the 20 patients who presented COVID-19 during the first year of transplantation, 3 did not seroconvert. The use of a depleting agent was not associated with no seroconversion (4 of 6 patients who had received a T- and/or

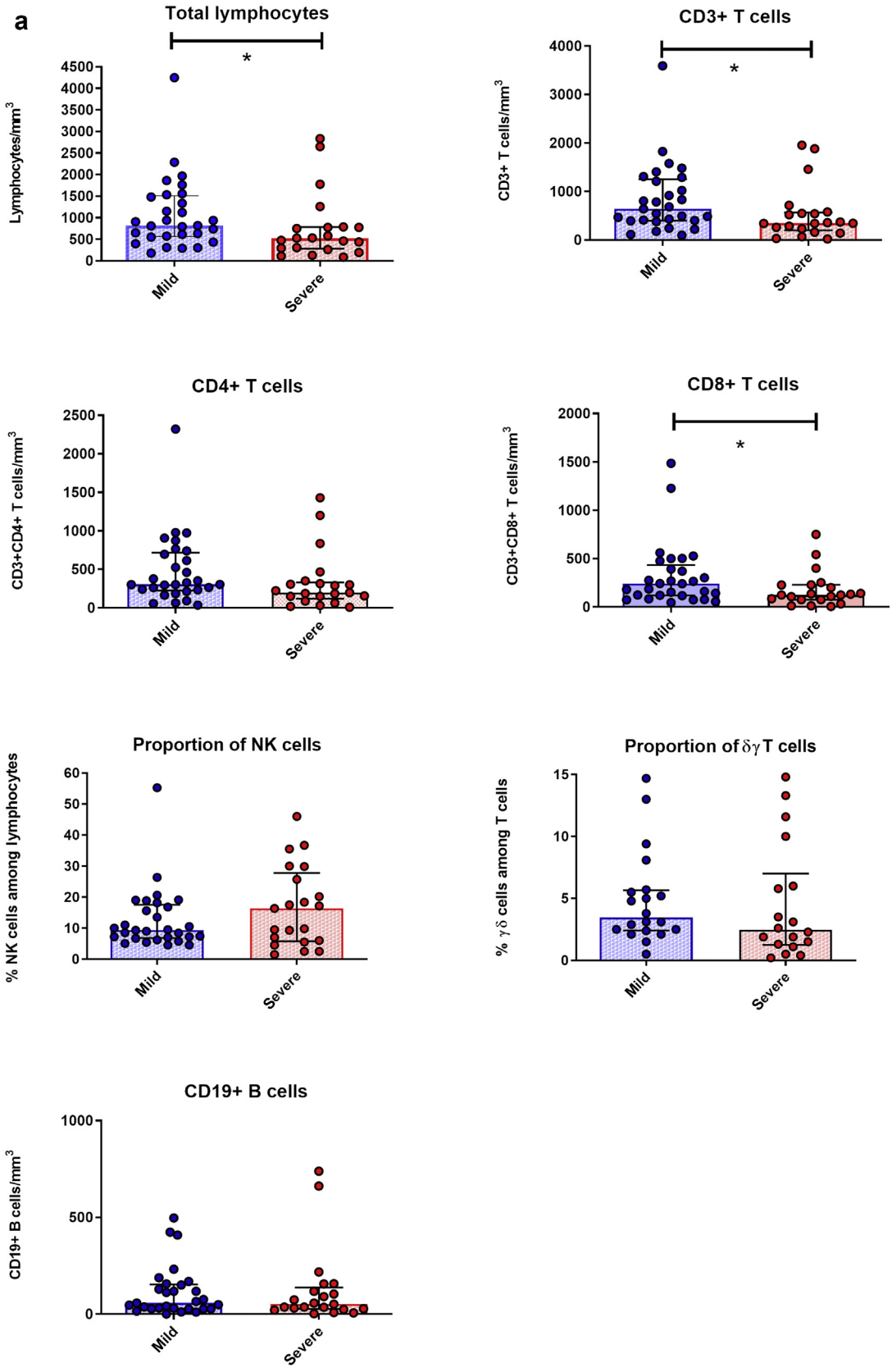

Figure 1 | (a–e) Comparison of natural killer (NK), conventional and regulatory T (Treg)–, and B-cell compartments in mild and severe forms of coronavirus disease 2019. Severe forms were analyzed using the immunological sample taken at the closest time before the worse clinical situation. (**a**) Total lymphocyte count; CD3+, CD4+, and CD8+ T-cell count; and proportion of NK cells, δγ T cells, and CD19+ B cells. (Continued)

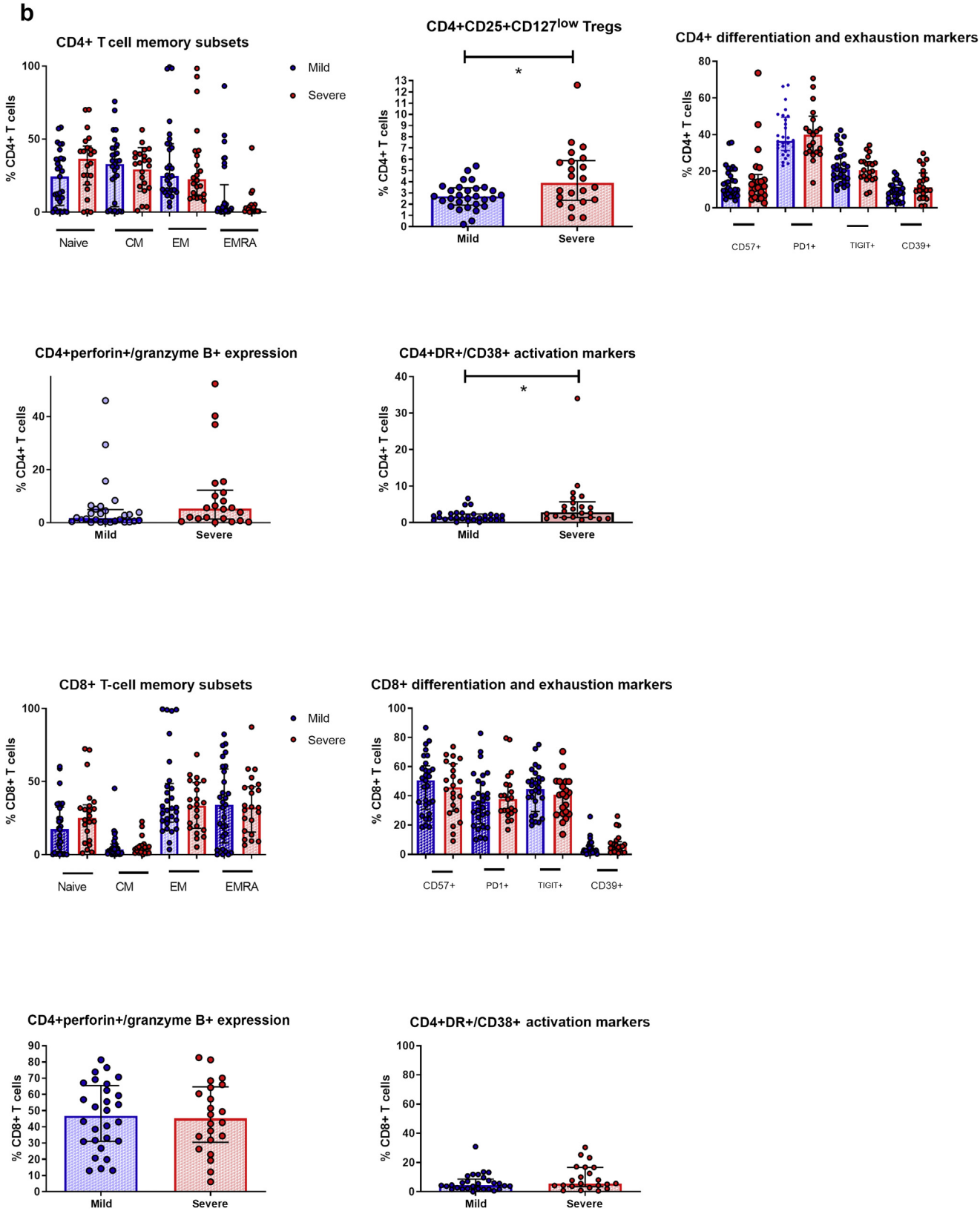

Figure 1 | (Continued) (**b**) T-cell compartment: CD4+ memory T-cell compartment, Tregs, activation senescence and exhaustion markers, and functional markers. CD8+ memory T-cell compartment, activation senescence and exhaustion markers, and functional markers. (Continued)

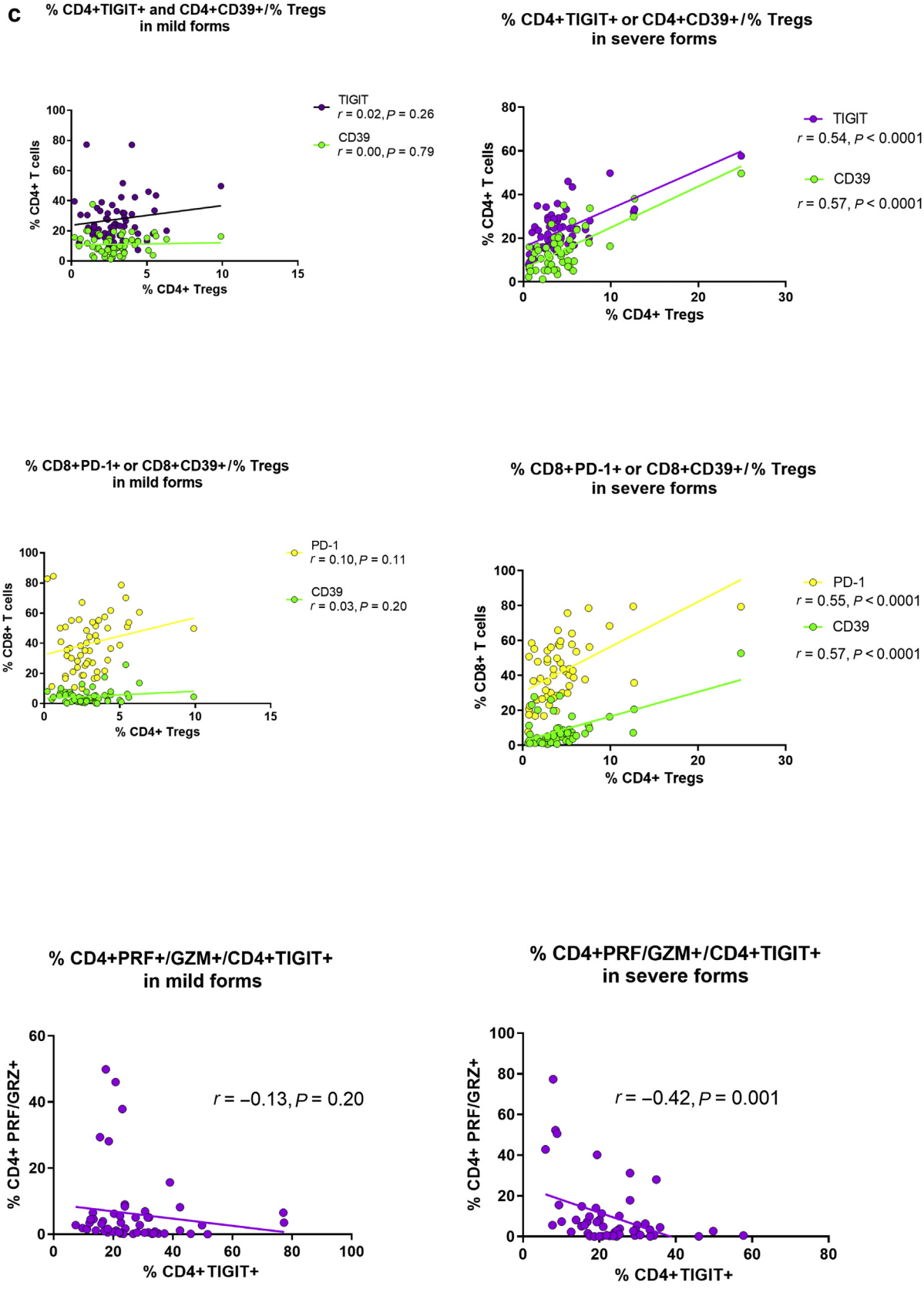

Figure 1 | (Continued) (**c**) Correlation between the percentage of Tregs and CD4+TIGIT+ or CD4+CD39+ cells, between the percentage of Tregs and CD8+PD-1+ or CD8+CD39+, and between the percentage of Tregs and CD4+TIGIT+ and CD4+perforin+/granzyme B+ (CD4+PRF+/GZM+) in mild and severe forms. The linear regression analysis was assessed using a Pearson correlation. (Continued)

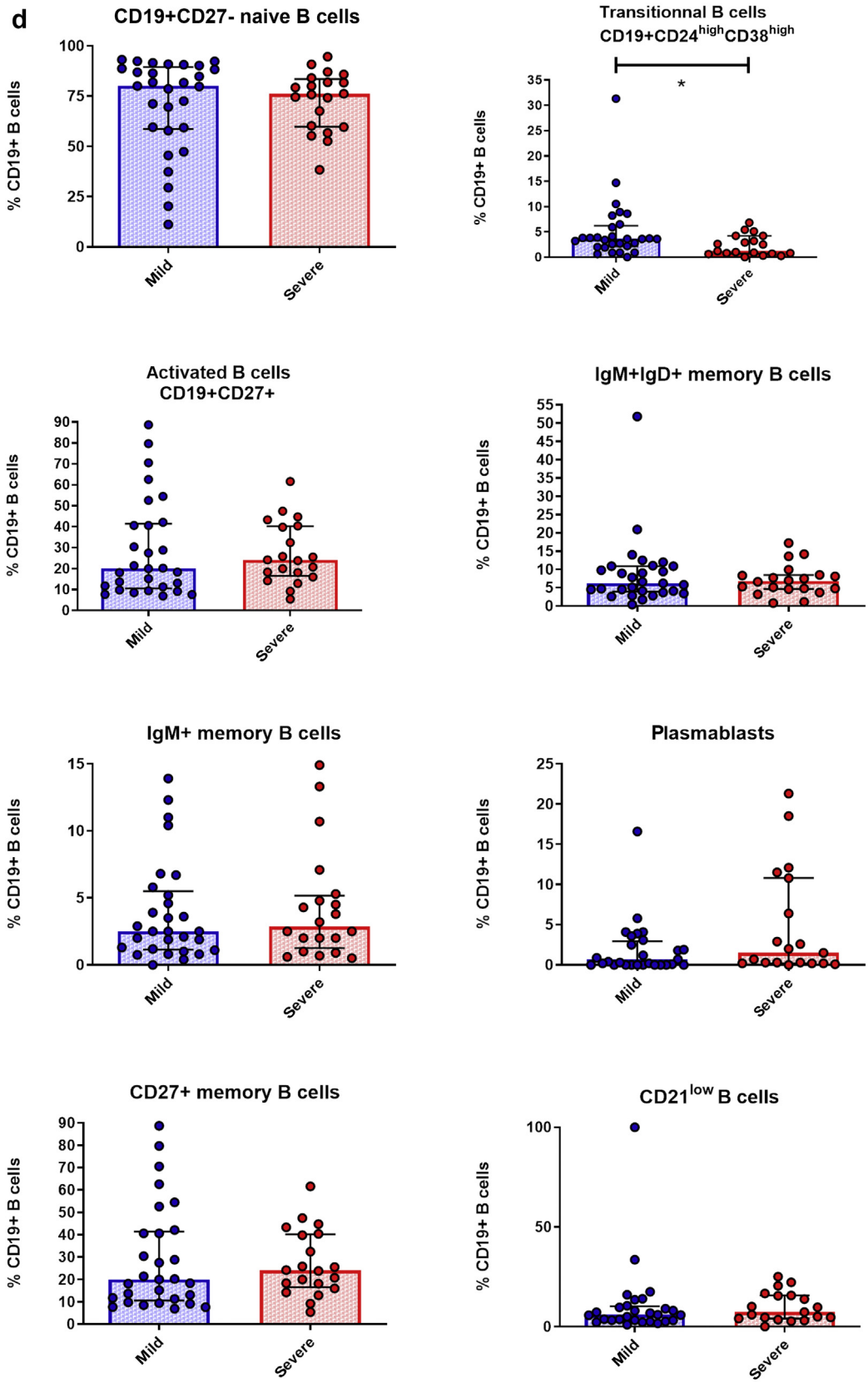

Figure 1 | (Continued) (**d**) B-cell compartment: naive, transitional, activated, memory B cells and plasmablasts. Data are expressed as median with interquartile range. (Continued)

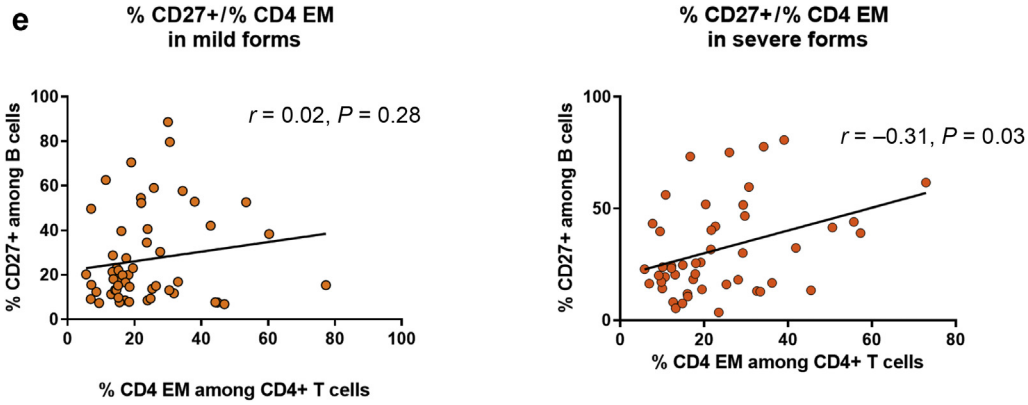

Figure 1 | (Continued) (**e**) Correlation between the percentage of CD27+ memory B cells and CD4+ effector memory (EM) T cells in mild and severe forms. The linear regression analysis was assessed using a Pearson correlation. *$P < 0.05$. CM, central memory; EMRA, effector memory re-expressing CD45RA; PD-1, programmed death receptor-1; TIGIT, T-cell immunoreceptor with Ig and ITIM domains.

B-cell depleting agent seroconverted vs. 13 of 14 patients without depleting agents; $P = 0.20$).

Patients with a negative serology presented a higher level of CD4+PD-1+ ($P = 0.06$) or CD8+PD-1+ ($P = 0.04$) than did patients who seroconverted (Supplementary Supporting Information S6).

DISCUSSION

In 2020, the pandemic caused by SARS-CoV-2 had a marked effect on SOT recipients. Although SARS-CoV-2 infection is frequently asymptomatic in the general population, severe forms seem to be higher by 13% to 50% in SOT recipients.[11] The course of infection in the general population is now well documented. A comprehensive understanding of immune responses in COVID-19 in 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.[12] 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, interferon regulatory factor 7 (IRF7)–dependent type I interferon, or neutralizing antibodies to interferon, was previously associated with severe forms of COVID-19.[5,13–15] 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.[16] As previously demonstrated in the immunocompetent population,[1,4,5,17] 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.[18,19] Memory CD8+ T cells can recognize the major histocompatibility complex I molecules of cells infected by viruses, leading to their elimination and subsequent clearance of many viruses including SARS-CoV-2.[20] 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.[18] 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[21] 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 Tregs in severe forms than in mild forms. Interestingly, we observed only in severe forms a correlation between the percentage of Tregs and expression of exhaustion-related markers TIGIT, CD39 in CD4+ T cells, and PD-1+ and CD39+ in CD8+ T cells. Conflicting results were observed concerning the frequency of Tregs and outcomes in the general population.[22–24] Exhaustion was suspected to be a mechanism to maintain immune cell homeostasis[25] and to participate in the progression of the disease severity.[9,20] The correlation between the percentage of Tregs and 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 the immunocompetent population,[20,26,27] 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.[28] In any event, the decrease in circulating NK cells during infection could facilitate viral spread.[28]

We observed changes in the B-cell compartment during infection that were similar to those described in non-transplanted immunocompetent patients.[29] First, we observed a sharp difference in $CD24^{high}CD38^{high}$ transitional B cells between mild and severe patients. A higher percentage of transitional B cells during mild COVID-19 infection than

during severe cases were previously reported in the general population.[29] Infectious diseases could promote the expansion of transitional B cells, which play a direct protective role by differentiation into antibody-secreting cells.[30] We also observed in severe forms a positive correlation between the proportion of memory B cells and CD4+ electron microscopy 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

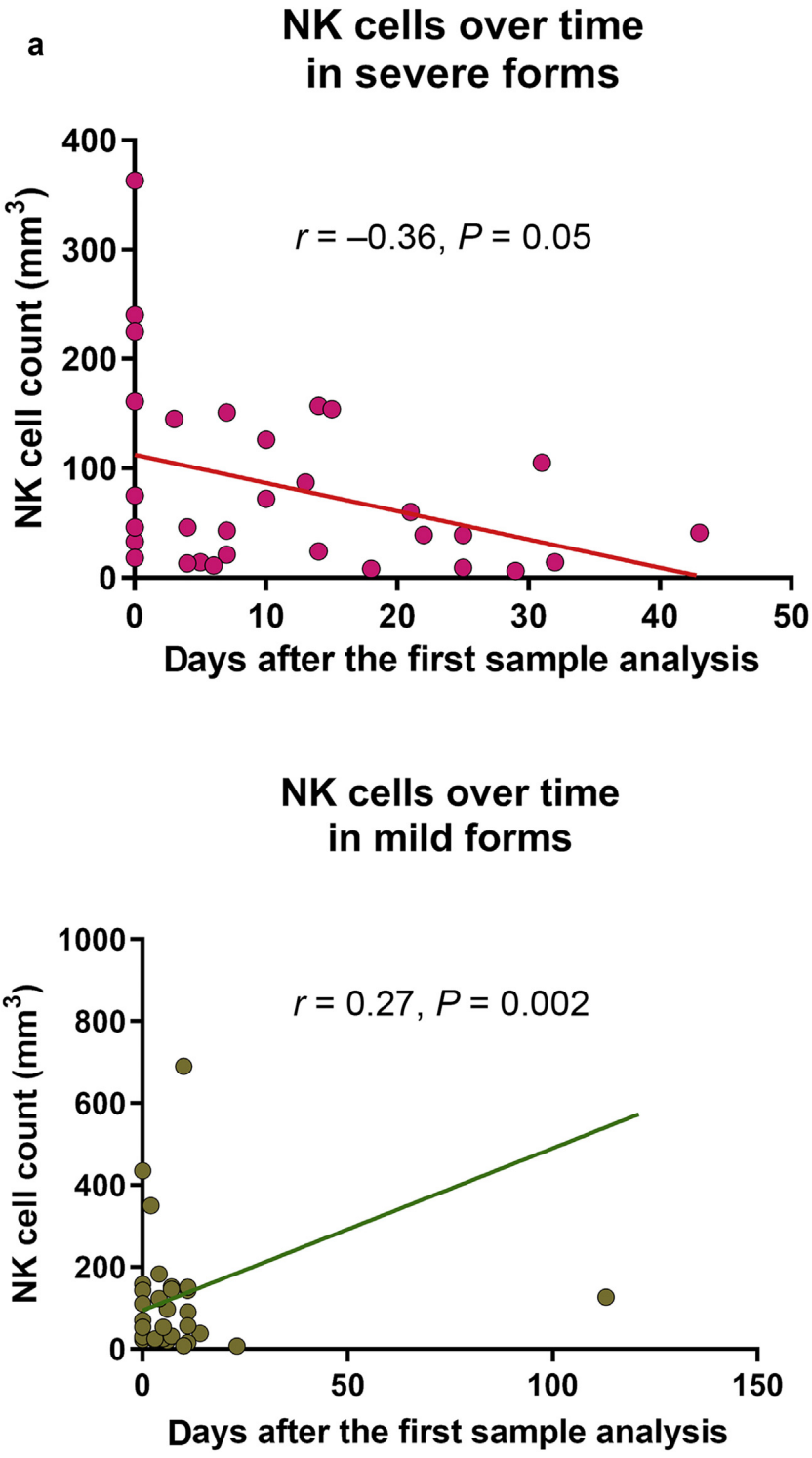

Figure 2 | (a,b) Comparison of natural killer (NK), conventional T-, and B-cell compartments in an initially mild case that became severe after the first blood test and those that remained mild. (**a**) NK cell count over time in mild and severe forms. (Continued)

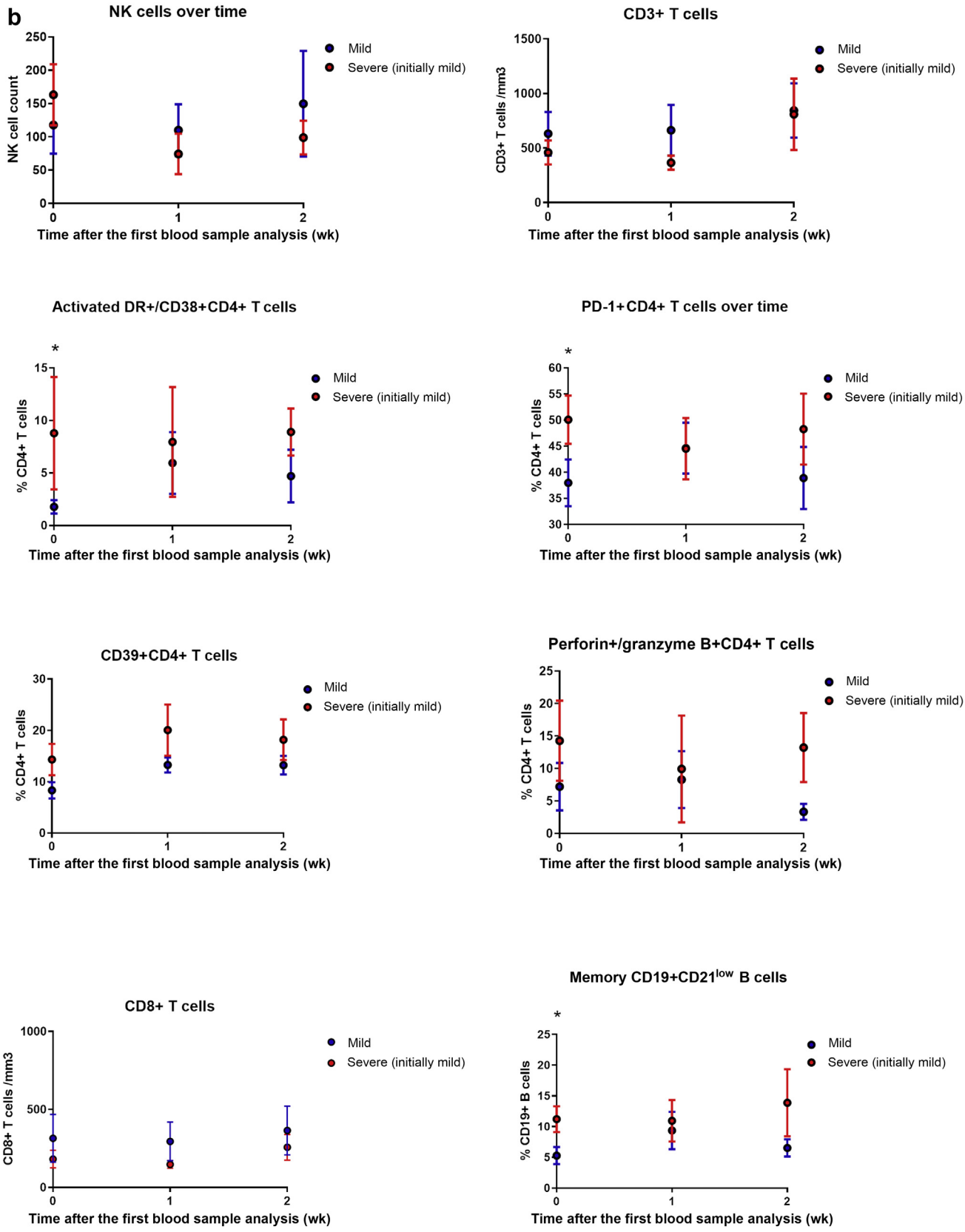

Figure 2| (Continued) (**b**) CD3+, activated PD-1+CD4+, and CD39+CD4+ T cells, perforin+/granzyme B+CD4+ T cells, CD8+ T cells, and memory CD19+CD21^{low} B cells. Data are expressed as mean with SEM. *$P < 0.05$. PD-1, programmed death receptor-1.

COVID-19, respectively). These numbers are higher than those described in the general population. Two of 9 seronegative patients had been treated with anti–T-lymphocyte globulin and/or anti-CD20 therapies and displayed no detectable circulating B cells. Recently, Burack *et al.*[31] reported similar results in a cohort of 70 SOT recipients, in which only 51% of patients developed antibodies after COVID-19. Interestingly, time post-transplantation (odds ratio 1.26; $P = 0.002$) and the use of >2 immunosuppressive agents (odds ratio 0.26; $P = 0.03$) were significantly associated with seroconversion.[31]

On the basis of these different observations, one could hypothesize that a delayed and weak specific T cell and neutralizing humoral response to SARS-CoV-2 caused by immunosuppression lead to virus immune neutralization escape[32] and prevent rapid clearance of the virus, leading to severe disease forms. Early reports investigating the response to vaccines tend to also demonstrate a weak and delayed response to vaccination in SOT recipients.[33,34]

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

Interestingly, as previously demonstrated in the general population, we observed a higher proportion of male recipients and lower kidney function in severe forms of COVID-19.[35] Acute kidney injury occurs frequently in patients with COVID-19 and is associated with poor prognosis.[36–38] Kidney susceptibility to SARS-CoV-2 infection is in part related to expression of angiotensin-converting enzyme 2 receptor, which is used as a port of the viral entry into the targeted cells.[39] Case series of naive kidney biopsies identified acute tubular necrosis as the main histological finding,[40,41] but some patients with genetic predisposition (i.e., apolipoprotein L1 G1 risk allele homozygosity) could develop collapsing focal segmental glomerulosclerosis.[42,43] Renal dysfunction could participate in dysregulation of inflammation (e.g., C-reactive protein and interleukin-6 levels) and nitrogen and carbon (glucose and free fatty acid) metabolism, fueling viral proliferation.[44]

Our study has various limitations. First, antirejection treatment used in our patients can have a dramatic effect 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, because of the relatively low number of patients recently transplanted who 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 effect of depleting agents and the durability of antibodies in patients having received depleting agents are required. Second, some patients with mild forms were not included in our study because of 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.[45] Third, because all patients received the same immunosuppressive treatment, with only the maintenance of a low dose of tacrolimus (and mammalian target of rapamycin 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–interleukin-6 receptor blockers), changed over time. However, because 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 SOT recipients. Prospective studies to investigate the effect of immunosuppression management are urgently needed.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

ADB, ET, NK, SF, and OM designed the study. ADB and AB collected the data. 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.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supporting Information S1. Registration information.

Supporting Information S2. Gating strategy.

Supporting Information S3. Comparison of NK, conventional and regulatory T, and B-cell compartment in mild and severe forms of COVID-19 in kidney transplant recipients.

Supporting Information S4. Clinical, biological, and radiological description of patients included in the kinetic evaluation of NK and conventional, T, and B-cell compartment during COVID-19.

Supporting Information S5. Kinetic of expression of CD4+ and CD8+ T-cell subsets during the hospitalization. Data are expressed as mean with standard error of the mean. $*P < 0.05$.
Supporting Information S6. Comparison between patients that developed or not a positive serology after COVID-19.

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