UNIVERSITAT DE BARCELONA

Clinical and immunological characterization of SARS-CoV-2 infection in Solid Organ Transplantation

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Clinical and immunological characterization of

SARS-CoV-2 infection in Solid Organ Transplantation

Doctoral thesis report submitted by **Alexandre Favà Buch**

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ORIOL BESTARD MATAMOROS, Doctor in Medicine and Surgery, Head of Nephrology and Kidney transplantation Department at Vall d'Hebron University Hospital,

CERTIFY

That Alexandre Favà Buch, graduated in Medicine and Surgery by the University of Barcelona, has carried out under my direction the research work to elaborate his Doctoral Thesis untitled **"Clinical and immunological characterization of SARS-CoV-2 infection in Solid Organ Transplantation",** and through this writing I authorize its presentation to achieve the degree of Doctor in medicine.

This is made evident to all effects in Barcelona, the 30th of September, 2022

Oriol Bestard Matamoros

Thesis Director

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Agraïments

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List of the articles that comprise the thesis (collection of published articles form).

This thesis comprises 4 objectives and 3 articles:

1.

Favà A, Cucchiari D, Montero N, Toapanta N, Centellas FJ, Vila-Santandreu A, Coloma A, Meneghini M, Manonelles A, Sellarés J, Torres I, Gelpi R, Lorenzo I, Ventura-Aguiar P, Cofan F, Torregrosa JV, Perelló M, Facundo C, Seron D, Oppenheimer F, Bestard O, Cruzado JM, Moreso F, Melilli E.

Clinical characteristics and risk factors for severe COVID-19 in hospitalized kidney transplant recipients: A multicentric cohort study

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SARS-CoV-2-specific serological and functional T cell immune responses during acute and early COVID-19 convalescence in solid organ transplant patients

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A comprehensive assessment of long-term SARS-CoV-2-specific adaptive immune memory in convalescent COVID-19 Solid Organ Transplant recipients

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RESUM DE LA TESI DOCTORAL (Català)

Títol: Caracterització clínica i immunològica de la infecció per SARS-CoV-2 en la població trasplantada d'òrgan sòlid.

Introducció: La malaltia per coronavirus 2019 (COVID-19) continua representant una de les causes més freqüents de mort precoç evitable entre els individus trasplantats d'òrgan sòlid (TOS). L'estudi detallat de la seva clínica i inmunobiologia és essencial per millorar el nostre coneixement sobre la interacció virus-hoste en el context de la immunosupressió crònica. Així mateix, la caracterització detallada de la qualitat, quantitat i durada de la resposta immunològica que genera la COVID-19 és imprescindible per a la presa de decisions en quant a les mesures preventives aplicables en aquesta població a risc.

Hipòtesi: La hipòtesi d'aquesta tesi és que l'estudi de les característiques clíniques i demogràfiques de la COVID-19, així com l'avaluació de les respostes immunològiques adaptatives SARS-CoV-2-específiques durant la infecció aguda i la convalescència dels pacients trasplantats d'òrgan sòlid en comparació a les presentades pels pacients immunocompetents, podria aportar evidència sobre els principals factors pronòstic i determinar el grau d'immunitat virus específica que assoleix aquest grup de població a risc.

Objectius:

- Analitzar els principals factors de risc associats al desenvolupament de la síndrome de dificultat respiratòria aguda (SDRA) i mortalitat en una cohort multicèntrica de trasplantats renals hospitalitzats per COVID-19 durant la primera onada de la pandèmia.
- Determinar la cinètica i la magnitud de la resposta immunològica adaptativa a diferents compartiments, cel·lular T i serològic, front el SARS-CoV-2 en pacients TOS hospitalitzats per COVID19, tot comparant-les amb les exhibides per un grup control de pacients immunocompetents.
- Investigar la persistència de la resposta adaptativa sis mesos després de la infecció mitjançant l'avaluació dels compartiments immunològics de memòria, serològic i

cel·lular (T i B) dels pacients TOS, utilitzant una cohort comparativa d'individus convalescents no immunodeprimits.

- Caracteritzar la magnitud i persistència de la immunitat adaptativa SARS-CoV-2 específica generada per les diferents formes clíniques de la COVID-19, des de els casos severs fins als asimptomàtics, en pacients convalescents TOS i immunocompetents.

Mètodes: Els tres estudis que constitueixen aquesta tesi es centraren en els pacients afectes per COVID-19 infectats durant la primera onada de 2020. Conseqüentment, inclouen pacients TOS i IC immunològicament *naïve*, no exposats prèviament al SARS-CoV-2. El primer article d'aquesta tesi consta d'una anàlisi observacional i retrospectiva d'individus trasplantats renals consecutivament hospitalitzats per COVID-19 durant la primera onada de la pandèmia en diferents centres trasplantadors, en el que a través de models de regressió logística examinarem els múltiples factors de risc clínics, demogràfics i immunològics predictius del desenvolupament de la SDRA i mortalitat conseqüència de la COVID-19. En el segon estudi vam realitzar una anàlisi prospectiva del desenvolupament de la immunitat adaptativa a nivell de la resposta cel·lular de memòria T i serològica durant la infecció aguda per SARS-CoV-2; mentre que al tercer avaluarem la immunitat adaptativa de memòria a llarg termini en una cohort transversal, sis mesos posteriors a la infecció. Els resultats obtinguts en la població trasplantada foren comparats amb els d'una cohort control d'individus immunocompetents; a més a més, en el tercer estudi vam classificar els pacients segons la gravetat de la infecció. L'avaluació de les respostes serològiques es va realitzar a través de diferents assaigs d'immunoabsorció lligats a enzims. Les respostes cel·lulars T de memòria/efectora productores de citocines (IFN-γ, IL-2, IFN-γ/IL-2, IL-21, IL-5, IL-6) contra les principals proteïnes estructurals del SARS-CoV-2 es van analitzar mitjançant FluoroSpot multicolor, mentre que l'ús de la tècnica d''ELISpot colorimètric per a cèl·lules B ens va permetre identificar les freqüències de cèl·lules B de memòria productores de IgG específiques, després del cultius in vitro de les PBMC.

Resultats principals: La mortalitat va ascendir fins gairebé un terç de la cohort estudiada en el primer treball. La SDRA fou la principal causa de mort per COVID-19, i el seu desenvolupament es va presentar a tots els grups d'edat, especialment entre els

pacients obesos. No obstant això, les taxes de mortalitat més importants convergiren en els ancians i aquells amb uns nivells més elevats de LDH a l'ingrés. Des d'un punt de vista immunològic, els pacients TOS trasplantats d'òrgans sòlid van mostrar un retràs significatiu en el desenvolupament de respostes adaptatives cel·lulars específiques detectables, sobretot en aquells amb pitjor pronòstic al final del seguiment. A més, vam objectivar que la COVID-19 moderada es caracteritza per un deteriorament funcional de la immunitat T de forma generalitzada, possiblement com a conseqüència d'un estat proinflamatori global, independentment de l'estat d'immunosupressió, donat que els pacients immunocompetents infectats també exhibiren aquest perfil d'anèrgia immunològica.

No obstant, hem observat que els individus TOS són capaços de desenvolupar una memòria immunològica específica robusta durant la convalescència, detectable més enllà dels sis mesos posteriors a la infecció i d'una magnitud comparable a les presentades pels seus homòlegs immunocompetents. De forma rellevant, aquesta magnitud sembla estar fortament influenciada per la severitat clínica de la infecció original, podent veure's més compromesa entre aquells pacients recentment trasplantats.

Conclusions: Més enllà de la immunosupressió crònica, diverses variables clíniques com la obesitat, la pneumopatia crònica o l'edat són determinants per al desenvolupament de formes greus de la COVID-19. A més a més, els pacients TOS presenten un retràs significatiu en el desenvolupament de la resposta antiviral adaptativa en les fases més inicials de la infecció, la qual cosa podria influir en les diferents trajectòries clíniques que presenten en relació a la població immunocompetent. Tot i això, durant la convalescència, aquests pacients són capaços de desenvolupar una memòria immunològica robusta i persistent, similar a l'exhibida per la població general, especialment aquells pacients convalescents d'infeccions moderades/severes. Aquesta observació podria contribuir a optimitzar l'estratificació del risc i les estratègies de vacunació en aquesta població.

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RESUMEN DE LA TESIS DOCTORAL (Castellano)

Título: Caracterización clínica e inmunológica de la infección por SARS-CoV-2 en la población trasplantada de órgano sólido.

Introducción: La enfermedad por coronavirus 2019 (COVID-19) sigue siendo una de las causas prevenibles de muerte temprana más comunes entre los individuos trasplantados de órgano sólido (TOS). El estudio detallado de su clínica e inmunobiología es esencial para mejorar nuestro conocimiento acerca de la interacción virus-huésped en el contexto de la inmunosupresión crónica. Así mismo, la caracterización detallada de la calidad, magnitud y duración de la respuesta inmune generada por la COVID-19 es imprescindible para la toma de decisiones en cuanto a las medidas preventivas aplicables en esta población a riesgo.

Hipótesis: La hipótesis de esta tesis es que el estudio de las características clínicas y demográficas de la COVID-19, así como la evaluación de las respuestas inmunológicas adaptativas SARS-CoV-2-específicas durante la infección aguda y la convalecencia de los pacientes trasplantados de órgano sólido en comparación a las presentadas por pacientes inmunocompetentes, podría generar evidencia entorno a los principales factores pronóstico y determinar el grado de inmunidad virus específica generada por este grupo de población a riesgo.

Objetivos:

- Analizar los principales factores de riesgo asociados al desarrollo del síndrome de dificultad respiratoria aguda (SDRA) y mortalidad en una cohorte multicéntrica de trasplantados renales hospitalizados por COVID-19 durante la primera ola de la pandemia.
- Determinar la cinética y la magnitud de la respuesta inmune adaptativa en distintos compartimentos, celular T y serológico, frente al SARS-CoV-2 en pacientes TOS hospitalizados por COVID-19, en comparación a las exhibidas por un grupo control de pacientes inmunocompetentes.
- Investigar la persistencia de la respuesta adaptativa seis meses después de la infección mediante la evaluación de los compartimentos inmunológicos de memoria, serológico y

celular (T y B) de los pacientes TOS, utilizando una cohorte comparativa de individuos convalecientes no inmunodeprimidos.

- Caracterizar la magnitud y persistencia de la inmunidad adaptativa SARS-CoV-2 específica generada por las distintas formas clínicas de la COVID-19, desde los casos severos a los asintomáticos, en pacientes convalecientes TOS e inmunocompetentes.

Métodos: Los tres estudios que componen esta tesis se centraron en los pacientes afectos por COVID-19 infectados durante la primera ola de 2020. En consecuencia, incluyen pacientes TOS e IC inmunológicamente *naïve*, no expuestos previamente al SARS-CoV-2. El primer artículo de esta tesis consta de un análisis observacional y retrospectivo de individuos trasplantados renales consecutivamente hospitalizados por COVID-19 durante la primera ola de la pandemia en distintos centros trasplantadores, en el que a través de modelos de regresión logística examinamos múltiples factores de riesgo clínicos, demográficos e inmunológicos predictivos para el desarrollo del SDRA y mortalidad. En el segundo estudio realizamos un análisis prospectivo del desarrollo de la inmunidad adaptativa a nivel de la respuesta celular de memoria T y serológica durante la infección aguda por SARS-CoV-2; mientras que en el tercero evaluamos la inmunidad adaptativa de memoria a largo plazo en una cohorte transversal, seis meses después de la infección. Los resultados obtenidos en población trasplantada fueron contrastados con una cohorte comparable de individuos inmunocompetentes; además, en el tercer estudio los sujetos fueron clasificados según la gravedad de la infección. Las respuestas serológicas se caracterizaron a través de distintos ensayos de inmunoabsorción ligado a enzimas. Las respuestas celulares T de memoria/efectora productoras de citoquinas (IFN-γ, IL-2, IFN-γ/IL-2, IL-21, IL-5, IL-6) contra las principales proteínas estructurales del SARS-CoV-2 se evaluaron mediante FluoroSpot multicolor, mientras que el empleo del ELISpot colorimétrico para células B nos permitió identificar las células B de memoria productoras de IgG específicas, tras cultivos in vitro de las PBMC.

Resultados principales: La tasa de mortalidad alcanzó prácticamente un tercio de la cohorte estudiada en el primer trabajo. El SDRA fue la principal causa de muerte por COVID-19, y su desarrollo se presentó en todos los grupos de edad, especialmente entre

los pacientes obesos. Sin embargo, las mayores tasas de mortalidad convergieron en los ancianos y en aquellos con niveles más elevados de LDH al ingreso. Desde un punto de vista inmunológico, los pacientes trasplantados de órgano sólido mostraron un retraso significativo en el desarrollo de respuestas adaptativas celulares y serológicas específicas detectables, en particular aquellos con peor pronóstico al final del seguimiento. Además, objetivamos que la COVID-19 moderada se caracteriza por un deterioro funcional de la inmunidad T de forma generalizada, posiblemente a consecuencia de un estado proinflamatorio global, independientemente del estado de inmunosupresión, puesto que los pacientes infectados inmunocompetentes presentaron también dicho perfil de anergia inmunológica.

Sin embargo, hemos observado que los pacientes TOS son capaces de desarrollar una memoria inmunológica específica robusta durante la convalecencia, detectable más allá de los seis meses posteriores a la infección y de una magnitud comparable a las presentadas por sus homólogos inmunocompetentes. Es importante destacar que dicha magnitud parece estar fuertemente influenciada por la severidad clínica de la infección, pudiéndose ver comprometida en aquellos pacientes recientemente trasplantados.

Conclusiones: Más allá de la inmunosupresión crónica, distintas variables clínicas como la obesidad, la neumopatía crónica o la edad son determinantes para el desarrollo de formas severas de COVID-19. Además, los pacientes TOS presentan un retraso significativo en el desarrollo de la respuesta antiviral adaptativa en las fases más iniciales de la infección, lo cual podría influir en las distintas trayectorias clínicas que presentan en relación a la población inmunocompetente. Sin embargo, durante la convalecencia, estos pacientes son capaces de desarrollar una memoria inmunológica robusta y persistente, similar a la exhibida por la población general, especialmente aquellos pacientes convalecientes de infecciones moderadas/severas. Esta observación podría contribuir a optimizar la estratificación del riesgo y las estrategias de vacunación en esta población.

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DOCTORAL THESIS SUMMARY (English)

Title: Clinical and immune characterization of SARS-CoV-2 infection in Solid Organ Transplantation.

Introduction: Coronavirus disease 2019 (COVID-19) became, and remains, one of the most common preventable causes of early death among solid organ transplant (SOT) patients. Exhaustive clinical and immune-biological evaluations are needed to improve our understanding of the virus-host interaction under the effects of chronic immunosuppression. More specifically, the quality, quantity and duration of infection-derived immune responses are key features that need to be thoroughly characterized to eventually guide preventive decision-making strategies in this high-risk patient population.

Hypothesis: The hypothesis of this thesis is that by investigating main clinical and demographic features of COVID-19 as well as by accurately assessing SARS-CoV-2-specific adaptive immunity, both during infection and convalescence in SOT patients as compared to immunocompetent (IC) patients, we would gain relevant insights on major determinants driving distinct clinical outcomes as well as on the degree of anti-viral immune protection achieved in this highly vulnerable group of patients.

Objectives:

-To analyze main clinical, demographic, and immunological risk factors associated with the development of adult respiratory distress syndrome (ARDS) and death in a large multicentric cohort of kidney transplant recipients hospitalized due to COVID-19 during the first wave of the pandemic.

-To characterize the kinetics and magnitude of SARS-CoV-2 specific adaptive immune responses at the T-cell and serological immune compartments, among SOT patients hospitalized due to COVID-19 and compare them to those exhibited by a matched group of IC patients.

- To investigate the relative persistence of peripheral adaptive immune memory specific to SARS-CoV-2 up to six months after COVID-19 by evaluating both serological and T and

B-cell memory/effector immune compartments in SOT recipients, compared to a matched cohort of IC convalescents patients.

- To comprehensively characterize the strength and durability of SARS-CoV-2-specific adaptive immunity across distinct clinical presentations of COVID-19, from severe to asymptomatic infections, in convalescent SOT and IC patients.

Methods: All 3 studies comprising this doctoral thesis focused on COVID-19 patients infected during the first pandemic wave in 2020 thus, among naïve SOT and IC individuals without any previous symptomatic exposure to SARS-CoV-2. The first article of this thesis is a multicenter, observational, retrospective cohort study of consecutive incident kidney transplant patients admitted with COVID-19, in which adjusted logistic regression models were performed to examine main risk factors predicting the development of ARDS and mortality. In the second study, we prospectively tracked the kinetics of de novo adaptive immune responses at the T-cell and serological level, during acute COVID-19 as compared to IC convalescent COVID-19 patients. Finally, in the third study of this thesis, we conducted a cross-sectional immune-monitoring analysis six months after COVID-19 in a group of SOT as well as IC convalescent individuals with distinct COVID-19 severity. Serological responses were assessed by distinct enzyme-linked immunosorbent assays. Multiple cytokine-producing T-cell responses (IFN-γ, IL-2, IFN-γ/IL-2, IL-21, IL-5, IL-6) against main structural SARS-CoV-2 proteins were evaluated using a multicolor FluoroSpot assay, whereas circulating viral-specific IgG-producing memory B cells were investigated using a colorimetric B-cell ELISpot assay, after in vitro B-cell cultures.

Main results: The mortality rate almost reached one-third of the kidney transplant cohort included in the first study. ARDS was the leading cause of COVID-19 deaths, and although its development occurred across all ages, obese transplant patients were the most affected. However, mortality converged in the elderly as well as those with higher levels of LDH at admission. Notably, SOT exhibited a significant delay in developing detectable adaptive immune responses specific against main immunogenic viral antigens, especially those with the poorest outcomes. In addition, we observed that moderate/severe COVID-19 is characterized by a non-specific global T-cell impairment,

which seemed to be independent of the immunosuppression state as though infected IC patients did also display such an immune anergic pattern.

Nonetheless, SOT recipients are capable of mounting robust SARS-CoV-2-specific immune memory over time during convalescence, which can be maintained beyond six months after infection and is comparable in magnitude to those responses presented by IC subjects. However, infection-derived immunity seems to be highly dependent on the clinical severity of COVID-19 and might be challenged in those more recently transplanted.

Conclusions: On top of chronic immunosuppression, other relevant clinical variables such as obesity, pulmonary baseline diseases and age are key factors favoring poor patient outcomes after COVID-19. Moreover, SOT seem to display a significant delay of ant-viral adaptive immune responses at the infection onset, which may also influence distinct clinical trajectories as compared to infected IC patients. At convalescence, however, SOT patients do develop robust and durable immune memory/effector responses, comparable to IC convalescent individuals, and especially those having experienced more severe forms of COVID-19. These observations could help improve current immune-risk stratification and guide vaccination policies in this vulnerable patient population.

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NOTE TO THE READER

The unprecedented and overwhelming evolution of the pandemic spread, the progress made in the understanding of the pathophysiology of this infection and how the scientific community has developed in a time-record successful new protective therapies, especially with the development of active immunization with effective vaccines, have made this pandemic unique in the history of infections ever.

Before going forward and read this thesis work, it is important to bear in mind that all the studies contained herein, were initiated during the first wave of the pandemic, when all hospitals and the entire world was knocked-down with the devastating effects of this infection leading to fatal outcomes.

These three works of this thesis were indeed conducted during the early phases of the infection onset with the main aim to try to better understand why a specific group of more vulnerable patients such as SOT seemed to be particularly exposed to the worst outcomes after infection, and how their immune protective responses behaved as compared to infected IC individuals. We would like to acknowledge the huge efforts put from multiple and diverse professionals that were directly impacted and touched by the pandemic and who made these studies possible.

These results helped us to better understand a number of important features, which may now seem somewhat evident, after this, albeit short, overwhelmingly fast period in which most of this patient population has been actively and effectively immunized with new vaccines.

Nonetheless, we believe this thesis retains its relevance in the current clinical scenario given the substantial proportion of SOT remaining poor or non-responder to the distinct vaccines, this most likely accounting for the higher odds of severe infection rates as compared to the general population. Furthermore, as the SOT population increasingly includes vaccinated individuals with prior or breakthrough infection history, it is necessary to delineate the precise effects of these recurrent antigenic challenges beyond the ongoing vaccination protocols. In fact, our studies put forward the different immunological behavior against SARS-CoV-2 of SOT as compared to IC, which seem to hold true for the immune responses achieved after active immunization.

Despite the significant improvements in SOT outcomes during this pandemic, further research is required to improve our understanding of viral-host interactions and gain insights into immunological protection among this group of vulnerable patient population.

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I. INTRODUCTION

A. The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and coronavirus disease 2019 (COVID-19)

1. Emergence and genomic identification of the new coronavirus

Coronaviridae is a family of enveloped, single-stranded positive-sense RNA viruses that cause respiratory and intestinal infections in animals and humans[1]. Since their first isolation in 1965[2], six human coronaviruses had been reported. Four of them (OC43, 229E, NL63, and HKU1) cause mild seasonal colds; however, the outbreak of *severe acute respiratory syndrome coronavirus* (SARS-CoV) in 2002 and the *middle east respiratory syndrome* (MERS) in 2012 raised significant alarm among the global health authorities due to their high fatality rates (9.6% and 34.3%, respectively)[3,4]. After the 2002 outbreak, many coronaviruses phylogenetically related to SARS-CoV (SARSr-CoVs) were found in bats from different countries, indicating their potential role as natural reservoirs[5]. Considering the great diversity and RNA recombination of SARSr-CoVs, spillovers to humans were expected to occur[1].

In late December 2019, a cluster of patients with severe pneumonia of unknown cause was reported in China[6]. Fifty-five percent of those infected before January 2020 were epidemiologically linked to the Huanan Seafood Wholesale Market, in Wuhan[7]. The pathogen of this pneumonia was a new coronavirus, firstly coined 2019 novel coronavirus (2019-nCov), sharing 96.2% of nucleotide sequence identity with RaTG13, a previously described bat-borne SARSr-CoV[8]. These facts suggest that the novel coronavirus emergence might have occurred through live wildlife trade, being the Huanan Market the most likely epicenter of the outbreak. Genetic analyses revealed that 2019-nCoV pertained to the genus Betacoronavirus, similarly to SARS and MERS. On February 11th, the International Committee on Taxonomy of Viruses named the novel coronavirus "*Severe Acute Respiratory Syndrome Coronavirus 2* (SARS-CoV-2)"[9], while the WHO designated the associated disease as "*coronavirus disease 2019* (COVID-19)". Given the large number of countries reporting human-to-human transmission, the WHO officially defined the COVID-19 outbreak as a pandemic on March 11th 2020[10].

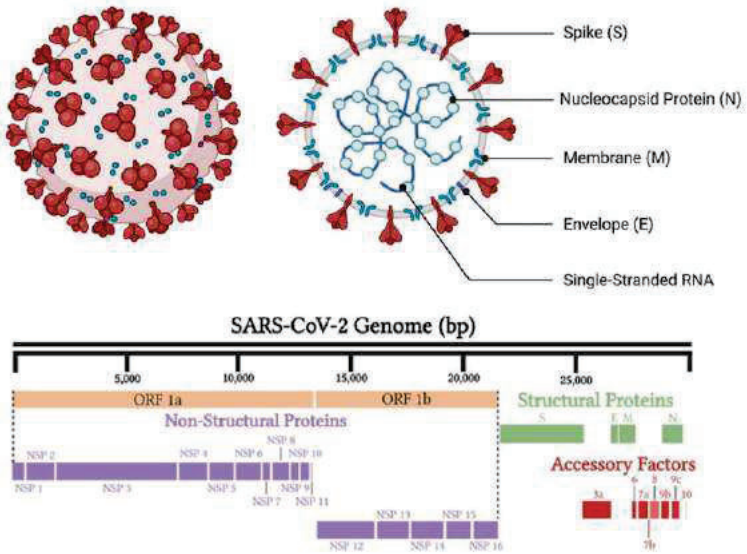
2. SARS-CoV-2 infection: From basic virology to clinical and epidemiological features

- *Viral structure, attachment, and entry*

SARS-CoV-2 is a single-stranded positive-sense RNA virus with a genome of 29.903 nucleotides[11], exhibiting 79% sequence homology with SARS and 50% with MERS. The viral genome includes 11 open reading frames (ORFs) encoding for 27 proteins[12] (**Figure 1**). Two-thirds of the SARS-CoV-2 orfeome account for 16 non-structural proteins (NSP) essential for viral replication. The remaining sequence is devoted to structural and accessory proteins involved in its infectivity. The structural proteins are the Spike glycoprotein (S), Membrane protein (M), Nucleocapsid (N) and Envelope (E), while the accessory proteins are the orf3a, orf6, orf7a, orf7b, orf8 and orf10. Notably, most of the structural divergence of SARS-CoV-2 to SARS-CoV relies on the Spike protein[8], which might explain its greater transmission.

The first step in SARS-CoV-2 infection involves the spike (S) interaction with the angiotensin-converting enzyme 2 (ACE2). The S protein has a trimeric composition, with distinct subunits S1 and S2. While the S1 subunit contains the receptor binding domain (RBD) that allows its attachment to ACE2, the S2 cleavage by host serine protease TMPRSS2 leads to the structural changes required for membrane fusion and virus entry[13]. TMPRSS2 and ACE2 are co-expressed along the respiratory tract, accounting for SARS-CoV-2 tropism[14].

Figure 1. SARS-CoV-2 structure (Jamison DA et al. 2022)[12]



- *Clinical and pathological features of COVID-19*

SARS-CoV-2 is transmitted through respiratory droplets and aerosols, firstly invading the ACE2+TMPRSS2+ ciliated cells of the nasal cavity epithelium[15], where significant viral replication occurs from 4 to 5 days before the symptoms onset[16]. In most cases, prompt immune responses clear the infection within ten days, causing cough and fever as the most common symptoms[17].

In some other individuals, SARS-CoV-2 disseminates into the lung, causing direct alveolar damage and inflammation through type II pneumocyte infection[18]. Clinically, most severe infections meet the criteria for acute respiratory distress syndrome (ARDS), defined by respiratory failure and bilateral radiographic opacities. Histological examination of COVID-19 patient lungs reveals diffuse alveolar damage (DAD) as the predominant tissue injury pattern[19], which is characterized by interstitial and intra-alveolar edema, hyaline membrane formation and type II pneumocyte hyperplasia and death. The disruption of the alveolar-endothelial interphase presumably triggers the prothrombotic milieu of COVID-19 pathology; in fact, 84% of autopsies show the presence of microthrombi within the alveolar capillaries[20]. The high levels of d-dimer exhibited by severely affected individuals are a biochemical surrogate of this phenomenon, constituting a prognostic factor of the disease[17].

Single-cell sequencing of bronchoalveolar lavage fluid cells show increased proportions of activated macrophages with low proportions of dendritic and T cells in critically affected patients, suggesting a highly proinflammatory macrophage microenvironment as a major contributor to tissue injury in critical COVID-19[21].

Aside from the viral cytopathic effects on the lung, severe COVID-19 is associated with systemic inflammation, as evidenced by elevated ferritin and C-reactive protein levels, whose values at admission and posterior dynamics define those patients with the poorest outcomes[17]. Also, serum levels of proinflammatory cytokines such as IL-6 or TNF-α have been established as survival predictors in some studies[22], supporting the pathogenic role of the inflammatory host responses.

Accordingly, severe COVID-19 may also lead to extra-pulmonary disease. Over a quarter of hospitalized patients with COVID-19 have been reported to develop acute kidney

injury, with up to 45% of patients in the intensive care unit (ICU) requiring kidney replacement therapy[23]. Besides, hypercoagulability and large vessel thrombosis have been reported in 10-20% of critically affected individuals. Although the specific mechanisms for such complications remain elusive, it is thought that hyperinflammation and endothelial dysfunction might be relevant contributors[24], altogether aggravating the morbidity and mortality of patients with SARS-CoV-2.

Therefore, COVID-19 displays a broad spectrum of clinical forms, ranging from asymptomatic infection to severe pneumonia. Defining those individuals at the highest risk for fatal outcomes has been of great importance from the beginning of the pandemic, due to its impact on guiding preventive measures.

- *COVID-19 epidemiology and vulnerable groups*

Given the absence of anti-viral immunity at the population level, SARS-CoV-2 infections spread rapidly all over the globe. By November 2021, 40% of the world's population had been infected[25]. With more than 6 million deaths officially reported[26], some studies revealed a three-fold higher toll when addressing the gap between estimated excess mortality and declared covid-19 deaths[27]. Fortunately, severe forms and death are not universal outcomes of COVID-19. Hence, a comprehensive evaluation of all the diagnoses is essential to estimate the actual impact of this emerging disease.

Due to the limited testing capacity during the pandemic's first months, initial reports underestimated the proportion of asymptomatic infections, accounting for 1-5% of the diagnosis[7]. However, recent studies indicate that up to 40% of the cases are asymptomatic[28,29], which is of utmost importance for public health strategies, given the high rates of pre-symptomatic viral transmission[16].

On the other hand, understanding the magnitude of COVID-19 mortality also has important implications. Because of the proportion of asymptomatic cases, accurate estimations of COVID-19 infection-fatality ratios (IFR) rely on sero-epidemiological surveys. During the pre-vaccine era, IFR was estimated to be 0.5-1%, with a significant heterogeneity led by age: while those aged 30 displayed an IFR of 0.057%, an exponential increase of IRF was found in people aged 60 (1%) and 90 (20%)[30,31]. Despite the lower fatality ratios as compared to MERS (34.3%) and SARS-CoV (9.6%), the 3

million daily infections estimated worldwide during 2020 posed an unprecedented challenge to global health systems[25].

These data show that **advanced age** was (and remains) a predominant risk factor for severe COVID-19, with the lowest mortality rates found among those aged 5-10 years[30]. To a lesser extent, underlying medical conditions such as **obesity**, **diabetes** and **chronic kidney disease** were also associated with disease progression[32], especially when combined in the same individuals[33]. In this regard, **solid organ transplant** (SOT) recipients were also rapidly identified as a vulnerable group; nonetheless, some authors questioned the impact of immunosuppression as an independent risk factor, given the significant prevalence of other comorbidities also within this group of patients[34,35]. Furthermore, the inflammatory features of severe COVID-19 raised the hypothesis of a protective role of immunosuppression in these individuals[36–40], altogether underscoring the need for better characterizing both innate and adaptive immune responses occurring in this specific group of patients.

B. The anti-viral immune response: General principles

1. Innate immunity and viral infections

The innate immune system provides the first line of defense against pathogens, rapidly identifying the infection and triggering the expression of a variety of inflammatory cytokines. Dendritic cells (DCs) are a major component of the innate anti-viral response involved in pathogen recognition, cytokine expression and antigen presentation, eventually limiting viral replication.

- *Dendritic cells, inflammation, and type I IFN production.*

Dendritic cells are bone marrow-derived cells found in blood, tissues, and lymphoid organs that initiate innate and adaptive immune responses to viruses by producing type I interferon (IFN-I) and delivering antigen presentation. Together with other numerous cells such as macrophages, lymphocytes and epithelial cells, they exhibit the capacity to recognize preserved molecular structures that are ubiquitous among microbes, known as pathogen-associated molecular patterns (PAMPs).

This recognition occurs through the Toll-like receptors (TLR), a group of membrane-bound proteins[41] that sense endocytic viral RNA/DNA and initiate the NF-Bk and the IFN response factors (IRFs) pathways, leading to nuclear transcription of inflammatory cytokines (TNF-α; IL-1 and IL-6) and type I interferons, respectively.

In particular, type I interferons are mainly produced by a subset of DC with a prominent endoplasmic reticulum known as plasmacytoid dendritic cell (pDC)[42]. Type I IFNs (α and β) exert their biological effects through the INFα/β receptor (IFNAR), which is expressed in all nucleated cells, ultimately interfering with viral replication by several mechanisms: from transient suppression of cellular protein synthesis to direct RNA degradation[43]. Additionally, IFN-I are potent activators of natural killer (NK) cells, a cytotoxic subset of cells that play essential roles in innate immune responses[44,45].

Overall, type I IFNs promote intracellular transcriptional changes in both infected and uninfected cells, leading the host to a protective biological milieu known as the "antiviral state"[46].

On the other hand, classical DC (cDC) are a subset of DC characterized by its capacity for antigen presentation via major histocompatibility complexes (MHC) type I and II expression. Therefore, cDC are key activators of naïve T cells. They are broadly present within the epithelia (skin, gastrointestinal tract, lung) and become activated upon viral recognition. Matured DC upregulate the expression of chemokine receptors[47,48], enabling its migration to local lymph nodes, where antigen presentation occurs.

In the context of a viral infection, type I INF upregulates the expression of MHC I/II complexes by cDC[49]. Furthermore, clonal expansion of anti-viral specific CD8 T cells (both memory and effector) strongly depends on the direct action of type I INF[50].

Hence, in addition to its innate effector function, DC play a central role in engaging innate recognition and adaptive immunity.

2. Adaptive immunity and viral infections

The adaptive immune system consists of two major functional categories: humoral (B-lymphocytes and antibodies) and cellular (T-lymphocytes) immunity. Contrary to innate immunity, the fundamental properties of the adaptive immune system are specificity, diversity and memory, which confers long-lasting protection against a virtually infinite range of antigenic structures.

- *Antigen presentation*

Initiation of adaptive immunity requires capturing and concentrating antigens in secondary lymphoid organs in a cell-associated manner[51], since T lymphocytes cannot recognize soluble antigens. Different cell types serve as antigen-presenting cells (APCs), namely the cDC mentioned above, macrophages, B lymphocytes and the vascular endothelial cells. T lymphocytes only recognize those peptides bound to the specialized Major Histocompatibility Complex (MHC) molecules, which are expressed on the surface of host cells[52]. Their function relies on binding antigens and displaying them for recognition. Whereas MHC I peptides are recognized by CD8+ T cells, MCH II proteins are identified by CD4+ T cells. Hence, for an antigen to be immunogenic, it must be processed into linear peptides associated with sufficient affinity to MHC molecules and be eventually recognized through MHC-TCR interactions. These linear series of amino acids bound to an MHC molecule and recognized by T cells are known as *epitopes*, and their characterization is relevant to precisely understand which specific protein regions elicit immune adaptive responses.

- *T-cell immunity*

Besides antigen presentation, APCs also provide the necessary molecules for T-cell activation[53], defining the classical two-signal model by which the TCR downstream pathways are amplified[54,55]. Among them, the autocrine effects of interleukin-2 (IL-2) and its cognate receptor (CD25) by T cells is essential for enhancing cell proliferation and clonal expansion; actually, it elicits a 50.000-100.000 fold increase among antigen-specific T cells in the acute infection setting[56].

On the other hand, during the earliest stages of T-cell development, a selection of TCR co-receptors occurs according to the recognition of class I or II self-MHC structures: the CD4+ (MHC-II) and CD8+ (MHC-I) co-receptors. During this process, these cells become committed to the CD4+ or CD8+ lineage, representing the two major classes of T lymphocytes.

There have been described four different subsets of CD4+ T cells according to their cytokine secretion signature: Th1 (IFN-γ, IL-2, TNF-α), Th2 (IL-4, IL-5, IL-13), Th17 (IL-17, IL-22) and Tfh; the former will be discussed elsewhere. Th1 differentiation is both driven and targeted to intracellular pathogens, including viruses. Interferon-γ, also known as type II interferon, is released in response to antigen recognition, amplifying antigen presentation by MHCI/II upregulation and mediating macrophage activation, ultimately leading intracellular microbe destruction. On the other hand, CD8+ are MHC class I restricted and its activation results in the release of perforins and cytotoxic granules over the target cell.

- *Humoral and B-cell immunity*

Humoral immune responses are mediated by circulating antibodies; however, their production and specificity rely upon a complex succession of events subject to lymphocyte cross-talking, antigen presentation and cellular migration.

In humoral responses, B cells are activated by the interaction of the B-cell receptor (BCR) with a cognate epitope. In contrast to TCR, BCR does not require MHC presentation; however, it is insufficient to initiate B-cell responses *per se*. Upon antigen recognition, BCR internalizes and processes the antigen into new peptides that eventually will be taken back to the B cell surface by MHC-II molecules. By doing so, epitope presentation to T helper lymphocytes occurs. Upon CD40 ligand (CD154) expression and co-stimulation, T helper lymphocytes induce B cell proliferation and its differentiation into short-lived plasmablasts secreting low-affinity antibodies. These relatively rapid interactions occur outside the B-cell follicles; thus, the name of extra-follicular B-cell activation.

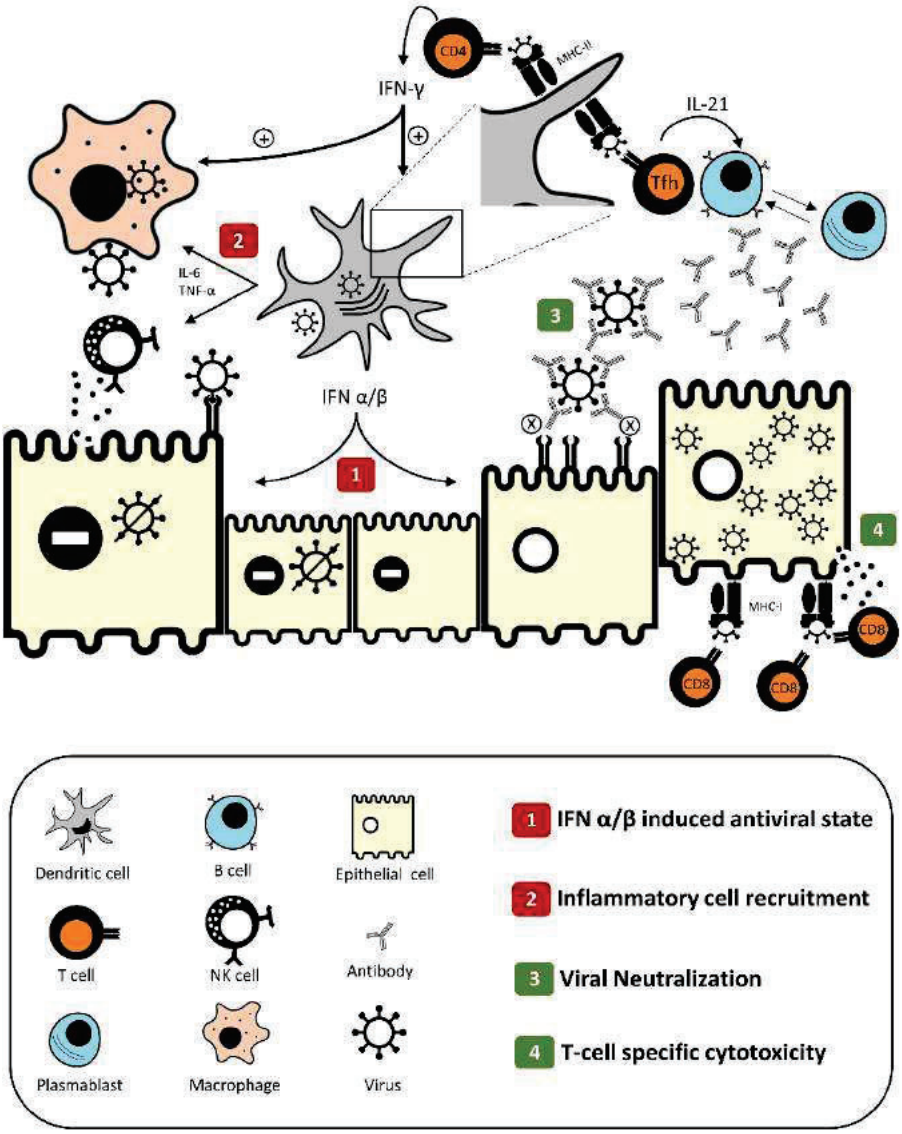
On the other hand, T follicular helper (Tfh) cells are a specific subset of CD4+ T cells that promote further B-cell differentiation, enhancing antibody maturation. IL-21 constitutes the defining cytokine of mature Tfh required for germinal center development[57], where activated B cells intensively proliferate and accumulate a high rate of mutations in the variable genes of heavy and light chains, ultimately leading to a wide range of BCR specificities. This process is called somatic mutation[58]. Thereafter B cells undergo a selection process in which those with the highest antigenic affinity survive, thereby improving antibodies specificity, breath and avidity, encompassing a process known as affinity maturation. Therefore, germinal center reactions are critical to developing effective humoral responses.

- *Main effector mechanisms of adaptive anti-viral immunity*

Circulating antibodies are considered the most important correlate of protection in infectious diseases[59]. Their main anti-viral function *in vivo* is the capacity to neutralize free viral particles[60]. Neutralization is the antibody's capacity to occupy a large portion of the virion surface, interfering with the host-cell fusion process and ultimately impeding viral entry to the host cells. Also, neutralizing antibodies can prevent viral release by cell-cell transmission[61,62]. Passive immunization strategies rely on these properties, being therefore indicated as prophylaxis or shortly after infection[63].

However, the mechanism of protection is not necessarily the mechanism of recovery from infection. Given the obligate intracellular nature of viruses, T-specific cell clones are the predominant effector mechanism for viral clearance [64–66]. Mice models of lymphocytic choriomeningitis virus (LCMV) reveal that CD4+ and CD8+ deficient animals are not able to attain viral control, developing persistent infections[65]. In humans, pre-existing Influenza CD4+ T cells are associated with lower viral shedding and less severe illness[67]. Conversely, the abrogation of CMV-specific T-cell immunity among solid organ transplant recipients promotes viral replication and disease in prior asymptomatic carriers[68]. Further, monitoring CMV-specific T-cell responses accurately predicts the risk of infection among these patients[69], altogether highlighting the pivotal role of T-cell immunity in viral control and infection recovery.

Figure 2. Integrated overwiew of anti-viral immunity



C. Immunity to SARS-CoV-2

1. Innate immunity and SARS-CoV-2

Defective type I IFN responses are a hallmark of severe COVID-19, accounting for the most relevant immunopathological feature of SARS-CoV-2 infection relative to the innate immune system. Preliminary whole blood transcriptomic analysis and cytokine measurements on 50 consecutive SARS-CoV-2 infected individuals revealed the lowest

IFN-α levels among the most critical COVID-19 cases[70]. Later longitudinal studies showed that mild SARS-CoV-2 infections are defined by an early and transient INF-I signaling[71–73], whereas severe infections exhibit profoundly impaired early induction, followed by a sustained up-regulation at disease progression[74,75]. Hence, it is likely that distinct temporal kinetics of type I interferon expression might account for the divergent outcomes observed in naïve individuals.

- *SARS-CoV-2 interferon antagonism and defective type I interferon responses*

Respiratory tract cell lines and animal models have shown that, compared to other respiratory viruses, SARS-CoV-2 is a poor inductor of IFN-I responses[76]. Its genome encodes interferon antagonists directly disrupting host responses, akin to previous SARS-CoV and MERS. Non-structural (NSP) and accessory proteins account for the vast majority of these suppressors. Among them, NSP1 and ORF6 are the most prominent viral components for suppressing IFN induction and signaling, promoting successfully viral evasion, replication and virulence[77,78].

In line with these data, Zhou et al. described a significant reduction in circulating DCs during acute severe infection compared to convalescent and healthy controls. Besides, ex-vivo stimulation of these DCs revealed a profound abrogation of IFN-α and IFN-β production and defective expression of the CD86 co-stimulatory molecule. In this regard, DCs from actively infected individuals were not capable of inducing CD4 and CD8 T-cell proliferation in mixed lymphocyte reaction assays, contrary to the controls[79]. These data support not only the defective IFN-I response as a hallmark of severe SARS-CoV-2 infections but also underline its influence over antigen presentation, thereby hampering adaptive immune priming and leading to delayed immune T-cell responses.

On the other hand, several reports revealed that a proportion of patients with life-threatening COVID-19 harbor autoantibodies against type I INF. A large cohort demonstrated a prevalence of 10%, being only detected among severe cases (mainly men and the elderly)[80], while absent among asymptomatic/mild cases. Also, a significant number of severely affected individuals (17 to 77 years) showed loss-of-function mutations on TLR and type-I IFN receptor genes[81], supporting the relevant implication of deficient type-I interferon responses in critical infections.

Notably, older individuals are known for displaying lower numbers of circulating pDCs with reduced capacity to type I IFN production[82]. In an experimental model of SARS-CoV-2 infection, transcriptomic analyses comparing type I interferon gene expression in lungs from young and old macaques revealed a severe downregulation of these pathways among the oldest subjects[83]. Additionally, prior mice SARS-CoV-1 models showed that lung DC from older animals displayed an impaired migration capacity, negatively affecting T-cell priming[84]. These studies shed some light on the innate determinants of SARS-CoV-2 progression among the elderly.

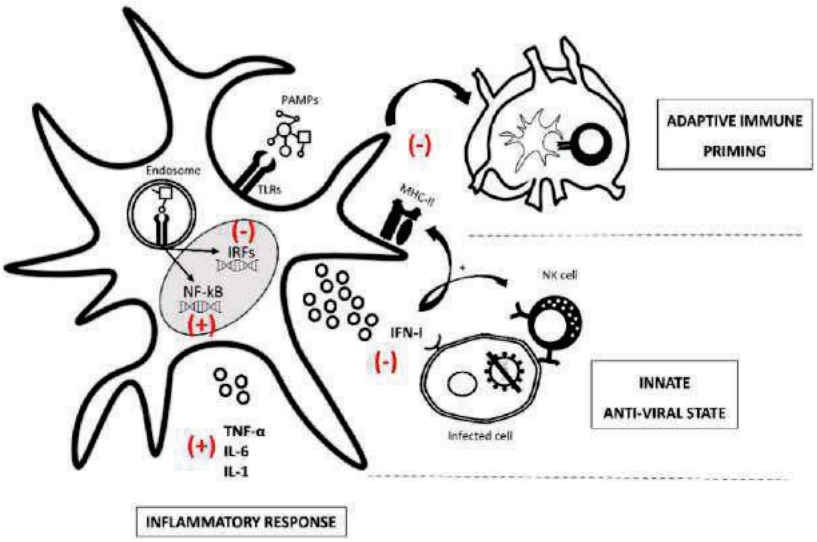
Therefore, the convergence of the viral escape mechanisms with some host predisposing factors might determine the risk of complications derived from SARS-CoV-2 infection.

- *Dysregulated inflammation in COVID-19*

SARS-CoV-2 infection promotes the expression of the NF-kB pathway's genes[70]. The subsequent production of inflammatory cytokines such as TNF-α, IL-6, CCL2 and CCL8 promotes endothelial permeability and monocyte migration[21], ultimately leading to pulmonary injury. In fact, serum TNF-α and IL-6 have been proposed as prognostic biomarkers[22], while IL-6 pathway inhibitors have been proved effective in severe COVID-19[85,86]. Remarkably, a longitudinal study in ferrets showed that these responses lasted beyond the viral clearance[76], supporting the overactive inflammation observed in critical infections. A direct role of SARS-CoV-2 viral proteins, both structural[87,88] and non-structural[89], has been suggested in activating NF-kB in vitro, thus providing insight into the molecular basis of the aberrant inflammatory responses observed in severe cases.

In sum, the imbalance between NF-kB and interferon response factors (IRFs) pathways upon viral recognition illustrates the innate immune signature of severe SARS-CoV-2 infections, which might ultimately interfere with the adaptive responses through defective antigen presentation (**Figure 3**).

Figure 3. Innate immunity and SARS-CoV-2



2. Adaptive immunity and SARS-CoV-2

2.1 - Immunological determinants of SARS-CoV-2 protection: experimental models

It is essential to distinguish between the role of the adaptive immunity developed de novo during the SARS-CoV-2 infection from that exhibited by *convalescent* individuals to delineate the relative contribution of all its components against the viral infection. Understanding which immunological mechanisms contribute to protection in these different settings is paramount for future SARS-CoV-2 vaccines and immunotherapeutics.

In this regard, because of ethical, biological and logistic constraints, it has been rather difficult to characterize host responses specific to SARS-CoV-2 in humans, nor its correlates of protection. However, animal models allow for deciphering important aspects of COVID-19 such as transmission, pathology and protection.

As for the primary infection, Israelow et al.[90] established the immunological determinants of viral clearance in naïve mice with various genetic immune deficiencies. They observed that RAG-1 deficient mice (deprived of B and T lymphocytes) were unable to clear acute SARS-CoV-2 infection. However, B-cell-deficient mice retained the ability to eliminate the viral RNA, whereas CD4+ and CD8+ deficient did not, suggesting that T-cell immunity might be capable of controlling the disease in the absence of humoral (B-

cell) responses. Overall, they established the pivotal contribution of the T-cell compartment for infection resolution. Accordingly, previous animal models based on the former SARS-CoV infection anticipated these results, highlighting the importance of T cells against human betacoronaviruses[91,92].

Furthermore, the importance of pre-existing humoral and cellular immunity by passive transfer of either specific T cells or sera before viral exposure was also investigated, and while T-cell infusion significantly attenuated viral replication, those treated with antibodies did not develop the infection. Accordingly, McMahan et al.[93] showed that convalescent immunoglobulin transfer prior SARS-CoV-2 challenge conferred dose-dependent protection (non-detectable RNA) in a group of naïve rhesus macaques. Therefore, both studies concluded that the presence of neutralizing antibodies might be sufficiently protective against infection, regardless of the T-cell compartment.

However, antibody titers waned over time in these convalescent macaques. Despite this, they all developed anamnestic responses at viral re-exposure (rise in both antibody titers and IFN-γ CD4+/CD8+ responses) that allowed these animals to clear the virus effectively. In order to evaluate the role of cellular responses in this setting, the authors depleted the CD8 T-cell compartment in these convalescent individuals displaying a waning humoral immunity. Although this group exhibited a significant increase in serologic and CD4+ T-cell responses, they all contracted an infection with longer persistence of respiratory RNA.

In conclusion, these animal models illustrate a synergic role of main adaptive immune components, with particular contributions:

- *Neutralizing antibodies are protective* by preventing viral attachment to the host cells, either by passive transfer (naïve animals) or by sustained intrinsic production (convalescent animals).

- Once infected by SARS-CoV-2, *T-cell responses* are key for achieving an *effective viral clearance* in both naïve and experienced animals.

- Convalescent animals are capable of developing *anamnestic immune responses* to prevent reinfection or limit its course.

2.2 - De novo adaptive immune responses to SARS-CoV-2

- *Epitope characterization and T-cell immunodominance*

Defining SARS-CoV-2 epitopes and their adaptive immune responses was crucial for assisting vaccination design and establishing mechanisms of effective host defense in naïve populations. Based on the high degree of similarity between SARS-CoV and SARS-CoV-2 sequences, and using bioinformatic predictive analyses, specific T-cell epitopes were initially defined[94], being afterwards confirmed and characterized by ex-vivo T-cell simulation assays[95,96] from convalescent donor's lymphocytes.

In these studies, epitope pools identified 100% and 70% of CD4+ and CD8+ specific responses in convalescent individuals, respectively. Whereas CD4+ exhibited a Th1 phenotype upon antigen stimulation (IFN-γ and IL-2 secretion), specific CD8+ expressed IFN-γ, granzyme B and tumor necrosis factor[95].

As for the antigenic profile, the Spike, Membrane, Nucleocapsid and the non-structural protein 3 (nsp3) were described as the most immunodominant proteins, representing more than 80% of the T-cell repertoire, both for the CD4+ and the CD8+ lineages. Notably, T-cell responses could recognize between 30-40 epitopes for each donor, showing minimal overlap with the relevant RBD epitopes of humoral responses.

- *B-cell and humoral immunity*

In those works evaluating humoral immunity, 90% of individuals seroconverted ten days after symptoms onset[97,98] with definite seroconversion rates ranging between 91-99% in large cohorts[99,100]. In a cohort of 647 convalescent individuals, more than 90% of serum neutralizing activity accounted for the receptor binding domain (RBD) antibodies, thus underscoring the specific structural target that prevents the Spike protein from its engagement with ACE receptors. Initial B-cell responses are predominantly mediated by short-lived plasmablasts generated by extrafollicular reactions, shifting towards a germinal center dependent maturation during convalescence[101]. Accordingly, despite a significant RBD-IgG decay in the following months, most individuals exhibited an increment in their neutralizing capacity, consistent with an ongoing affinity maturation of the B-cell responses[102].

- *Adaptive immunity and disease severity: the importance of early kinetics*

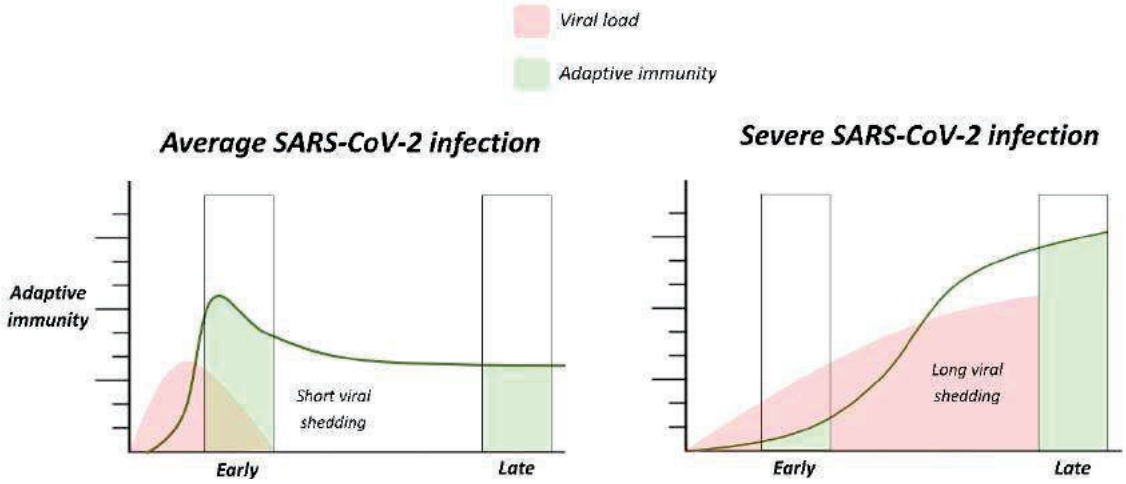
Several works consistently reported higher IgG and IgA titers among moderate/severely affected patients, compared to mildly symptomatic subjects[103–105]. Furthermore, 77% of hospitalized individuals' serum was neutralizing, whereas it was the case in only 18% and 11% of the mild and asymptomatic groups, respectively[102]. Therefore, disease severity determines the magnitude and affinity of humoral responses against the RBD domain. A similar pattern has also been identified for the T-cell[106–110] and B-cell compartments[110–112]with more significant responses following the most severe infections.

Therefore, given the robust adaptive responses exhibited by critical COVID-19, some authors proposed that overactive adaptive immunity might be in itself involved in the pathogenesis of COVID-19[113].

Notwithstanding, a longitudinal study including 229 COVID-19 subjects[105] revealed that while anti-S IgG levels correlated with disease severity, *such correlation was time-dependent*: deceased patients mounted a robust *yet delayed* humoral response compared to survivors, which was also described by others[114,115]. Moreover, longitudinal analyses of SARS-CoV-2 T-cell responses revealed rapid and specific cell proliferation as a feature of non-severe infections[98,116–118] with adequate viral clearance, describing detectable CD4+/CD8+ responses from day four after symptoms onset. In a thorough evaluation of 53 acute patients, those eventually developing a severe COVID19 presented delayed and uncoordinated humoral and T-cell responses at the most initial time points[98].

In summary, the available data underscores the importance of immune trajectories, suggesting a critical time window in which prompt innate and adaptive responses improve viral control and disease outcomes[75,119]. Delayed adaptive priming and innate dis-regulation at advanced stages of the disease could lead to hyper-inflammatory responses, higher viral loads and tissue injury (**Figure 4**). Whether these defects rely on innate deficiencies[80,81] impaired antigen presentation[120] or intrinsic adaptive immune dysfuntion[121], separately or in a combined fashion, remains uncertain.

Figure 4: *De novo* adaptive immunity to SARS-CoV-2 (Sette A et al. 2021)[75]



2.3 - Immune memory to SARS-CoV-2

SARS-CoV and Middle East Respiratory Syndrome (MERS) survivors showed persistent specific T-cell immunity up to 11 years after the infection[122], whereas circulating antibodies waned over time[123]. Given the high similarity of the SARS-CoV-2 protein sequence to the other two zoonotic betacoronaviruses, similar immune kinetics were expected from COVID-19 convalescent subjects.

- *T-cell immune memory*

Specific T-cell responses peak after infection and contract thereafter, consistent with the canonical dynamics following the effector anti-viral phase[124]. Nonetheless, IFN-γ-producing T-cell frequencies may be detected by ELISpot assays in up to 90-95% of convalescent patients after 6 to 12 months, showing a preferential recognition for the structural antigens (S,M,N) in comparison to the non-structural SARS-CoV-2 peptides[107,110]. Importantly, and in line with humoral immunity, the duration of anti-viral T-cell responses is highly influenced by disease severity.

Activation-induced markers (AIM) T-cell assays have revealed that specific CD4+ T cells prevail over the CD8+ T cells in the long term. CD4+ T cells display a central memory phenotype (CD45RA-CCR7+) and a Th1 polyfunctional profile with specific IFN-γ, IL-2 and

TNF-α secretion. Phenotypic markers indicate that SARS-CoV-2 specific CD8+ T cells are terminally differentiated effector memory cells (T_{EMRA}) secreting IFN-γ, TNF-α and granzyme B[125,126].

Notably, several studies reported the presence of SARS-CoV-2 specific T-cells in a certain proportion of un-exposed donors ranging from 20-50%[127–130]. Hence, it was speculated that pre-existing T-cell immunity might originate from prior exposures to "common cold" coronaviruses. Given the high rates of asymptomatic infection, only pre-pandemic biological sample analyses could rule out prior exposures; however, most of the studies enrolled contemporary "un-exposed" donors in their cohorts, thus making it difficult to differentiate between eventual *in-vitro* cross-reactivity from true previous antigen-specific memory T cells[129,131]. In addition, long-lasting PBMC's stimulation cultures during weeks have been employed in some works[130], which may trigger in vitro differentiation of de novo memory T cells instead of providing the functional assessment of circulating, freshly retrieved PBMC. While several works considering these methodological aspects demonstrated the presence of cellular cross-reactivity to SARS-CoV-2 in some donors[127,132], the clinical relevance of these pre-existing T cells remains unproven, being still a subject of debate.

- *B-cell and humoral immune memory*

As for the humoral immune kinetics, several studies have described a bi-phasic pattern consisting of an initial peak 1-2 months after the symptoms onset, followed by a rapid decrease afterwards and a stable plateau lasting for more than one year[110,125,133,134]. This pattern is consistent with transitioning from an early burst of extrafollicular plasmablasts producing antibodies with little to no somatic hypermutation[101,102,135] to constitutive antibody production by long-lived plasma cells (LLPC). Accordingly, one study reported a positive correlation between specific LLPC frequencies and antibody titers seven months after the infection[136]. Moreover, these authors identified Spike-specific memory B-cells in circulation, altogether indicating that SARS-CoV-2 infection triggers a robust germinal center reaction. Interestingly, different kinetics on antibody decay has been reported, being those Spike and RBD long-lasting compared to the nucleocapsid ones, which show an accelerated decrease.[133,137].

As described for the de novo anti-viral immune responses, some other works have also reported a much faster antibody waning among those asymptomatic infections[138,139]. Indeed, in several studies[110,125,133,140], disease severity was consistently found of relevance in determining not only the magnitude (peak) but also the durability of humoral responses.

Conversely, RBD-specific mBC trajectories are characterized by a progressive expansion up to eight months post-infection, before reaching a plateau[110,125,141]. The antigenic persistence in the host might explain these observations; indeed, viral nucleic acids have been identified in the gut for at least two months after infection[141].

These data confirm that adaptive responses after SARS-CoV-2 infection are long-lasting, encompassing several immune compartments with distinct kinetics. However, infection-derived immunity is highly heterogeneous between individuals, with some viral and host-related factors influencing both early and persistent immune kinetics.

2.4 - Protective immunity

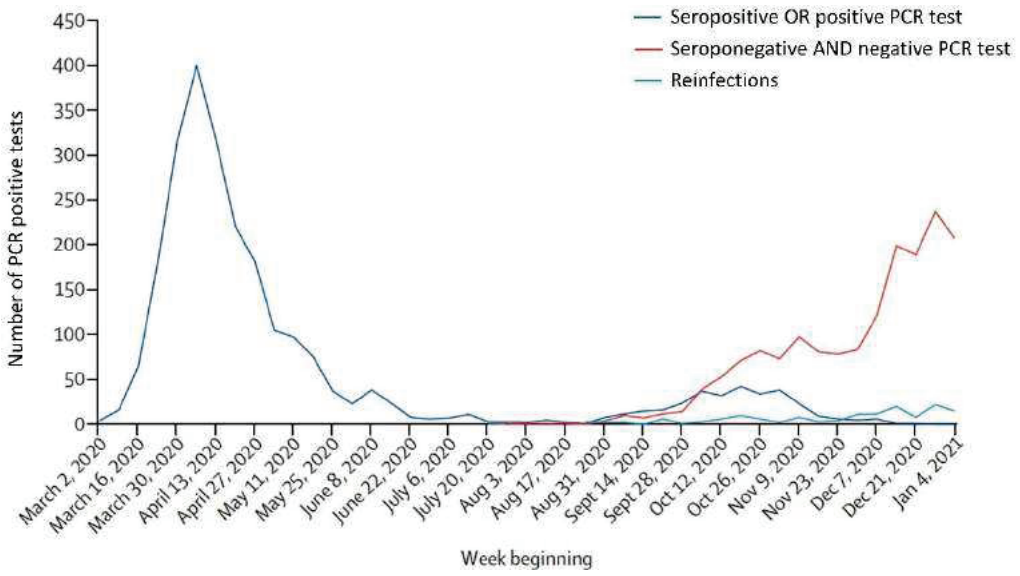
- *Reinfection risk among convalescents*

Protective immunity to most pathogens does not refer to a binary outcome but reflects a spectrum of expressions. Thus, considering the different outcomes of COVID-19 (transmission, infection, disease and death) when evaluating immunological protection is of utmost importance to improve our understanding of the main immune mechanisms behind[142].

Several studies addressing the protective immunity derived from prior SARS-CoV-2 infected (non-vaccinated) individuals have been published, showing that infection provided long-lasting protection to subsequent COVID-19 (asymptomatic included) with 85-95% lower risk of reinfection over the following 7-8 months[143–145], especially among those patients with detectable antibodies[144]. In a prospective cohort of 25.661 healthcare workers attending regular SARS-CoV-2 PCR testing, the median interval between primary and secondary infections was longer than 200 days (**Figure 5**)[145].

More recently, a population-based retrospective work including more than 2 million convalescent, unvaccinated individuals revealed even longer protection, with 87% lower risk of hospitalization due to COVID-19 up to 20 months of follow-up, compared to naïve (unvaccinated/uninfected) people[146]. Notably, the Alpha and Delta strain outbreaks occurred during the follow-up, suggesting a preserved heterologous protection against the distinct variants of concern (VOC).

Figure 5. Weekly frequency of positive PCR according to prior infection (Hall.V.J et al. 2021)[145]



- *Infection-derived immunity and the new VOC: insights into protective mechanisms*

In late December 2021, the Omicron variant emerged, causing a large number of infections and currently becoming dominant in many countries. A recently published case-control study from Qatar[147], which excluded vaccination status from the analysis, revealed that prior SARS-CoV-2 conferred an 87-92% protection against Alpha, Beta and Delta variants infection, while dropping to 56% against Omicron. Remarkably, none of the reinfections progressed to critical/fatal COVID-19, providing an 87% protection against hospitalization, consistently with another report from South Africa[148].

The significant antigenic changes within the Omicron RBD domain are the result of dominant humoral response against those sites[149]. Consequently, antibodies from recovered individuals have shown a dramatic neutralizing reduction compared to former strains[150]. Nonetheless, SARS-CoV-2 specific CD4+ and CD8+ T cells elicited by prior infection remain largely preserved against distinct VOC[137], including Omicron[151].

Since neutralizing antibodies are highly predictive of immune protection[152], decoupling severe outcomes from the high number of reinfections is likely to rely on the T-cell immune compartment[119,148,153,154].

This concept is of great interest, especially for those groups remaining vulnerable to severe COVID-19 as the immunocompromised patients. In particular, given the T-cell targeted therapies as the mainstay of solid organ recipients management, it is necessary to characterize the infection-derived responses in this vulnerable group of patients.

3. Immune phenotypes in vulnerable individuals

- *The elderly*

De novo specific T-cell responses are highly dependent on the circulating pool of naïve T cells, which correlate with the TCR repertoire. However, TCR diversity declines with age[155]. In an elegant study[98], Moderbacher et al. showed that low proportions of circulating naïve CD8+ T-cell populations were associated with COVID-19 severity[156], while confirming a negative correlation between age and naïve T compartment. On the other hand, those individuals aged >65 displayed more delayed and uncoordinated adaptive responses between CD4+ T cell and antibody responses than younger patients, contrary to other reports on mildly symptomatic infections. Therefore, delayed, uncoordinated and restricted T-cell immunity might define the dysregulated adaptive responses in the elderly, leading to enhanced viral replication and disease progression.

In this regard, the elderly, immunosuppressed patients, and those with severe infection phenotypes have been associated with longer SARS-CoV-2 shedding[157]. On the contrary,

asymptomatic patients display much earlier viral clearance[158], thus supporting the influence of early de novo responses over the viral kinetics.

Strikingly, age has been associated with stronger immune responses at convalescence[110,137,159]. In a cross-sectional study including 31.426 convalescent subjects[160], antibody titers displayed a U shape trend, with the lowest antibody titers among those aged 19-30. These paradoxical observations might rely on the higher viral loads and prolonged shedding reported among the elderly and the higher proportions of severe infections occurring among this group of patients.

While confirmatory data is lacking in this regard, it is essential to consider the distinct kinetics in a longitudinal manner (infection-convalescence) to properly understand the immune phenotypes reported at the distinct time points (**Figure 4**).

- *Immunocompromised Solid Organ transplant patients*

Discriminating the role of chronic immunosuppression from that exerted by other often concomitant comorbidities such as age, obesity, or cardiovascular disease has been a challenging issue. Initial works draw discordant conclusions on the effects of immunosuppression in hospitalized, already severely affected individuals[34,35]. Nonetheless, population-based studies encompassing the full spectrum of COVID-19 proved the deleterious impact of immunosuppression on disease progression[161]. It was the case for solid organ transplantation, showing an increased likelihood of hospitalization and death during the pre-vaccination era.[32,162–164]

Other works on immunosuppressed individuals revealed significant insights on the relative contributions of adaptive immunity on SARS-CoV-2 clearance and disease severity. In particular, studies on chronically infected people with the human immunodeficiency virus (HIV) determined that those living with <200 CD4+ T cells / mm3 were at the highest risk for hospitalization, ICU admission and lower survival[165,166]. Likewise, individuals with hematologic malignancies with fewer CD8+ T cells presented higher viral loads and reduced survival rates after the infection[167]. Overall, these data underscored the impact of baseline T-cell depletion on COVID-19 progression risk.

Another observation supporting the deleterious impact of immunosuppression is the prolonged viral shedding exhibited by patients with hematologic malignancies[168] and solid organ transplants. In kidney transplant series[169–171], 20-25% of individuals present viable SARS-CoV-2 one month after the symptom onset. Remarkably, in another KT cohort, when participants were reevaluated at 28 days of infection with a follow-up SARS-CoV-2-IgG test and PCR, 43.2% of seronegative individuals still tested positive[172]. These findings are consistent with those reporting a relationship between late-onset SARS-CoV-2 T-cells and longer viral shedding[116], suggesting a dynamic interplay between delayed adaptive immunity and inadequate viral control (**Figure 4**), confirming preliminary observations in animal models[90].

Despite these insights, the inflammatory profiles triggered by severe SARS-CoV-2 infections fostered the hypotheses of the potential benefits of using immunosuppressive treatments during acute infection. Therefore, defining the immunobiology of the disease and the implication of the T-cell compartment on SARS-CoV-2 specific cytokine production was particularly important in solid organ transplantation, given the consequences of immunosuppression adjustment. Moreover, considering the long-lasting immune protection achieved by convalescent patients in the general population, characterizing the infection-elicited immune memory in SOT was considered an urgent need.

Indeed, the first report on adaptive immunity in a SOT recipient was published by Babel et al. in June 2020, describing the presence of SARS-CoV-2 reactive T cells and the production of specific anti-Spike antibodies in an infected kidney-pancreas transplant recipient[173]. Shortly after, Candon et al. characterized seven convalescent KTs, demonstrating comparable IFN-γ producing T-cell frequencies against Spike, Membrane, Nucleocapsid and ORF3 to non-immunosuppressed patients in chronic hemodialysis, between 17-42 days after infection[174]. A larger cohort of 35 kidney transplant recipients showed a 100% detection of recombinant Spike and Nucleocapsid antibodies from day 14 onward[175]; however, six months after COVID-19, detectable rates of humoral immunity seemed to decline, ranging from 50% to 85% of seropositive convalescents[176–178].

Despite the small sample size and the lack of standardized cut-offs that hindered direct comparisons with the general population, a relatively preserved immune memory profile was shown by these works, at least among hospitalized patients. Nevertheless, larger and more comprehensive studies with extended follow-up were necessary to confirm these preliminary results and improve our understanding of infection-derived immune responses in SOT.

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II.HYPOTHESIS

The hypothesis of this thesis is that by investigating main clinical and demographic features of COVID-19 as well as accurately characterizing SARS-CoV-2-specific adaptive immunity, both during infection and convalescence in solid organ transplant patients, as compared to immunocompetent patients, we would gain relevant insight on major determinants driving distinct clinical outcomes as well as on the degree of anti-viral immune protection achieved in this highly vulnerable group of at-risk patients.

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III. OBJECTIVES

The main objectives of this thesis were:

1. To analyze main clinical, demographic and immunological risk factors associated with the development of adult respiratory distress syndrome (ARDS) and death in a large multicentric cohort of kidney transplant recipients hospitalized due to COVID-19 during the first wave of the pandemic.

2. To characterize the kinetics and magnitude of SARS-CoV-2 specific adaptive immune responses at the T-cell and serological immune compartments, among SOT patients hospitalized due to COVID-19 and compare them to those exhibited by a matched group of immunocompetent patients.

3. To investigate the relative persistence of peripheral adaptive immune memory specific to SARS-CoV-2 up to six months after COVID19 by evaluating both serological and T and B cell memory/effector immune compartments in SOT recipients, compared to a matched cohort of IC convalescents patients.

4. To comprehensively characterize the strength and durability of SARS-CoV-2-specific adaptive immunity across distinct clinical presentations of COVID-19, from severe to asymptomatic infections, in convalescent SOT and immunocompetent patients.

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IV. MATHERIALS, METHODS AND RESULTS

Article 1.

Clinical characteristics and risk factors for severe COVID-19 in hospitalized kidney transplant recipients: A multicentric cohort study

Am J Transplant. 2020 Nov;20(11):3030-3041; doi: 10.1111/ajt.16246

Objective:

To analyze main clinical, demographic and immunological risk factors associated with the development of adult respiratory distress syndrome (ARDS) and death in a large multicentric cohort of kidney transplant recipients hospitalized due to COVID-19 during the first wave of the pandemic.

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ORIGINAL ARTICLE

AJT

Clinical characteristics and risk factors for severe COVID-19 in hospitalized kidney transplant recipients: A multicentric cohort study

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Kidney transplant recipients might be at higher risk for severe coronavirus disease 2019 (COVID-19). However, risk factors for relevant outcomes remain uncertain in this population. This is a multicentric kidney transplant cohort including 104 hospitalized patients between March 4 and April 17, 2020. Risk factors for death and acute respiratory distress syndrome (ARDS) were investigated, and clinical and laboratory data were analyzed. The mean age was 60 years. Forty-seven patients (54.8%) developed ARDS. Obesity was associated to ARDS development (OR 2.63; P = .04). Significant age differences were not found among patients developing and not developing ARDS (61.3 vs 57.8 years, P = .16). Seventy-six (73%) patients were discharged, and 28 (27%) died. Death was more common among the elderly (55 and 70.8 years, P < .001) and those with preexisting pulmonary disease (OR 2.89, P = .009). At admission, higher baseline lactate dehydrogenase (257 vs 358 IU/mL, P = .001) or ARDS conferred higher risk of death (HR 2.09, P = .044). In our cohort, ARDS was equally present among young and old kidney recipients. However, the elderly might be at higher risk of death, along with those showing higher baseline LDH at admission.

KEYWORDS
clinical research/practice, complication: infectious, epidemiology, infectious disease, kidney transplantation/nephrology, patient survival

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; AKI, acute kidney injury; ARB, angiotensin receptor blocker; ARDS, acute respiratory distress syndrome; ARDS, acute respiratory distress syndrome; CI, confidence interval; CNI, calcineurin inhibitor; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; FIO_2, fraction of inspired oxygen; HR, hazard ratio; KT, kidney transplant recipient; LDH, lactate dehydrogenase; MMF, mycophenolate mofetil; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; rATG, rabbit antithymocyte globulin; rt-PCR, reverse transcriptase–polymerase chain reaction; SD, standard deviation; SpO_2, blood oxygen saturation measured by pulse oximetry; TAC, tacrolimus.

Alexandre Favà and David Cucchiari contributed equally to this work.

Francesc Moreso and Edoardo Melilli are co-senior authors.

1 | INTRODUCTION

In December 2019, severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2) emerged in China, which was shortly recognized as the pathogen of a new cluster of respiratory illness designated as coronavirus infectious disease 2019 (COVID-19).[1]

The clinical course and prognosis of COVID-19 have been thoroughly described, identifying older age and the presence of comorbidities as the main risk factors for mortality and acute respiratory distress syndrome (ARDS) development.[2-4] Nevertheless, whether these clinical manifestations and risk factors are valid in renal transplant or other solid organ recipients remains unclear. The role of immunosuppressive therapies, its management during the course of the active viral infection and their potential interactions with currently compassionate treatments used for COVID-19 represent a unique clinical scenario that deserves to be characterized.

Among the 33 766 prevalent kidney transplant recipients (KTs) in Spain,[5] 433 COVID-19 infection cases had been reported up to April 25, 2020.[6] The KT population constitutes a large group of patients considered to be at high risk of complications due to the maintenance of chronic immunosuppression. Single-center case-reports and small series of KT have been published[7-9] with divergent results and recommendations. Actually, in those studies sample size was small and most patients remained admitted at the end of the follow-up.

Here, we report clinical data and outcomes of 104 consecutive KTs with confirmed COVID-19 infection hospitalized in 5 different Spanish kidney transplant units. Our main objectives were to evaluate main risk factors of ARDS and death and to delineate the clinical course and biological profile in this setting.

2 | METHODS AND MATERIALS

This is a retrospective multicentric observational cohort study. Our study enrolled all KTs with COVID-19 infection and hospitalized between March 4 and April 17, 2020, in participant centers. All of them had confirmed SARS-CoV-2 infection by real-time reverse transcriptase–polymerase chain reaction (rt-PCR) analysis performed on nasal or pharyngeal swab samples. The hospital admission criteria were need of oxygen therapy, x-ray infiltrates, renal dysfunction, or those with recent (<7 days) symptom onset regardless of its severity (i.e., fever without pneumonia or diarrhea). All patients included had a complete follow-up until discharge (curation or clinical improvement) or death. COVID-19 KTs with exclusively outpatient care were excluded from the analysis because of the potential not-reported cases and the lack of follow-up data. Data were obtained and recorded in a common data collection form in all transplant centers. The study was approved by all hospital ethical review boards (PR173/20).

We collected the following baseline data: age, race/ethnicity, gender, time after KT, first or repeat transplant, type of transplant (kidney or combined pancreas with kidney or liver with kidney), type of donor (deceased or living), primary end-stage renal disease (ESRD), maintenance immunosuppression, induction therapy, basal graft function (serum creatinine and estimated glomerular filtration rate by CKD-EPI [eGFR]), comorbidities such as heart disease (heart failure, coronary artery disease, atrial fibrillation or valvular heart disease), hypertension (type of treatment before illness), obesity (body mass index ≥30), pulmonary disease (chronic obstructive pulmonary disease, bronchiectasis, asthma, or sleep apnea-hypopnea syndrome), active neoplasm, or lymphopenia before admission. Initial clinical symptoms (fever defined by a temperature >37.5°C, respiratory status recorded through the pulse oximetry saturation/fraction of inspired oxygen ratio [Spo_2/Fio_2]) and x-ray evaluation and analytical assessment that were carried out at admission and 3, 6, 9, 12, and 15 days after the admission were also recorded. Individuals considered to have a COVID-19 nosocomial infection were patients in these 2 clinical scenarios: a diagnosis of COVID-19 while being hospitalized due to a different clinical reason or COVID-19 infection in patients who had been discharged from the hospital within the preceding 14 days. Missing data were recovered and inconsistencies were corrected through online interaction.

The primary endpoints were death and ARDS defined by the World Health Organization interim guidance (bilateral opacities not explained by volume overload and Spo_2/Fio_2 ratio <315).[10] The secondary endpoints were acute kidney injury (AKI) using KDIGO definition,[11] number and type of immunosuppression withdrawal, use of anti–COVID-19 therapies, and associated adverse events (including gastrointestinal, cutaneous rash, QT prolongation [considered prolonged if QTc values are >450 milliseconds in males or >470 milliseconds in females], hepatitis [defined as an elevation of alanine transaminase and aspartate transaminase greater than twice the normal values], and tacrolimus intoxication defined by plasmatic levels of ≥20 ng/mL regardless of nephrotoxicity or neurotoxicity). Anti–COVID-19 protocols in all hospitals were similar and regularly updated according to newly published information. Generally, these included first hydroxychloroquine and lopinavir/ritonavir, darunavir/ritonavir, darunavir/cobicistat, and then remdesivir, interferon-β1a, intravenous steroid therapy, and tocilizumab in case of clinical deterioration.

2.1 | Statistical analysis

Continuous variables were expressed as mean ± SD or median and IQR and categorical variables as number of total (n) and percentage (%). Comparison between groups was performed using Pearson' χ^2 test for categorical data or Fisher exact test was applied when the number of cases was < 5. One-way analysis of variance and *t* tests were used for normally continuous distributed data, and nonparametric Kruskal–Wallis test and Mann–Whitney *U* test were used for nonnormally distributed variables.

Both univariate and multivariate logistic regression models were performed to examine the risk factors associated with ARDS. To explore the risk factors associated with patient survival, a Cox regression model was used to estimate hazard ratios in an univariate and

multivariate analyses, missing data were excluded listwise. The analyses of patient's survival were censored for death (death certificate date) or recovery (day of discharge and clinical recovery). Due to the relatively small number of death (25) events to avoid overfitting in the model, just 4 variables were chosen for multivariable analysis on the basis of previous findings and clinical constraints.

All *P*-values were 2-tailed and statistical significance level was fixed at $P < .05$. SPSS 20.0 software (SPSS Inc), STATA16, and GraphPad Prism version 6.0 (GraphPad Software) were used for data management and analysis.

3 | RESULTS

We followed the STROBE Guidelines to report this observational study. Data from 5 Spanish kidney transplant units were obtained (Hospital Universitari de Bellvitge, Hospital Clínic, Fundació Puigvert, Complejo Hospitalario Universitario de Albacete, and Hospital de Vall d'Hebron). Among the 7092 KTs followed in these units, 112 had COVID-19 infection during the study period, 109 patients required hospitalization, and 104 fulfilled inclusion and exclusion criteria (Figure 1). Considering the prevalent KT population followed in the 5 hospitals, the admission for COVID-19 rate was 0.23 per 1000 patient-days. The median follow-up of the entire cohort was 14.5 (IQR 8-96) days.

Baseline characteristics of the study population are shown in Table 1. The mean age was 59.7 ± 12.48 years. The majority of the patients were male (55.7%) with a high prevalence of hypertension (85.6%); 35.6% of the cohort were treated with angiotensin -converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs). Diabetes was present in 30.8%, and 15.4% of patients had previous pulmonary disease. The most frequent immunosuppressive drug used was tacrolimus in 85.5% of the cohort, and 19.3% of patients were maintained using a mTOR inhibitor–based strategy. The mean serum creatinine levels before admission were 158.6 ± 79.1 μmol/L.

There were 14 nosocomial COVID-19 infections. Clinical characteristics and outcomes of individuals with nosocomial COVID-19 infection are presented in detail in Table S1.

The median time between appearance of symptoms and admission was 5 (IQR 2-10) days. The most frequent initial clinical manifestation was fever (77.9%), followed by: cough (68.3%), dyspnea (36.5%), myalgia (32.7%), and diarrhea (30.8%) (Table 1, Figure 2). Analytical parameters at the admission showed a general inflammatory status with elevation of lactate dehydrogenase (LDH) with a mean of 317.46 ± 147.44 IU/mL, C-reactive protein (CRP) of 78.7 mg/L (IQR 31.9-137.15), D-dimer of 614 ng/mL (IQR 400.75-1344.5), ferritin levels of 574.5 μg/L (IQR 309.75-933.5), and lymphopenia with a median of 650 cells/mm^3 (IQR 400-1000). Seventeen patients (16%) were admitted without oxygen requirement or x-ray abnormalities.

3.1 | ARDS

Oxygen supply was required at any time point in 85.6% of all the included patients, 54.8% met ARDS criteria, and 16.3% were treated with invasive and/or noninvasive ventilation (13.6% and 15.3%, respectively). The median time of appearance was 3 (IQR 3-6) days after admission (7 days after symptoms onset). Those who died presented

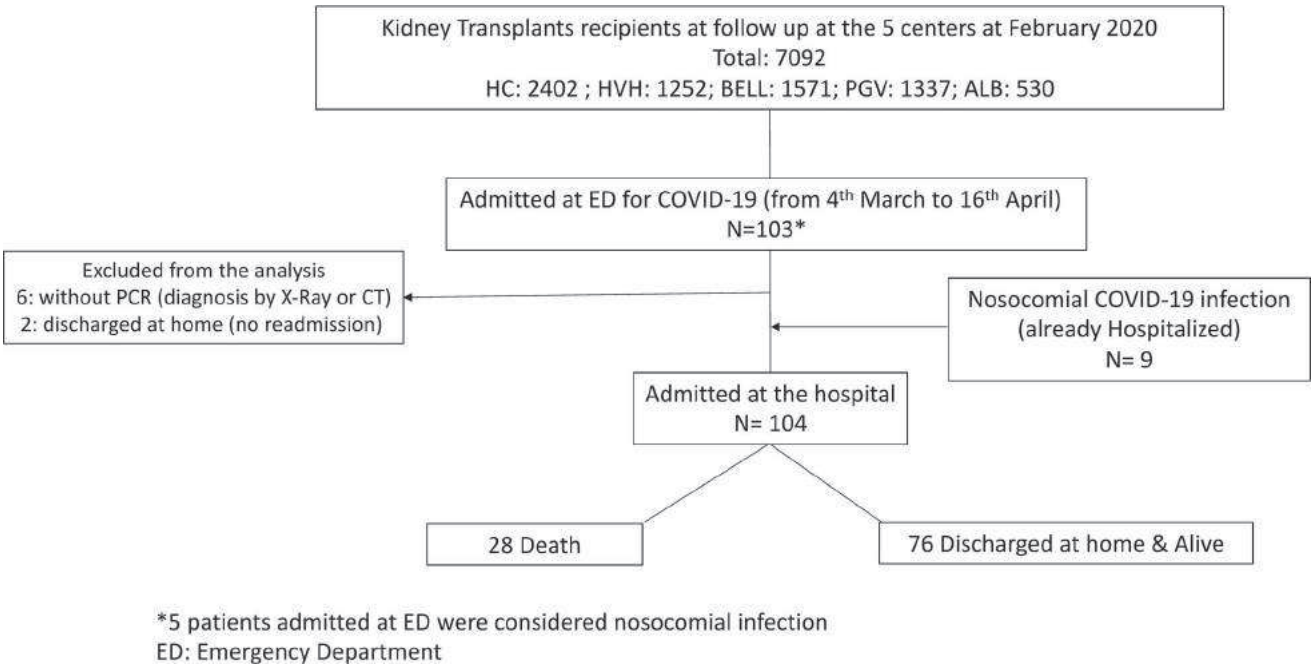

FIGURE 1 Flowchart of the study population. We excluded nonhospitalized and nonconfirmed by real-time RT-PCR COVID-19 kidney recipients. ED, emergency department

TABLE 1 Demographic and clinical characteristics of kidney transplant patients with coronavirus disease 2019 pneumonia

Patient characteristics	
Age (y, mean ± SD)	59.7 ± 12.48
Sex: male/female (n, %)	60/44 (55.7/42.3)
Race (n, %)	
Caucasian	90 (86.5)
African/African American	4 (3.8)
Latin American	9 (8.7)
Asian	1 (1)
Primary end-stage renal disease (n, %):	
Nephroangiosclerosis	12 (11.5)
Diabetic nephropathy	17 (16.3)
Glomerulonephritis	30 (28.8)
Polycystic kidney disease	13 (12.5)
Other	11 (10.6)
Uncertain	21 (20.2)
Comorbidities (n, %)	
Diabetes	32 (30.8)
Arterial hypertension	90 (86.5)
Obesity	28 (26.9)
Pulmonary disease	16 (15.4)
Heart disease	31 (29.8)
Active neoplasm	8 (7.7)
Lymphopenia before admission	45 (43.3)
ACEI/ARB use (n, %)	37 (35.6)
Nosocomial COVID-19 infection (n, %)	15 (14.4)
Transplant characteristics	
Time after transplant <6 mo (n, %)	15 (14.4)
Time (mo, median, IQR)	59 (18-130)
Type of transplant (n, %)	
KT/combined transplant[a]	100/4 (96.2/3.8)
First KT/repeat transplant	88/16 (84.6/15.4)
Type of donor (n, %)	
Deceased/living	90/14 (86.5/13.5)
Standard criteria/expanded criteria[b]	48/42 (46.1)/ (40.3)
Induction therapy (n, %)	
None	11 (10.6)
Rabbit antithymocyte globulin	37 (35.6)
Basiliximab	56 (53.8)
Maintenance therapy (n, %)	
TAC use	89 (85.5)
Cyclosporine use	3 (2.88)
mTOR inhibitor use	20 (19.28)
MMF/MPA use	87 (83.6)
Prednisone use	96 (92.3)
Basal serum creatinine (μmol/L) (mean ± SD)	158.6 ± 79.1
Basal eGFR CKD-EPI (mL/min/1.73 m^2) (mean ± SD)	48.2 ± 21.9
Initial clinical symptoms	
Cough (n, %)	71 (68.3)
Dyspnea (n, %)	38 (36.5)
Diarrhea (n, %)	32 (30.8)
Myalgias (n, %)	34 (32.7)
Fever (n, %)	81 (77.9)

Abbreviations: CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; MMF/MPA, mycophenolate mofetil or mycophenolic acid; mTOR, mammalian target of rapamycin; TAC, tacrolimus.

[a]Multiorgan transplant: 1 pancreas–kidney and 3 liver–kidney.

[b]Expanded criteria donor refer to older kidney donors (≥60 years old) or donors who are aged 50-59 years and have 2 of the following 3 features: hypertension, terminal serum creatinine >1.5 mg/dL, or death from cerebrovascular accident.

ARDS before those who were alive at the end of follow-up (mean difference −1.44 days, $P = .04$). Patients with ARDS showed 11.4 times higher death risk than those without ARDS (95% CI 3.181-41.26, $P < .001$). Thirty-two of 58 patients who developed ARDS survived; among them, the mean time to resolve ARDS was 20.5 (IQR 14.2-30.7) days. The analysis of clinical and biological characteristics among patients with or without ARDS is shown in Table 2. By univariate analysis, we found an increased odd for obesity (OR 2.63, 95% CI 1.034-6.714, $P = .04$) and LDH at admission (OR 1.006, 95% CI 1.001-1.011, $P = .01$) and a decreased odds for $PaFI/Spo_2$ (partial pressure of oxygen/fraction of inspired oxygen ratio) (OR 0.991, 95% CI 0.985-0.997, $P = .005$). No differences were found in terms of age, type of maintenance immunosuppression use, prevalence of previous lymphopenia, pulmonary disease, baseline graft function, or AKI for ARDS. The antiviral therapy did not impact on ARDS outcomes either.

3.2 | Mortality

The overall mortality was 26.9%. All deaths were due to ARDS except one that was due to sudden death and another one that occurred after an aspiration pneumonia. We found that age was related to mortality with an HR of 1.101 (95% CI 1.057-1.157, $P < .001$). The mean age for those who survived was 55 ± 11.4 years, and for those who died, 70.8 ± 9.4 years ($P < .001$) (Table 2). There was also an increased risk of mortality for patients presenting ARDS at admission (HR 3.923, 95% CI 1.641-3.942, $P = .002$), patients with previous pulmonary disease, increased levels of LDH, CRP, and ferritin, and low lymphocyte count (Tables 2-4). Other significant differences in the evolution of analytical parameters between survivors and nonsurvivors are shown in Figure 3. In the multivariate Cox regression model, we found that age, ARDS, and higher baseline LDH were associated with increased risk of death (Table 4). No differences in terms of patient survival were found depending on baseline graft function, time after transplant, presence or absence of AKI, and

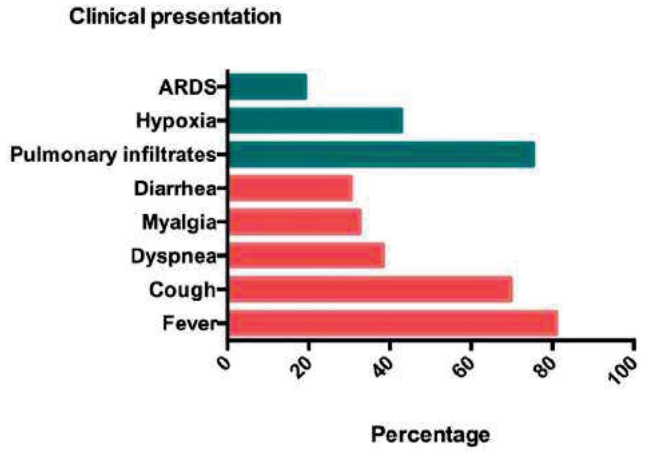

FIGURE 2 Clinical presentation of coronavirus disease 2019 pneumonia. Figure shows proportion of pulmonary and extrapulmonary manifestations at admission. ARDS, acute respiratory distress syndrome [Color figure can be viewed at wileyonlinelibrary.com]

type of maintenance immunosuppression used. None of the antiviral therapies used had any impact on patient survival.

Mortality was different depending on patient baseline characteristics. Of the total cohort, 23.1% of patients needed to be admitted to the intensive care unit and 15 of 24 (62.5%) died. Of the 17 patients who were admitted without oxygen requirement and no x-ray abnormalities, 8 (47%) progressed to ARDS, 4 (23%) needed ICU admission, 6 (46%) developed AKI, and 1 patient (5.9%) died. Of these 17 patients, those eventually requiring ICU admission presented a significant rapid increase in their CRP levels until day 6 after admission, unlike patients who did not need ICU admission (Figure S1). Furthermore, patients with COVID-19 nosocomial infection had a high mortality rate of 57.14% (8/14) (Table S1).

3.3 | Acute kidney injury

AKI was present in 47% of the included cohort (Table 5). Four patients were excluded from this analysis. The majority of patients presented with AKI stage 1 (30%). No differences in terms of age or antiviral use were found. Interestingly, AKI stage 3 presented a higher median tacrolimus through levels compared with other AKI stage patients ($P < .001$). Mortality was higher in AKI stage 3 patients compared with the rest of the cohort ($P < .05$), although in Cox regression analysis, the presence of AKI at any stage or AKI stage 3 compared to no AKI was not a risk factor associated with death or ARDS. There were no acute graft rejection episodes during the follow-up.

3.4 | Immunosuppression, other treatments, and safety endpoints

At least 1 immunosuppressive drug was withdrawn in 91.3% of patients (Table S2). Intravenous steroid treatment (methylprednisolone 0.5-1 mg/kg/d) was used in 52.9% of cases. CNI withdrawal was higher in patients who developed ARDS ($P = .018$), as well as in patients taking an mTOR inhibitors ($P = .028$). We did not find any relationship between type of immunosuppression modification and mortality.

Regarding anti–COVID-19 therapies, different drugs were used (Figure 4A). Hydroxychloroquine was given to 97.1% and lopinavir/ritonavir to 48.1% of patients. Azythromycin was used in 63.5% of patients. None of these strategies showed any impact on mortality or ARDS, except interferon-β1a or tocilizumab, which were associated with worse outcomes for ARDS (Table S2). Importantly, these investigational treatments were related to 28.8% incidence of adverse effects such as hepatitis (20.2%), tacrolimus toxicity (14.4%), QT prolongation (observed in 5 patients), or gastrointestinal (12.5%) (Figure 4B).

4 | DISCUSSION

In early 2020, Spain emerged as one of the most affected countries by the COVID-19 pandemic.[12] This situation forced the discontinuation of many transplant programs worldwide.[13] Transplant units faced a significant number of infected recipients without evidence-based strategies and many uncertainties regarding the clinical course and prognosis of this novel infection. Here, we report the clinical characteristics and risk factors associated with the development of ARDS and death in a cohort of 104 consecutive kidney transplant patients hospitalized for COVID-19 infection in 5 different Spanish centers.

In agreement with previous reports of an immunocompetent infected population, the most common symptom reported at admission was fever,[3] although one-third of patients were admitted with gastrointestinal complaints, as already described in other transplant reports.[14] X-ray abnormalities preceded hypoxemia onset, which accounts for the natural history of pulmonary involvement on the general population.[4]

ARDS is considered a severe form of COVID-19 infection and entails greater mortality risk,[2] which was also confirmed in our cohort. Half of our COVID-19 cohort progressed to ARDS, and 50% of them had a fatal outcome. Recently, case report series of in-hospital kidney and other solid organ transplants described similar ARDS incidence.[14,15] Early observations among hospitalized general population reported a 41.8% ARDS incidence,[2] which is in line with our results with KTs. However, in our cohort no age differences were described among patients with and without ARDS, contrary to immunocompetent published cohorts.[2]

It has been suggested that KTs encompass a susceptible group for aggressive manifestations of COVID-19 infection[7,16] due to the ongoing immunosuppression. In our current study, we report an overall mortality rate of 26.9% in consonance with recent reports on kidney and other solid organ transplant patients showing similar fatality rates, ranging from 6% to 30%.[7,9,14,16] General population fatality rates were initially described as

TABLE 2 Main clinical characteristics associated with patient death and acute respiratory disease distress syndrome

	Mortality			ARDS		
	Alive (n = 76)	Death (n = 28)	*P*-value	No (n = 47)	ARDS (n = 57)	*P*-value
Clinical characteristics						
Age (y, mean ± SD)	55 ± 11.4	70.8 ± 9.4	<.001	57.8 ± 12.4	61.3 ± 13.2	.16
Sex (n, %): female/male	31/45 (40.8/59.2)	13/15 (46.4/53.6)	.60	21/26 (40.7/55.3)	23/34 (40.4/59.6)	.65
Race (n, %): Caucasian/other	64/12 (84.2/15.8)	27/1 (96.4/3.6)	.17	40/7 (85.1/14.9)	51/6 (89.5/ 10.5)	.5
Comorbidities (n, %)						
Hypertension (n) (no/ACEI/ARB/other)	10/6/21/38 (13/8/28/50)	4/2/8/14 (14/7/28/50)	.98	7/5/14/20 (15/10/30.4/43.5)	7/3/15/32 (12.3/5.3/23.3/56.1)	.51
Diabetes	21 (27)	11 (39)	.25	12 (25.5)	20 (35.1)	.29
Obesity	17 (22.4)	11 (39.3)	.08	8 (17)	20 (35.1)	.03
Cardiac disease	20 (19.4)	11 (39.3)	.2	16 (34.8)	15 (26.3)	.35
Pulmonary disease	6 (7.9)	10 (35.7)	<.001	5 (10.6)	11 (19.3)	.28
Active cancer	3 (3.9)	5(17.9)	.03	3 (6.4)	5 (8.8)	.64
Lymphopenia before admission	31 (41.3)	14 (50)	.43	22 (47.8)	23 (44.4)	.44
Days from symptoms onset to admission (median, IQR)[a]	7 (3-10)	6 (4-10)	.76	7 (3-10)	6 (4-10.7)	.77
Initial symptoms (n, %)						
Fever	60 (78.9)	21 (75)	.66	39 (83.1)	42 (73.7)	.3
Cough	52 (68.4)	19 (67.9)	.95	30 (63.8)	41 (71.9)	.29
Dyspnea	21 (27.6)	17 (60.7)	.002	9 (19)	29 (50.9)	<.001
Myalgia	25 (32.9)	9 (31.1)	.94	14 (29.8)	20 (35.1)	.56
Diarrhea	22 (28.9)	10 (35.7)	.50	12 (25.5)	20 (35.1)	.29
Nosocomial COVID-19 infection (n, %)	6 (7.9)	8 (28.6)	.01	6 (12.8)	9 (15.8)	.66
Initial Spo_2 (%, mean ± SD)	96.4 ± 2.4	94.8 ± 3.6	.12	96.6 ± 2.2	95.3 ± 3.3	.03
Initial Spo_2/Fio_2 (mean ± SD)	407.3 ± 97.3	353.2 ± 123.4	.03	432.1 ± 76.6	357.4 ± 118.5	.001
Any radiography infiltrate initially (n, %)	53 (69.7)	23 (82.1)	.23	33 (70.2)	43 (75.4)	.55
Transplant characteristics						
Type of transplant (n, %)						
First kidney transplant	65 (85.5)	23 (82.1)	.76	41 (87.2)	47 (82.5)	.50
Type of donor (n, %)						
Cadaveric	62 (81.6)	28 (100)	.01	37 (78.7)	53 (93)	.04
ECD	22 (37.9)	16 (66.7)	.02	13 (35.1)	25 (55.6)	.06
Induction therapy (n, %)						
None	8 (10.5)	3 (10.7)	.88	4 (8.5)	7 (12.3)	.73
rATG	26 (34.2)	11 (39.3)		16 (34)	21 (36.8)	
Basiliximab	42 (55.3)	14 (50)		27 (57.4)	29 (50.9)	
Maintenance therapy (n, %)						
TAC use	66 (86.8)	23 (82.1)	.54	40 (85.1)	49 (86)	.9
mTORi use	22 (21.6)	4 (3.9)	.19	15 (31.9)	11 (20)	.1
MMF/MPA use	61 (80.3)	26 (92.9)	.14	38 (80.9)	49 (86)	.48
Prednisone use	71 (93.4)	24 (85.7)	.21	44 (93.6)	51 (89.5)	.4

(Continues)

TABLE 2 (Continued)

	Mortality			ARDS		
	Alive (n = 76)	Death (n = 28)	*P*-value	No (n = 47)	ARDS (n = 57)	*P*-value
Time after transplant						
<6 mo (n, %)	6 (11.8)	6 (21.4)	.21	5 (10.6)	10 (17.5)	.40
Time (mo, median, IQR)	56.5 (20-130.5)	71.6 (6-135)	.8	65 (24-133)	57 (12.5-127)	.33

Abbreviations: ARDS, acute respiratory distress syndrome; CI, confidence interval; F_{IO_2}, fraction of inspired oxygen; MMF/MPA, mycophenolate mofetil or mycophenolic acid; mTORi, mammalian target of rapamycin inhibitors; rATG, rabbit antithymocyte globulin; SD, standard deviation; Spo_2, blood oxygen saturation measured by pulse oximetry; TAC, tacrolimus.

[a]Those who were hospitalized because of other reasons were excluded from the analysis.

TABLE 3 Laboratory findings at the time of hospital admission among patient deaths and patients with or without acute respiratory disease distress syndrome

	No. of patients tested	Mortality			ARDS		
		Alive (n = 76)	Death (n = 28)	*P*-value	No (n = 47)	Yes (n = 57)	*P*-value
Basal laboratory findings							
Basal serum creatinine (μmol/L, mean ± SD)	95	152.8 ± 77	170.5 ± 86	.35	159.4 ± 74.2	155.1 ± 85.2	.84
Basal eGFR (mL/min/1.73 m^2, mean ± SD)	95	50 ± 19.8	48.3 ± 23.5	.45	47.7 ± 23	48.9 ± 20	.57
Initial laboratory findings							
Serum creatinine (μmol/L, median, IQR)	95	160 (120-221.2)	202 (143-164)	.08	167 (104-232)	164.5 (124.5-164.5)	.67
CK (IU/mL, median, IQR)	32	59 (38.7-169.5)	49.5 (31.7-129.5)	.54	50 (30-169)	59 (38-140)	.77
White blood cells ($\times 10^3$/cmm, mean ± SD)	103	6 ± 2.6	6.9 ± 3.4	.18	5.5 ± 2.4	6.8 ± 3.2	.032
Hemoglobin (g/dL)	103	12.2 ± 1.94	11.5 ± 2.0	.1	12.1 ± 1.9	12.0 ± 2.0	.97
Platelets ($\times 10^3$/cmm)	103	172 ± 68	186 ± 75	.39	168 ± 64	182 ± 73	.32
Lymphocytes (cells/mm, median, IQR)	103	680 (400-1000)	560 (325-711)	.14	690 (400-910)	600 (400-1000)	.68
D-dimer (ng/mL, median, IQR)	78	574 (324-1081)	850 (610-2599)	.004	606.5 (288-1337.5)	626.5 (424.2-1375.7)	.25
ALT (IU/mL, median, IQR)	94	23.5 (15-35.5)	18.5 (11.5-27.5)	.06	21 (16-31)	22 (13-39)	.73
LDH (IU/mL, median, IQR)	89	257 (212-332)	358.5 (258-522.5)	.001	255 (203-317.5)	278.5 (242.2-448.2)	.007
CRP (mg/L, median, IQR)	101	56 (27.3-132)	114-2 (62.5-199.5)	.006	62.8 (22.5-114.8)	87 (44.5-153.7)	.07
Serum ferritin (pg/L, median, IQR)	62	559.5 (301.7-812.7)	1030 (350.5-1952)	.13	478 (301.7-932)	631 (330.5-1140)	.47

Abbreviations: ARDS, acute respiratory distress syndrome; CI, confidence interval; CK, creatinine kinase; eGFR, estimated glomerular filtration rate measured by CKD-EPI; LDH, lactate dehydrogenase; cmm, per cubic millimeter of whole blood; CRP, C-reactive protein.

2.3% in China,[17] whereas in Spain, it has reached around 10%.[12] It should be emphasized, though, that these figures relate to both hospitalized and nonhospitalized infected patients. Hence, since published kidney transplant cohorts are mainly composed of hospitalized individuals, these comparisons might be inaccurate. Furthermore, admission criteria are likely to differ between solid organ recipients and the general population (in fact, 16% of our patients were admitted without pneumonia or hypoxia in our cohort). Nonetheless, recently accepted for publication OpenSAFELY trial suggests a higher HR for mortality among solid organ recipients.[18]

In terms of AKI, nearly half of our patients developed renal dysfunction, according to recently published kidney transplant cohorts.[15,19] AKI occurrence in general population studies ranges from 5% to 10%[20,21]; therefore, KTs entail a group of risk for this complication.

The etiology of AKI in patients with COVID-19 remains elusive, and several conditions might act as major contributors, beyond the

TABLE 4 Risk factors associated with mortality in kidney transplant patients hospitalized for COVID-19

	Univariate HR (95% CI)	*P*-value	Multivariate HR (95% CI)	*P*-value
Age	1.101 (1.057-1.157)	<.001	1.103 (1.048-1.162)	<.001
ARDS day 0	3.923 (1.641-3.942)	.002	2.091 (1.031-8.233)	.044
Pulmonary disease	2.891 (1.311-6.392)	.009	1.544 (0.592-4.026)	.375
LDH day 0	1.004 (1.002-1.006)	<.001	1.003 (1-1.005)	.024
Day 3	1.003 (1.000-1.006)	.016		
Day 9	1.002 (1.000-1.004)	.03		
Day 15	1.004 (1.000-1.007)	.026		
CRP day 0	1.003 (1.002-1.005)	<.001	—	
Ferritin day 0	1.001 (1.000-1.001)	.056	—	
Lymphocytes				
Day 6	0.998 (0.996-1)	.018	—	
Day 9	0.997 (0.995-0.999)	.007		
Day 12	0.997 (0.995-0.999)	.014		

Note: Adjusted and nonsignificant for sex; race; repeat transplant; induction therapy; maintenance immunosuppression without mTOR inhibitors; heart disease; AKI stage 3 vs others; AKI 2 and 3 stages vs others; hypertension; use of ACEi/ARB; diabetes; obesity; basal lymphopenia; lymphocyte days 0, 3, and 15; serum creatinine; white blood cells; hemoglobin; D-dimer; ALT; LDH days 6-12; ferritin rest of the days; platelets; tacrolimus levels; diarrhea at admission; fever at admission; cough at admission; myalgia at admission; anticoagulation; time after transplant; days from symptoms onset to admission; nosocomial infection.

Abbreviations: ARDS, acute respiratory distress syndrome; CRP, C-reactive protein; CI, confidence interval; LDH, lactate dehydrogenase; HR, hazard ratio.

virus in itself.[22] As a matter of fact, AKI severity was related to tacrolimus trough levels, especially in those with the most severe disfunction (AKI stage 3). No relationship was found between COVID-19 severity and AKI in our cohort, and no associations with mortality were identified.

A relevant concern derived from SARS-CoV-2 transmissibility is the presymptomatic disease stage,[23] thereby resulting in health care professionals' contagion and nosocomial patient infection. Fourteen patients were infected within our facilities, with 8 deaths in this group. Most nosocomial infections occurred at the beginning of the pandemic, and all these patients were admitted before the implementation of measures of isolation. Taking into account the inherent limitations of this sample size, these outcomes might be explained by the intrinsic morbidity associated with the ongoing admission in itself.[24] It is of utmost importance to assess the benefits and potential consequences of admission amid this pandemic, which have become one of the reasons for decreased transplant activity in our country in the past months.[13]

The vast majority of our patients had at least 1 of their immunosuppressants withdrawn, in consonance with already published works.[9,16] Mycophenolate mofetil was the most frequently withdrawn medication, regardless of infection severity. In contrast, CNI and mTOR inhibitors were withheld more frequently in the ARDS group, restricting this strategy to those patients with severe pulmonary involvement.

Steroid withdrawal was, however, exceptional, and its administration as intravenous treatment was used in more than half (52%) of patients. Our study reports cases detected in the early phase of the COVID-19 pandemic, when the efficacy of anti-inflammatory therapies such as steroids was speculative. Thus, in our cohort, steroidal use was mainly reactive to clinical worsening, to ensure immunosuppression after CNI, mTOR inhibitor, and antimetabolite withdrawal. However, recently published results from the RECOVERY trial[25] have shown that dexamethasone use reduced mortality in severe COVID-19 cases in the general population, which might support, to some extent, our adopted strategy.

In terms of antiviral treatments, the World Health Organization[10] claimed that there is no existing evidence to recommend any treatment in this regard. However, the use of compassionate treatments has become a widespread practice during the pandemic. Accordingly, a high proportion of our cohort was treated with some of these drugs (Table S1). We did not find any differences in terms of outcomes among different treatments, although our study does not allow, by nature, this type of analysis.

Initial reports suggested that the combination of hydroxychloroquine and azithromycin might provide superior viral clearance and improved clinical outcomes, despite significant limitations in its design.[26] However, one of the major concerns about these therapies combinations is cardiotoxicity.[27] In fact, QT prolongation was recorded in 5 individuals, of whom 1 had sudden death while presenting with a mildly symptomatic COVID-19 case that was treated with hydroxychloroquine and azithromycin. Moreover, recently published data from large trials show the absence of clinical benefit from the use of hydroxychloroquine in COVID-19 patients. Because of the

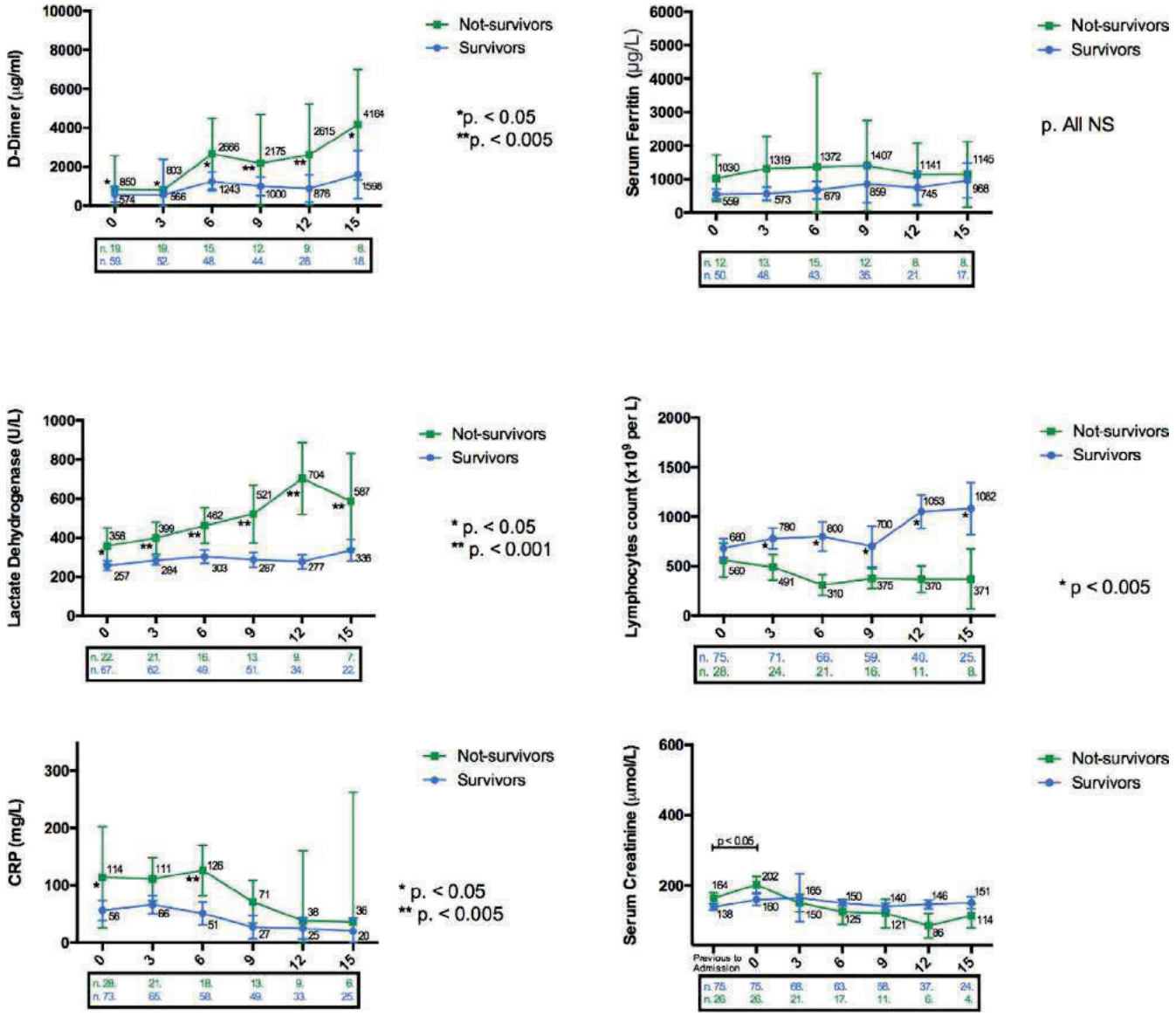

FIGURE 3 Dynamic profile of laboratory markers in kidney transplant recipients with COVID-19. Differences between survivors and non survivors are shown; significant differences are indicated. Box below each graph detail the number of patient at risk and/or availability of the test. CRP, C-reactive protein [Color figure can be viewed at wileyonlinelibrary.com]

current available data,[25,28] along with the adverse effects reported in our cohort, we advise against the use of hydroxychloroquine in COVID-19 KTs.

On the other hand, more than half of our patients received protease inhibitors as adjunctive therapy, resulting in 15.7% of tacrolimus intoxications. Additionally, severe AKI were significantly prevalent among those patients exhibiting tacrolimus overexposure. Thus, given the lack of evidence supporting its use[28] and the concurrent risk of the above-mentioned adverse effects, we support the idea that the use of investigational anti–COVID-19 therapies must be restricted to randomized controlled trials, as it has recently shown in a randomized controlled trial of remdesivir that resulted in U.S. Food and Drug Administration approval.[29]

At present, there are no available data in terms of risk stratification in KTs affected by COVID-19. As aforementioned, significant rates of COVID-19 progression among patients without pneumonia nor hypoxemia at admission were observed. Therefore, given the unpredictable clinical course of this infection, discharge criteria should differ from the general population at early stages regardless of age, and a strict follow-up must be provided if an outpatient approach is agreed on.

Despite this, we were able to identify certain risk factors for ARDS and death among KTs. We found that obesity was independently associated with ARDS. Likewise, in the 2009 H1N1 pandemic, an association between hospitalization and obesity was described.[30] Interestingly, although we did not identify older age as a risk factor for ARDS, it was certainly associated with mortality. These data suggest that ARDS might develop indistinctly among young and old KTs; however, once it is established, the elderly would be at most risk for death. Likewise, the preexisting pulmonary disease did not

TABLE 5 Acute kidney injury stages according to KDIGO definition and clinical outcomes

	NO AKI (n = 53)	AKI stage 1 (n = 30)	AKI stage 2 (n = 7)	AKI stage 3 (n = 10)
Age (y, mean ± SD)	60.4 ± 13	56.3 ± 13	63.4 ± 10	64 ± 11
Tacrolimus levels day 6 (ng/mL, median, IQR)[a]	5.6 (3.3-8.5)	6.6 (4.7-10.1)	10.4 (6.7-22.8)	24.3 (16.9-44)**
Antiviral use (n, %)	23 (43.4)	18 (60)	3 (42.9)	6 (60)
ARDS (n, %)	25 (47.2)	18 (60)	6 (85.7)	6 (60)
Death (n, %)	12 (22.6)	6 (20)	3 (42.9)	6 (60)***

Note: Antiviral use included lopinavir/ritonavir-darunavir/ritonavir or darunavir/cobicistat use. Four patients were excluded from the analysis: 2 with delayed graft function in dialysis after kidney transplant and 2 because were their basal eGFR was inferior to 10 mL/min before admission (one pending to start hemodialysis and the other with obstructive AKI due to lymphocele).

Abbreviations: AKI, acute kidney injury; AKI stage 1, rise in serum creatinine ≥26.5 μmol/L in 48 h or rise 1.5-1.9 times from baseline; AKI stage 2, rise in serum creatinine 2.0-2.9 times from baseline; AKI stage 3, rise in serum creatinine 3 times from baseline or increase in serum creatinine to ≥353.6 μmol/L or initiation of renal replacement therapy irrespective of serum creatinine; ARDS, acute respiratory distress syndrome.

[a]Number of patients with data of tacrolimus levels available: no AKI = 20, AKI stage 1 = 18, AKI stage 2 = 5; AKI stage 3 = 4.

**$P < .001$ no AKI vs AKI stage 1.

***$P < .05$ no AKI vs AKI stage 1.

confer additional risk for ARDS development in our cohort, but it was associated (in the univariate analysis) with mortality.

Among laboratory markers, our analysis showed that higher LDH levels at admission were associated with increased odds for both ARDS and death, which might be useful to identify the KTs at higher risk from the admission.[19]

We have to acknowledge some limitations in our study. First, our cohort is not representative of the whole kidney transplant population, because outpatient individuals were not included. Second, we did not consider postdischarge follow-up data; therefore, long-term conclusions cannot be drawn. On the other hand, biochemical data (CRP, D-dimer, ferritin) were not available for all established time-points, which may undervalue their association with the main outcomes. Last, our findings might be limited and our results underpowered because of the small sample size. As far as we are concerned, however, this is one of the largest published cohorts of COVID-19 infection of a homogeneous cohort of KTs. Additionally, the exclusive inclusion of patients with definite outcomes in our analysis provides more reliable and clearer information regarding these population outcomes.

In conclusion, older age, obesity, and pulmonary disease, along with high baseline LDH levels at presentation and ARDS, were associated with poorer outcomes in KTs affected by COVID-19. Half of our population developed ARDS, even those without pneumonia at admission. In terms of pharmacologic strategies, steroids arose as the most commonly used antirejection drug during the infection, especially in severe forms, whereas compassionate anti–COVID-19 treatments lead to remarkable rates of adverse effects. A larger study with a longer-term follow-up for COVID-19 transplant recipients could answer some of the remaining questions, particularly concerning the treatment, long-term prognosis, and most suitable strategy in terms of immunosuppression management in this scenario.

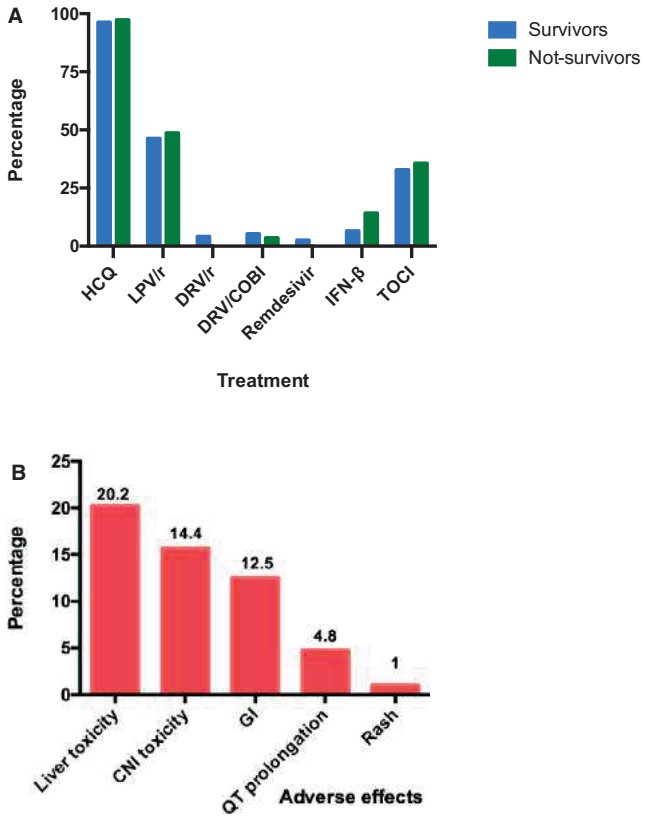

FIGURE 4 A, Proportion of antiviral therapies use and associated adverse effects. Distribution among survivors and nonsurvivors is shown. B, Associated adverse effects. AR, acute graft rejection; CNI, calcineurin inhibitor; DRV/r, darunavir/ritonavir; DRV/COBI, darunavir/cobicistat; GI, gastrointestinal; HCQ, hydroxychloroquine; IFN-β, interferon-beta; LPV/r, lopinavir/ritonavir; TOCI, tocilizumab [Color figure can be viewed at wileyonlinelibrary.com]

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

AUTHOR CONTRIBUTIONS

FA: designed the study, collected the data, analyzed the data, interpreted the data, drafted the article and revised the article critically. MN: designed the study, collected the data, analyzed the data, interpreted the data, drafted the article and revised the article critically. CD: collected the data and revised the article critically. TN: collected the data and revised the article critically. CJ: collected the data and revised the article critically. VA: collected the data and revised the article critically. CA: collected the data and revised the article critically. MM: collected the data and revised the article critically. MA: collected the data and revised the article critically. SJ: collected the data and revised the article critically. TI: collected the data and revised the article critically. GR: collected the data and revised the article critically. FC: collected the data and revised the article critically. LI: collected the data and revised the article critically. VP: collected the data and revised the article critically. CF: collected the data and revised the article critically. TV: collected the data and revised the article critically. PM: revised the article critically. MF: revised the article critically. SD: revised the article critically. OF: revised the article critically. OB: revised the article critically. CJM: interpreted the data and revised the article critically. ME: designed the study, collected the data, analyzed the data, interpreted the data, drafted the article and revised the article critically.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Zhu NA, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;382(8):727-733.
2. 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.
3. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;395(10229):1054-1062.
4. Wang D, Hu BO, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 2020;323(11):1061-1069.
5. Informe de Diálisis y Trasplante. 2018. https://www.senefro.org/contents/webstructure/SEN_2019_REER_modificada.pdf. Accessed May 2, 2020
6. Informe 5 (18 marzo - 25 abril) Registro COVID-19. https://mailchi.mp/senefro/registro-epidemiolgico-vhc-vhb-vih-1314562. Accessed May 2, 2020
7. Alberici F, Delbarba E, Manenti C, et al. A single center observational study of the clinical characteristics and short-term outcome of 20 kidney transplant patients admitted for SARS-CoV2 pneumonia. *Kidney Int*. 2020;97(6):1083-1088.
8. Zhu L, Xu X, Ma KE, et al. Successful recovery of COVID-19 pneumonia in a renal transplant recipient with long-term immunosuppression. *Am J Transplant*. 2020;20(7):1859-1863.
9. Columbia University Kidney Transplant Program. Early description of coronavirus 2019 disease in kidney transplant recipients in New York. *J Am Soc Nephrol*. 2020;31(6):1150-1156.
10. Clinical management of severe acute respiratory infection when COVID-19 is suspected. https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected. Accessed May 2, 2020
11. KDIGO Clinical Practice Guideline for Acute Kidney Injury. https://kdigo.org/wp-content/uploads/2016/10/KDIGO-2012-AKI-Guideline-English.pdf. Accessed May 2, 2020
12. Situación de COVID-19 o Coronavirus en España. https://covid19.isciii.es/. Accessed May 2, 2020
13. Domínguez-Gil B, Coll E, Fernández-Ruiz M, et al. COVID-19 in Spain: transplantation in the midst of the pandemic [published online ahead of print 2020]. *Am J Transplant*. https://doi.org/10.1111/ajt.15983
14. Fernández-Ruiz M, Andrés A, Loinaz C, et al. COVID-19 in solid organ transplant recipients: a single-center case series from Spain. *Am J Transplant*. 2020;20(7):1849-1858.
15. Bossini N, Alberici F, Delbarba E, et al. Kidney transplant patients with SARS-CoV-2 infection: the brescia renal COVID task force experience [published online ahead of print 2020]. *Am J Transplant*. https://doi.org/10.1111/ajt.16176
16. Akalin E, Azzi Y, Bartash R, et al. Covid-19 and kidney transplantation. *N Engl J Med*. 2020;382(25):2475-2477.
17. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *JAMA*. 2020;323(13):1239.
18. Williamson EJ, Walker AJ, Bhaskaran K, et al. OpenSAFELY: factors associated with COVID-19 death in 17 million patients. *Nature*. 2020;1-11: https://doi.org/10.1038/s41586-020-2521-4
19. Cravedi P, Mothi SS, Azzi Y, et al. COVID-19 and kidney transplantation: results from the TANGO International Transplant Consortium [published online ahead of print 2020]. *Am J Transplant*. https://doi.org/10.1111/ajt.16185
20. Hirsch JS, Ng JH, Ross DW, et al. Acute kidney injury in patients hospitalized with COVID-19. *Kidney Int*. 2020;98(1):209-218.

21. Pei G, Zhang Z, Peng J, et al. Renal involvement and early prognosis in patients with COVID-19 pneumonia. *J Am Soc Nephrol*. 2020;31(6):1157-1165.
22. Delsante M, Rossi GM, Gandolfini I, Bagnasco SM, Rosenberg AZ. Kidney Involvement in COVID-19: need for better definitions. *J Am Soc Nephrol*. 2020. https://doi.org/10.1681/ASN.2020050630
23. He X, Lau EHY, Wu P, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nat Med*. 2020;26:672-675.
24. Pascual J, Melilli E, Jiménez-Martín C, et al. COVID-19–related mortality during the first 60 days after kidney transplantation. *Eur Urol*. 2020. https://doi.org/10.1016/j.eururo.2020.06.036
25. Low-cost dexamethasone reduces death by up to one third in hospitalised patients with severe respiratory complications of COVID-19. https://www.recoverytrial.net/files/recovery_dexamethasone_statement_160620_v2final.pdf. Accessed July 13, 2020.
26. Gautret P, Lagier J-C, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents*. 2020;56(1):105949.
27. Ray WA, Murray KT, Hall K, Arbogast PG, Stein CM. Azithromycin and the risk of cardiovascular death. *N Engl J Med*. 2012;366(20):1881-1890.
28. Solidarity clinical trial for COVID-19 treatments. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments. Accessed July 13, 2020.
29. Coronavirus (COVID-19) Update: FDA Issues Emergency Use Authorization for Potential COVID-19 Treatment | FDA. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-emergency-use-authorization-potential-covid-19-treatment. Accessed May 4, 2020.
30. Dietz W, Santos-Burgoa C. Obesity and its implications for COVID-19 mortality. *Obesity*. 2020;28(6):1005.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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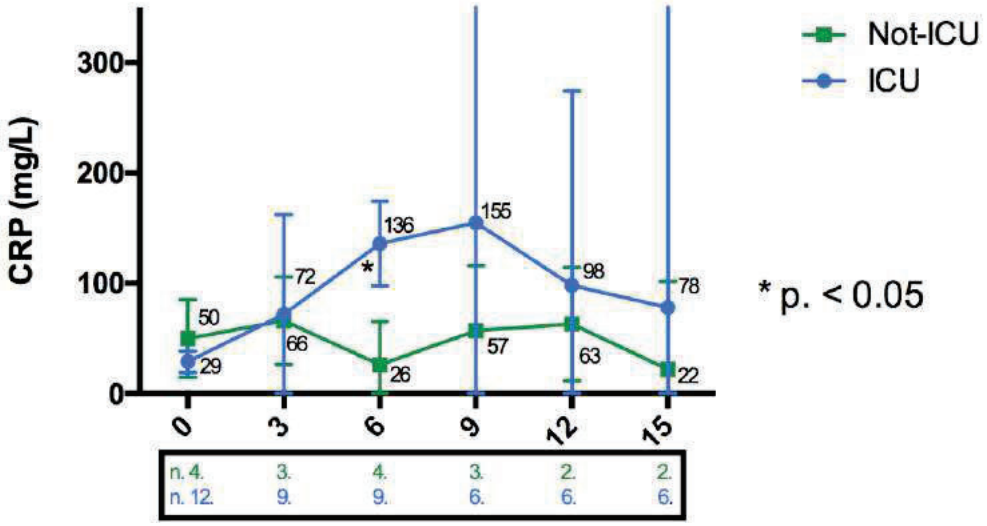
SUPPLEMENTAL DIGITAL CONTENT

Supplementary Figure 1. C-Reactive Protein levels depending on the final need or no need of Intensive Care Unit admission in patients without need of oxygen or x-ray infiltrates at admission.



** CRP: C-Reactive Protein*

Supplementary Table 1. Evolution of patients classified as nosocomial Sars-Cov-2 infection

Patient	Gender	Age	KT Date	Admission's date	Date Swab Sars-Cov-2	Cause of Admission	Creat (µmol/L at Swab Date
1	FEMALE	45	28-Mar-2012	09-Mar-2020	09-apr-2020	Congestive heart failure	205
2	FEMALE	61	18-feb-2020	18-Feb-2020	17-Mar-2020	Kidney transplant	DGF*
3**	MALE	70	10-Mar-2020	08-Apr-2020	08-Apr-2020	AKI & COVID-19	660
4	FEMALE	74	09-Feb-2019	09-Apr-2020	14-Apr-2020	Pyelonephritis	123
5**	MALE	71	25-Feb-2020	25-Feb-2020	1-apr-2020	Kidney Transplant	194
6	FEMALE	77	10-Mar-2020	10-Mar-2020	30-Mar-2020	Kidney Transplant	135
7	FEMALE	72	27-Feb-2020	27-Feb-2020	23-Mar-2020	Kidney transplant	DGF*
8**	FEMALE	74	04-Feb-2020	18-Mar-2020	28-Mar-2020	AKI & COVID-19	416
9**	MALE	49	01-Jan-2002	23-Mar-2020	23-Mar-2020	Coronary revascularization	363
10	MALE	64	26-Jun-2012	28-Feb-2020	28-Feb-2020	Non-covid respiratory infection	126
11	MALE	79	15-Jun-1997	11-Feb-2020	31-Mar-2020	Congestive heart failure	254
12	FEMALE	75	26-Feb-2012	12-Mar-2020	28-Mar-2020	Pyelonephritis (with renal abscess)	267
13**	FEMALE	63	10-Mar-2020	18-Mar-2020	23-Mar-2020	Pyelonephritis	300
14	MALE	75	03-Jul-1996	20-Mar-2020	26-Mar-2020	Pyelonephritis	424

** AKI: Acute Kidney Injury; ARDS: Acute Respiratory Distress Syndrome; DGF: Delayed graft function; KT: kidney transp*

***Patients 3, 5, 8, 9, 13 were classified as Nosocomial infection since they were discharged from the same Hospital < 2 time to readmission 4 days IQR 3-10)*

Supplementary Table 2. Therapeutic strategies used in patients with fatal outcome and in those developing acute respiratory disease syndrome (ARDS).

	Mortality			**ARDS**		
	Alive (n=76)	**Death (n=28)**	**p-value**	**no (n=47)**	**ARDS (n=57)**	**p-value**
Hydroxychloroquine	74 (97.4)	27 (96.4)	1	45 (95.7)	56 (98.2)	0.44
Azithromycin	46 (60.5)	20 (71.4)	0.30	28 (59.6)	38 (66.7)	0.45
Antiviral treatment						
Lopinavir/ritonavir	37 (48.7)	13 (46.4)	0.83	19 (40.4)	31 (54.5)	0.15
Darunavir/Ritonavir	3 (4.2)	0	0.5	1 (2.2)	2 (3.8)	0.65
Darunavir/Cobicistat	4 (5.4)	1 (3.6)	1	1 (2.1)	4 (7.3)	0.23
Remdesivir	2 (2.6)	0	1	1 (2.1)	1 (1.8)	1
Interferon-Beta-1a	5 (6.6)	4 (14.3)	**0.24**	0	9 (15.8)	**0.004**
Tocilizumab	25 (32.9)	10 (35.7)	0.78	6 (12.8)	29 (50.9)	**<0.001**
Changes in immunosuppression						
Immunosuppression withdrawal	**68 (89.5)**	**27 (96.4)**	**0.43**	**39 (83)**	**56 (98.2)**	**0.01**
CNI withdrawal	35 (69)	15 (68)	0.61	18 (48.6)	32 (74.4)	**0.018**
mTORi withdrawal	9 (52.9)	3 (100)	0.24	4 (36.4)	8 (88.9)	0.028
MMF/MPA withdrawal	49 (73.1)	22 (84.6)	0.28	29 (69)	42 (82.4)	0.13
Steroid withdrawal	1 (1.4)	1 (3.7)	0.47	2 (4.5)	0	0.19

**ARDS: Acute Respiratory Distress Syndrome; CNI: calcineurin inhibitor; MMF/MPA: mycophenolate mofetil or mycophenolic acid; mTORi: mammalian target of rapamycin inhibitor.*

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IV. MATHERIALS, METHODS AND RESULTS

Article 2.

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. 2021 Aug;21(8):2749-2761. doi: 10.1111/ajt.16570

Objective:

To characterize the kinetics and magnitude of SARS-CoV-2 specific adaptive immune responses at the T-cell and serological immune compartments, among SOT patients hospitalized due to COVID-19 and compare them to those exhibited by a matched group of immunocompetent patients.

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DOI: 10.1111/ajt.16570

ORIGINAL ARTICLE

SARS-CoV-2-specific serological and functional T cell immune responses during acute and early COVID-19 convalescence in solid organ transplant patients

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The description of protective humoral and T cell immune responses specific against SARS-CoV-2 has been reported among immunocompetent (IC) individuals developing COVID-19 infection. However, its characterization and determinants of poorer outcomes among the at-risk solid organ transplant (SOT) patient population have not been thoroughly investigated. Cytokine-producing T cell responses, such as IFN-γ, IL-2, IFN-γ/IL-2, IL-6, IL-21, and IL-5, against main immunogenic SARS-CoV-2 antigens and IgM/IgG serological immunity were tracked in SOT (n = 28) during acute infection and at two consecutive time points over the following 40 days of convalescence and were compared to matched IC (n = 16) patients admitted with similar moderate/severe COVID-19. We describe the development of a robust serological and functional T cell immune responses against SARS-CoV-2 among SOT patients, similar to IC patients during early convalescence. However, at the infection onset, SOT displayed lower IgG seroconversion rates (77% vs. 100%; p = .044), despite no differences on

Alexandre Favà and Laura Donadeu equally contributed as first authors to this work.

IgG titers, and a trend toward decreased SARS-CoV-2-reactive T cell frequencies, especially against the membrane protein (7 [0–34] vs. 113 [15–245], p = .011, 2 [0–9] vs. 45 [5–74], p = .009, and 0 [0–2] vs. 13 [1–24], p = .020, IFN-γ, IL-2, and IFN-γ/IL-2 spots, respectively). In summary, our data suggest that despite a certain initial delay, SOT population achieve comparable functional immune responses than the general population after moderate/severe COVID-19.

KEYWORDS
adaptive immunity, basic (laboratory) research / science, clinical research / practice, COVID-19 infection, heart transplantation / cardiology, infection and infectious agents, kidney transplantation / nephrology, liver transplantation / hepatology, solid organ transplantation, T cell biology

1 | INTRODUCTION

A novel coronavirus, designated as SARS-CoV-2, emerged in Wuhan, China, at the end of 2019 and has spread all over the globe in a logarithmic manner. The increasing number of fatal outcomes related to the Coronavirus Disease-2019 (COVID-19) has put global health institutions on high alert.

While most people remain asymptomatic or develop only mild symptoms during COVID-19,[1,2] some specific group of patients seem to be at significantly higher risk of fatal outcomes,[3] and among them recipients of solid organ transplants (SOT) most likely because they receive chronic immunosuppressive therapy that predominantly targets T cell adaptive immunity.[4] Importantly, SOT patients represent an important prevalent high-risk population in whom the biology of the adaptive immunity specific to SARS-CoV-2 during COVID-19 has not yet been thoroughly investigated.

First studies evaluating immunocompetent (IC) convalescent individuals have shown the induction of neutralizing antibodies after primary infection[5-8] which seem to be detectable essentially among patients with more severe forms of COVID-19.[9,10] Conversely, robust anti-viral T cell responses have been described after SARS-CoV-2 infection, which seem to correlate with the magnitude of SARS-CoV-2-specific IgG and IgA titers during the initial phase of convalescence[11] and with the severity of COVID-19 infection.[12] Interestingly, SARS-CoV-2-reactive T cell immunity seems to last for a longer period of time, even among seronegative convalescent patients[13] and can discriminate those patients with the poorest outcomes.[14]

In this study, we aimed at investigating the IgM and IgG serological antibody responses as well as the SARS-CoV-2-reactive T cell responses against main four different structural viral proteins, Spike (S), Nucleocapsid (N), Membrane (M), and Envelope (E), in SOT recipients as compared to matched hospitalized IC healthy individuals due to COVID-19, both at the time of the acute infection phase and over the convalescent clinical course after infection, in order to provide mechanistic insights that could explain the recent epidemiological observations of a higher risk of poorer outcomes in SOT as compared to IC-infected patients.

2 | MATERIAL AND METHODS

2.1 | Patients of the study and clinical definitions

In this study, we evaluated 44 consecutive patients hospitalized between March 15 and April 18, 2020, at Bellvitge University Hospital (Barcelona, Spain) and Montpellier University Hospital (Montpellier, France) due to COVID-19 infection, and in whom peripheral blood mononuclear cells (PBMCs) and serum samples were available. All patients had been tested positive for SARS-CoV-2 infection by a RT-PCR analysis on nasopharyngeal swab samples. Among these 44 patients, 28 were SOT recipients and 16 IC patients, who were matched for age, gender, and severity of COVID-19 at study inclusion (Figure 1; Table 1).

A total of 113 serially collected peripheral blood samples at three different time points of the disease were analyzed in this study—during the acute phase of infection (T1; median 16, IQR 12–19 days after symptom onset) and at two convalescence periods (T2; median 32, IQR 25–37 days, and T3; 49 days, IQR 43–53), which represented a median of 7 days, IQR 4–11 and 23 days, IQR 20–27 and 40 days, and IQR 37–44, after first positive PCR, respectively.

Additionally, PBMC samples from 16 non-immunosuppressed patients on the waiting list for kidney transplantation that were obtained 2 years before the COVID-19 outbreak (November 2018) and were stored in our biobank facilities were used as healthy controls (HC).

All clinical, demographic, and immunological patient characteristics as well as the main outcomes, such as mortality, or the need of invasive/non-invasive mechanical ventilation (MV) were recorded. COVID-19 disease severity was defined according to the level of oxygen support during hospitalization according to the World Health Organization interim guidance to define Acute Respiratory Distress Syndrome (bilateral opacities not explained by volume overload with an oxygen saturation/fraction of inspired oxygen ratio <315).[15]

The study was approved by the Ethical Review Boards (PR115/20) at each center and patients were recruited in the study after providing a signed informed consent.

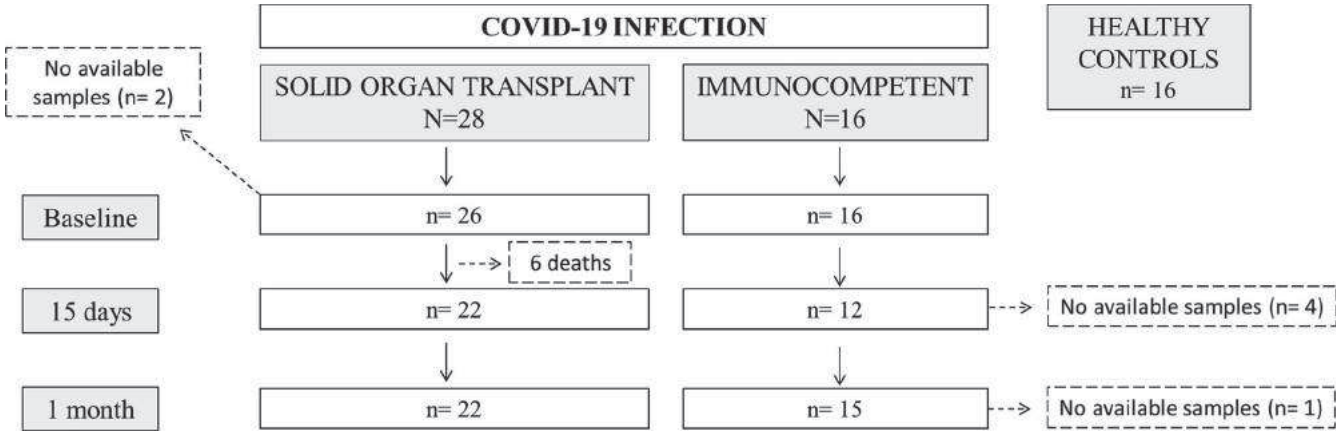

FIGURE 1 Flowchart of the study

2.2 | Collection and management of serum and PBMC samples

Detailed description is depicted in Data S1.

2.3 | Assessment of SARS-CoV-2-specific antibodies

IgM and IgG antibodies against SARS-CoV-2 were detected by a chemiluminescence technique, using the MaglumiTM 2019 nCov-IgM and the MaglumiTM 2019 nCov-IgG tests (Snibe Diagnostic) on a Maglumi 2000® analyzer (Snibe Diagnostic), according to the manufacturer's instructions. Detailed information is provided in Data S1.

2.4 | Assessment of cytokine-producing SARS-CoV-2-reactive T cell responses

SARS-CoV-2-reactive T cell responses were evaluated using a multicolor FluoroSpot Immune assay kit (AID® Gmbh). Distinct cytokine-producing T cell frequencies were assessed: effector (IFN-γ), proliferative (IL-2) and central (IFN-γ/IL-2) memory Th1 responses, IL-5 and IL-21 Th2 responses, and IL-6 pro-inflammatory T cell responses. The main four structural SARS-CoV-2 proteins, Spike Glycoprotein (S), Membrane Protein (M), Nucleoprotein (N), and Envelope Small Membrane Protein (E) (JPT®), were used for stimulation in the multicolor FluoroSpot Immune assay individually. Overlapping peptide pools covering the whole Influenza virus antigen length (AID® Gmbh) were also tested. In each test, complete medium alone and Pokeweed (PWM) mitogen were used as negative and positive controls, respectively. Any antigen-specific ELISPOT test with less than 5 spots/2 × 10^5 PBMC was considered as negative when assessed in a qualitative manner. Precise information is provided in Data S1.

2.5 | Statistics

Continuous variables were expressed as mean ±SD or median and IQR and categorical variables as number of total (n) and percentage (%). A comparison between groups was performed using Pearson's χ^2 test for categorical data. Continuous measurements were compared among groups using Kruskal-Wallis and Mann-Whitney U test for non-normally distributed data, while ANOVA and t tests were used when data were normally distributed. *p*-values <.05 were considered statistically significant. SARS-CoV-2-reactive cellular and humoral responses were centered and scaled and heatmap was built by means of the pheatmap R package [16] using Euclidean distance and complete method as agglomeration method. R package version 1.0.12 was used https://CRAN.R-project.org/package=pheatmap. All other analyses were performed using SPSS version 26 software, and graphs were generated using GraphPad Prism version 8.0 software (GraphPad Software).

3 | RESULTS

3.1 | Patients of the study

Forty-four hospitalized patients with COVID-19 disease confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR) were included: 28 SOT recipients and 16 IC patients. Eighteen (64.3%) kidney, five (17.9%) heart, and five (17.9%) liver transplants composed the SOT group, with a median time after transplantation of 9 ± 7 years (IQR 3–14) and were receiving a calcineurin inhibitor (CNI)-based immunosuppressant scheme (67.9%). Also, 16 individuals in whom PBMC samples were retrieved and stored at our biobank facilities in 2018 were included in the study (Figure 1).

Main clinical, demographic, and immunological characteristics are depicted in Table 1. As shown, SOT and IC patients of the study were matched for age, sex, and main comorbidities, but IC patients were less diabetic. The degree of COVID-19 severity and time of

TABLE 1 Demographic and clinical characteristics of patients infected by SARS-CoV-2

	SOT (N = 28)	IC (N = 16)	HC (n = 16)	P value
Age (years, mean ± SD)	59.4 ±13.6	59.4 ± 11.3	63.4 ± 10	0.531
Sex (Female) (n, %)	7 (25)	7 (44)	5 (31.3)	0.437
Comorbidities (n, %)				
Diabetes	11 (39.3)	1 (6.3)	N/A	0.032
Arterial hypertension	19 (67.9)	6 (37.5)	N/A	0.051
Obesity [a]	6 (21.4)	3 (18.8)	N/A	0.868
Pulmonary disease [b]	2 (7.1)	2 (12.5)	N/A	0.614
Heart disease [c]	6 (21.4)	2 (12.5)	N/A	0.689
Active neoplasm	4 (14.3)	1 (6.3)	N/A	0.638
ACEi/ARB use	10 (35.7)	2 (12.5)	N/A	0.116
Previous Influenza vaccine (yes)	22 (78.6)	7 (43.8)	12 (75)	0.082
Clinical symptoms at onset (n, %)				
Cough	18 (64.3)	13 (81.3)	N/A	0.314
Dyspnea	10 (35.7)	7 (43.8)	N/A	0.749
Diarrhea	14 (50)	6 (37.5)	N/A	0.534
Myalgias	11 (39.3)	7 (43.8)	N/A	1.000
Fever	23 (82.1)	16 (100)	N/A	0.141
Disease severity at enrollment (n, %)				
No oxygen therapy needed	5 (17.9)	1 (6.2)	N/A	0.276
Oxygen requirement (NO ARDS)	8 (28.6)	6 (37.5)	N/A	0.738
ARDS	15 (53.6)	9 (56.3)	N/A	1.000
Outcomes at the end of follow-up (n, %)				
Death	6 (21.4)	0 (0)	N/A	0.072
MV or Death	9 (32.1)	1 (6.2)	N/A	0.05
Sampling time points (days)				
Days from symptom onset to first time-point PBMC collection (median, IQR)	15 (12–20)	17 (10–18)	N/A	0.794
Days from symptom onset to second time-point PBMC collection (median, IQR)	31 (25–40)	32 (26–37)	N/A	0.711
Days from symptom onset to third time-point PBMC collection (median, IQR)	48 (42–53)	50 (44–54)	N/A	0.225
Days from positive PCR to first time-point collection (median, IQR)	7 (5–12)	6 (4–10)	N/A	0.15
Days from positive PCR to second time-point collection (median, IQR)	23 (20–28)	24 (20–26)	N/A	0.762
Days from positive PCR to third time-point collection (median, IQR)	40 (36–44)	41 (38–44)	N/A	0.556

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ARDS, acute respiratory distress syndrome; HC, healthy controls; IC, immunocompetent; MV, mechanical ventilation (invasive or non-invasive); PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; SOT, solid organ transplant.

[a]Obesity: body mass index >30.

[b]Pulmonary disease: chronic obstructive pulmonary disease, asthma, bronchiectasis, or sleep apnea-hypopnea syndrome.

[c]Heart disease: congestive heart failure, coronary artery disease, atrial fibrillation, or valvular heart disease.

assessment were not different between groups. After a follow-up of 40 days (37–44), six (13.6%) patients passed away, they were all SOT (three liver, two kidney, and one heart transplant recipient). The composite outcome depicted as requirement of MV or death did also occur more frequently among SOT (9 [32.1%] SOT vs. 1 [6.2%] IC; $p = .05$). First time-point blood samples were retrieved prior to this composite outcome.

We further evaluated 16 healthy control (HC) individuals in whom PBMC samples had been retrieved in 2018, before the SARS-CoV-2 pandemic, and were also matched for age and gender with the

other two study groups. As expected, previous influenza vaccination rate was lower among the IC group (43.8%) as compared to SOT (78.6%) and HC (75%) groups (p = .082).

3.2 | Circulating lymphocytes and functional adaptive immunity during acute and convalescent COVID-19 infection

Our first analysis showed that while both SOT and IC patients displayed abnormally low total lymphocyte counts, this lymphopenia was more pronounced for SOT recipients (866 ± 427 vs. 1531 ± 490 in IC; p < .001). Total lymphocyte counts in HC were 1564 ±427 and were significantly higher than SOT at T1 (p < .001) (Figure S2).

As shown in Figure 2A and Figure S3A, during acute infection (T1), SARS-CoV-2-reactive T cell responses against four main viral antigens were more predominantly detected among IC patients than within SOT and especially among those with higher severity index. Notably, no SARS-CoV-2-reactive responses were observed among HC. IgG and IgM serological immunity against SARS-CoV-2 was detected within both SOT and IC. At the last convalescent period (T3) (Figure 2B and Figure S3B), SARS-CoV-2-reactive T cell immune responses were now detectable within the SOT group while they had faded in IC patients. Likewise, more predominant IgM responses were observed among SOT than IC, whereas IgG-specific antibodies were similarly detected.

Conversely, non-SARS-CoV-2-reactive T cell immune responses against influenza and a polyclonal stimuli (PWM) were significantly weaker within both SOT and IC as compared to HC at baseline, which persisted during the convalescence period.

3.3 | SARS-CoV-2-reactive T cell immunity during acute and early convalescent COVID-19 infection

No correlation was observed between absolute lymphocyte counts and SARS-CoV-2-reactive T cell frequencies for each antigen-specific cytokine-producing T cell (IFN-γ, IL-2, IFN-γ/IL-2, IL-6, IL-21, and IL-5) at any time point of the study (Table S1).

3.3.1 | SARS-CoV-2-reactive T cell function during acute COVID-19 infection

A strong correlation was observed between all four SARS-CoV-2 antigen responses (Table S2), showing a wide and different range of T cell frequencies.

As illustrated in Figure 3A and described in Table S3, as compared to IC individuals, SOT displayed numerically lower IFN-γ, IL-2, and IFN-γ/IL-2-producing T cell frequencies, although being statistically significant only for antigen M (7 [0–34] vs. 113 [15–245], p = .011; 2 [0–9] vs. 45 [5–74], p = .009, and 0 [0–2] vs. 13 [1–24], p = .020, for IFN-γ, IL-2, and IFN-γ/IL-2 spots in SOT and IC, respectively). A certain detectable IL-6 stimulation was widely detected in all evaluated patients, including HC thus suggesting a general non-antigen-specific immune response. Notably, IL-21 and IL-5-producing T cells against SARS-CoV-2 were barely detectable in both SOT and IC patients at this time point. As also illustrated, the highest frequencies were observed for T cells only producing IFN-γ, whereas the lowest for those polyfunctional IFN-γ/IL-2-producing T cells.

While IC patients showed similarly high T cell immune responses against both antigens S and M, the highest immune response among SOT was only against antigen S. Of note, T cell responses against antigen E were barely detectable in all infected patients (Figure S4A).

As illustrated in Figure S5A, a higher proportion of SARS-CoV-2 T cell non-responders was observed among SOT as compared to IC, and especially those IFN-γ/IL-2-producing T cells.

3.3.2 | Progression of SARS-CoV-2-reactive T cell immunity during COVID-19 convalescence

We next sequentially monitored these patients at two consecutive time points during convalescence periods: at T2; 32 (IQR 25–37) and T3; 49 (IQR 43–53) days after symptom onset, which represents a median of 11 (IQR 3–16) and 27 (IQR 22–30) days after discharge, respectively. Similar to T1, a strong correlation of T cell responses was observed between the different SARS-CoV-2 antigens at both time points (Tables S4–S5).

Unlike during acute infection, there were in general no longer differences between SOT and IC regarding the distinct SARS-CoV-2-reactive T cell responses (Figure 3B; Tables S6–S7). However, at T3, while no statistically significant differences were noted between groups, numerically higher SARS-CoV-2-reactive T cell responses in SOT as compared to IC patients were observed, and particularly against antigen S for IL-2 and IL-21 (425 [242–606] vs. 181 [58–289], p = .07 and 107 [36–212] vs. 10 [2–83], p = .025, respectively) (Figure 3C). Similarly, as during the acute infection phase, while the strongest T cell responses among IC were driven against SARS-CoV-2 antigens S and M, the predominant T cell response among SOT was against antigen S but not to antigen M (Figure S4B,C). Also, almost no detectable T cell responses were observed against SARS-CoV-2 antigen E. As also illustrated in Figures S5B,C, now at T2 and T3, the great majority of both SOT and IC patients showed detectable SARS-CoV-2-reactive T cell frequencies.

To examine the kinetics of SARS-CoV-2-reactive T cell responses over time in the two groups, we assessed the global SARS-CoV-2-reactive T cell immune responses by means of the median T cell frequencies against the three main immunogenic antigens (S, M, and N) in each patient and at each time point. As shown in Figure 4, both SOT and IC developed a rapid increase of global SARS-CoV-2-reactive T cell responses until T3. Notably, these functional changes were more evident among SOT as compared to IC patients, which fundamentally occurred between T1 and T2. As previously described at the single antigen level, SOT displayed weaker global SARS-CoV-2-reactive T cell frequencies at baseline than IC patients (11 [1–42] vs. 90 [26–143] spots, p = .003 and; 6 [0–15] vs. 30 [4–60] spots, p = .049; 1 [0–2] vs. 9 [0–16], p = .050; for IFN-γ, IL-2, and IFN-γ/IL-2, respectively).

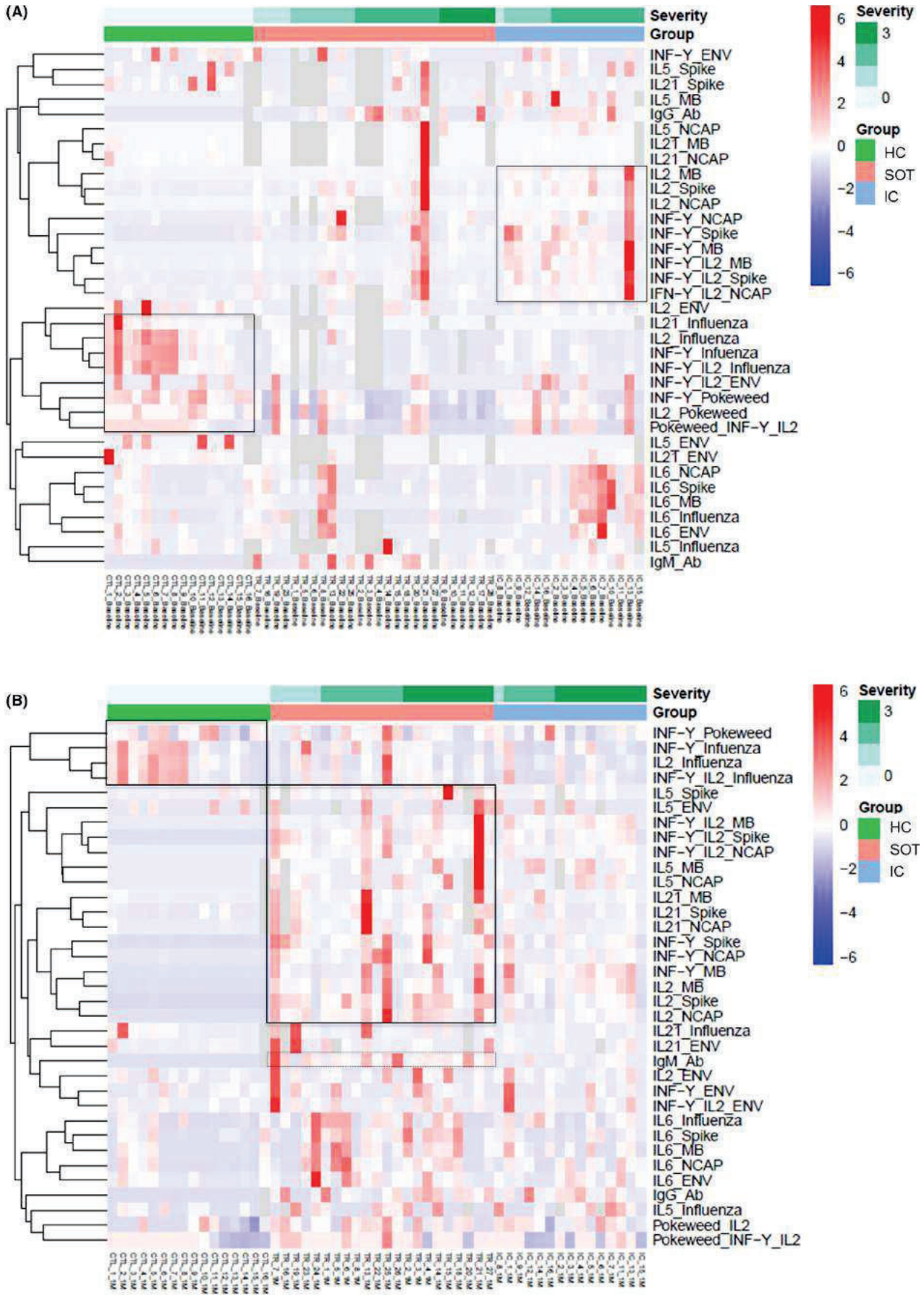

FIGURE 2 Heatmaps generated by hierarchical clustering of SARS-CoV-2-specific and non-specific immune responses for SOT, IC patients, and HC, according to the COVID-19 disease severity (0 = no oxygen need; 1 = oxygen need; 2 = acute respiratory distress syndrome, 3 = death). Immune responses used for clustering were differentially expressed (fold change >2, false discovery rate $p < .05$). Gray fields indicate missing values. (A) Heatmap performed at first time point during acute COVID-19 infection (7; 4–11 days after the diagnosis) among 26 SOT, 16 IC, and 16 HC. (B). Heatmap performed during the early convalescent period (40; 37–44 days after the diagnosis) of COVID-19 disease in 22 SOT, 15 IC, and 16 HC [Color figure can be viewed at wileyonlinelibrary.com]

3.4 | SARS-CoV-2-specific serological immunity in SOT and IC with severe COVID-19

All infected patients showed detectable SARS-CoV-2-specific IgM titers at baseline (Figure 5A) and remained detectable in the following two time points. Conversely, while all 16 IC patients showed detectable virus-specific IgG titers already at T1, 6/26 (23%) SOT did not ($p = .044$). All SOT seroconverted at T2 and remained positive until T3. Nevertheless, while no differences were observed regarding quantitative IgG titers between the two groups at any time point, IgM titers, albeit detectable, seemed to be cleared from the circulation much faster among IC than in SOT over time (Figure 5B). Indeed, at T2 and T3, IC showed significantly lower IgM titers than SOT patients (1.6 [0.75–3.1] vs. 5.3 [3.7–7.7] UA/ml, $p = .001$ at T2 and 0.8 [0.6–1.6] vs. 3.5 [1.9–5.3] UA/ml; $p < .001$ at T3).

Of note, patients without IgG class-switch seroconversion displayed lower SARS-CoV-2-reactive IL-2-producing T cell frequencies against antigens S and M than patients with IgG serology (6 [1–9] vs. 28 [4–98], $p = .073$ and 1 [0–5] vs. 7 [2–63], $p = .067$ for IL-2-producing T cells against antigens S and M, respectively).

3.5 | T cell immunity against influenza and polyclonal stimulation during COVID-19

To investigate the degree of general immune impairment in patients developing moderate/severe COVID-19 infection, we assessed non-SARS-CoV-2-reactive T cell responses to influenza peptides and to a strong polyclonal T cell stimulation with PWM. To note, a correlation was found between these antigens, mainly for IFN-γ-producing T cells at the two first time points of evaluation, T1 ($r = .403$, $p = .015$) and T2 ($r = .403$ $p = .015$). No differences were observed between SOT and IC patients regarding both influenza and PWM T cell responses at any time point. Remarkably, both SOT and IC individuals displayed significantly lower IFN-γ, IL-2, IFN-γ/IL-2, and IL-21 T cell responses against both stimuli as compared to HC, which lasted in some cases until T3 (Figure 6), despite significant vaccination rates.

3.6 | Baseline SARS-CoV-2-reactive T cell immunity and clinical outcomes among SOT

In our study, 10 (22.7%) patients required MV or died during the follow-up, being nine SOT. As depicted in Table S8, we did not find any differences regarding main clinical or demographic within the whole study population. Likewise, no differences were observed when analyzing SARS-CoV-2-reactive T cell responses and outcomes (data not shown). However, and since almost no fatal events occurred within the IC group in our study, we then focused on the SOT group. Also, no clinical nor demographical variables discriminated a poorer clinical evolution. Nevertheless, while no differences were observed regarding most SARS-CoV-2-reactive T cell responses, SOT with the poorest outcomes displayed lower IL-2-producing T cell frequencies against main three immunogenic SARS-CoV-2 antigens as compared to those with better clinical results (0 [0–3] vs. 10 [4–60] $p = .003$; 6 [0–13] vs. 28 [4–110] $p = .085$; and 0 [0–3] vs. 4 [0–22] $p = .075$ for antigens N, S, and M, respectively) (Figure 7A). Intriguingly, the only patient of the IC group who required MV showed robust IL-2-producing T cell frequencies against the three viral antigens (Figure 7B). Furthermore, the proportion of IgG seroconversion was numerically lower among those with worse outcomes (80% vs. 62.5%, $p = .245$).

In terms of immunosuppression, while mycophenolate was broadly withdrawn in our cohort (Table S9), no differences were found between patients with or without CNI-based immunosuppressive regimens at T1. Also, no differences were observed at the successive time points for those patients who had the CNI withdrawn during the infection phase (data not shown).

4 | DISCUSSION

In this study, we investigated the magnitude and kinetics of adaptive immunity, both serological and specific T cell responses to main four immunogenic SARS-CoV-2 antigens among chronically immunocompromised SOT recipients and compared them to matched IC individuals developing the same moderate/severe COVID-19 infection. Here, we show that SOT patients achieve a similarly robust serological and functional T cell immune response comparable to that of IC patients during early COVID-19 convalescence. Nonetheless, a certain delay achieving such strong immune responses was observed among SOT, depicted by lower IgG seroconversion rates and cytokine-producing T cell frequencies, especially against the membrane antigen, as compared to IC patients during the acute infection onset. Moreover, we also describe that among SOT, those patients developing the worst clinical outcomes displayed more deprived SARS-CoV-2-reactive IL-2-producing T cell immune responses as compared to patients with better clinical results.

A widely reported viral-related effect is the severe peripheral lymphopenia observed during COVID-19 infection.[17-19] Indeed, it

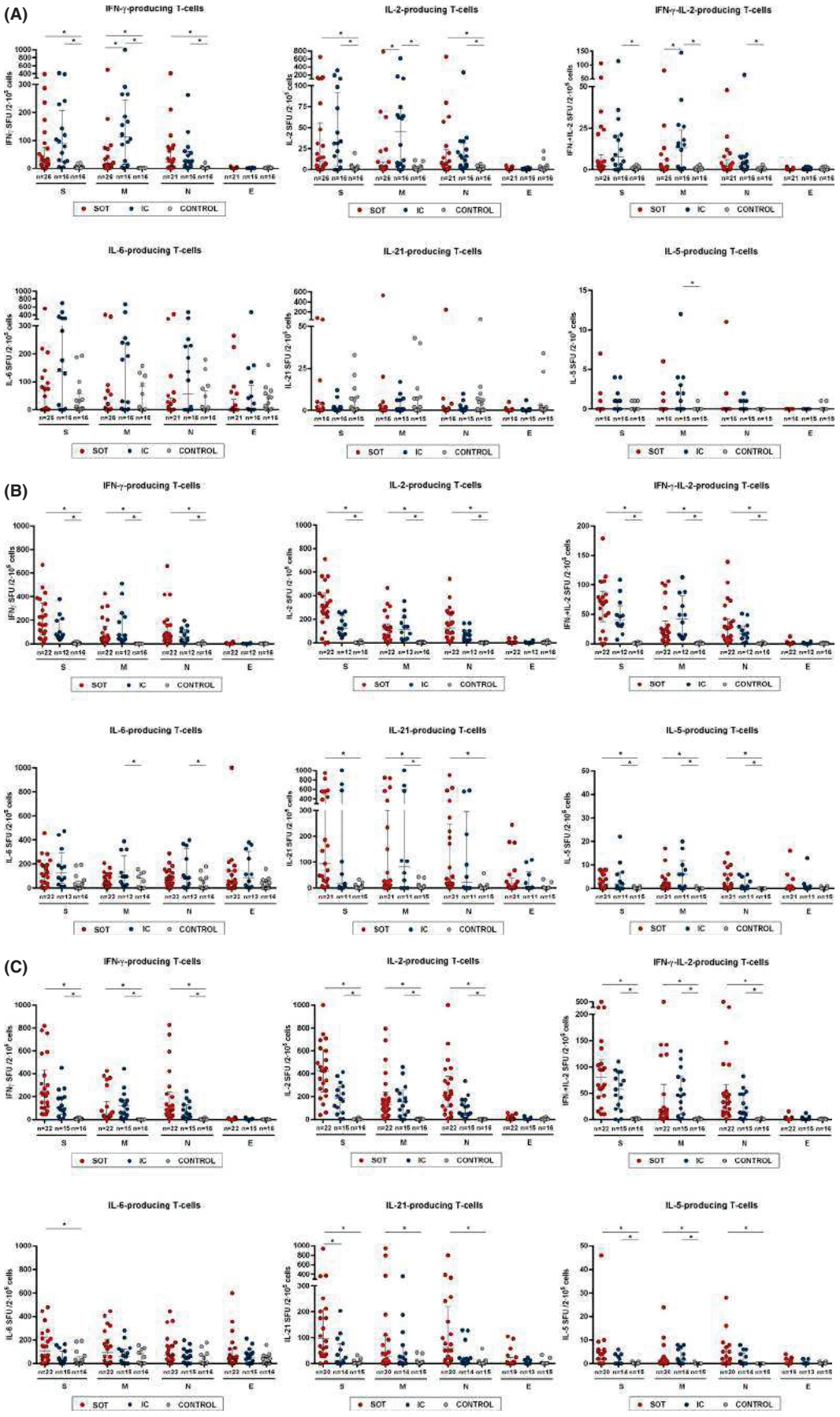

FIGURE 3 Cytokine profile of T cell responses against main structural SARS-CoV-2 proteins Spike (S), Membrane (M), Nucleoprotein (N), and Envelope (E). Frequencies of IFN-γ, IL-2, IFN-γ/IL-2, IL-6, IL-5, and IL-21-producing T cells were assessed among the three study group samples at different time points. *p < .05, calculated with Kruskal-Wallis test. (A) T1 = 16; 12–19 days. (B) T2 = 32; 25–37 days. (C) T3 = 49; 43–53 days after symptom onset [Color figure can be viewed at wileyonlinelibrary.com]

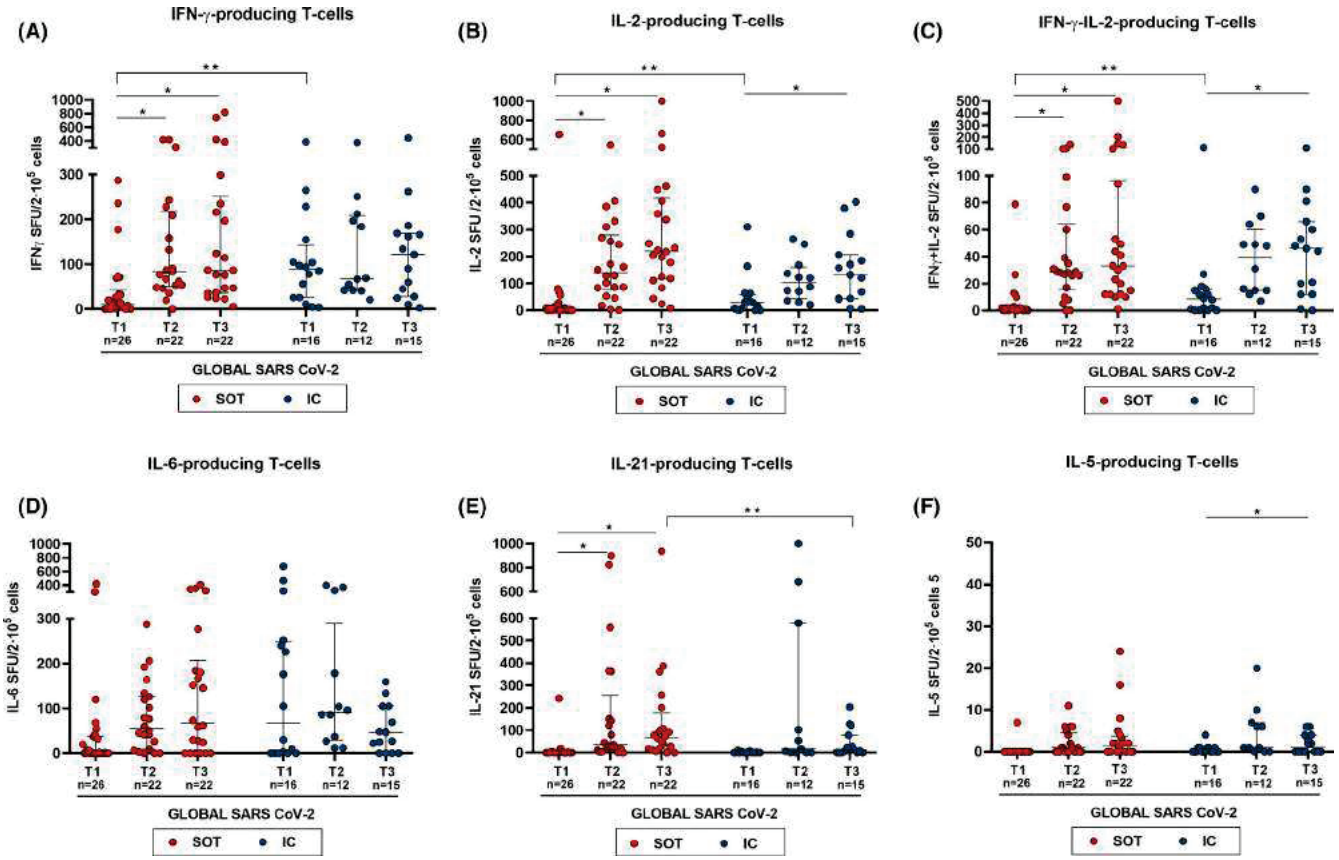

FIGURE 4 Global T cell responses specific to SARS-CoV-2 at different time points (median T cell frequencies against the three SARS-CoV-2 immunogenic antigens: S, M, and N). At T1, N = 42 (SOT = 26, IC = 16); T2, N = 34 (SOT = 22, IC = 12), and T3, N = 37 (SOT = 22, IC = 15). Median and IQR are shown. Intragroup paired analysis; *p < .05 evaluated with Friedman's test. Significant intergroup differences (IC vs. SOT) are also shown; **p < .05 (analyzed by Mann-Whitney U test) [Color figure can be viewed at wileyonlinelibrary.com]

was particularly severe among SOT as compared to IC patients, a finding that would seem to be most likely favored in this group of patients by the chronic immunosuppressive therapy these patients follow. However, we did not observe any correlation between total lymphocyte counts and the different SARS-CoV-2-reactive T cell responses, thus illustrating the importance of not only measuring total cell numbers but also their antigen-specific function.

So far, a number of studies have shown the contribution of T cell immunity specific to SARS-CoV-2 in COVID-19 patients.[20] However, most of them have exclusively focused in patients without previous underlying immune condition such as SOT, and have not assessed the magnitude and relevance of different peripheral T cell immune subsets against the distinct viral antigens both during the acute infection phase as well as during the convalescence period.[11,13,21] Herein, we first show that an important proportion of patients, both SOT and IC, display a wide range of SARS-CoV-2-reactive T cell responses, already in a very early phase of the disease. Globally, and as previously reported, main functional T cell responses were observed against three viral antigens: Spike (S), Membrane (M), and Nucleocapsid (N),[11,22-24] but not against Envelope (E).

Different studies have described the significantly higher risk of fatal outcomes among SOT developing COVID-19 infection as compared to healthy population.[4,25-27] While the main hypothesis for these poorer outcomes is sustained on their T cell immunocompromised status, no evaluation of their anti-viral immune response, both at the time of acute infection and during convalescence, has been reported yet. In our study, the lower IFN-γ, IL-2, and IFN-γ/IL-2-producing T cell frequencies against SARS-CoV-2, especially against antigen M, along with the higher proportion of patients with no detectable SARS-CoV-2-reactive T cell responses and the lower IgG seroconversion rates at the infection onset in SOT as compared to IC patients, suggest a certain delay of SOT to achieve a similarly robust

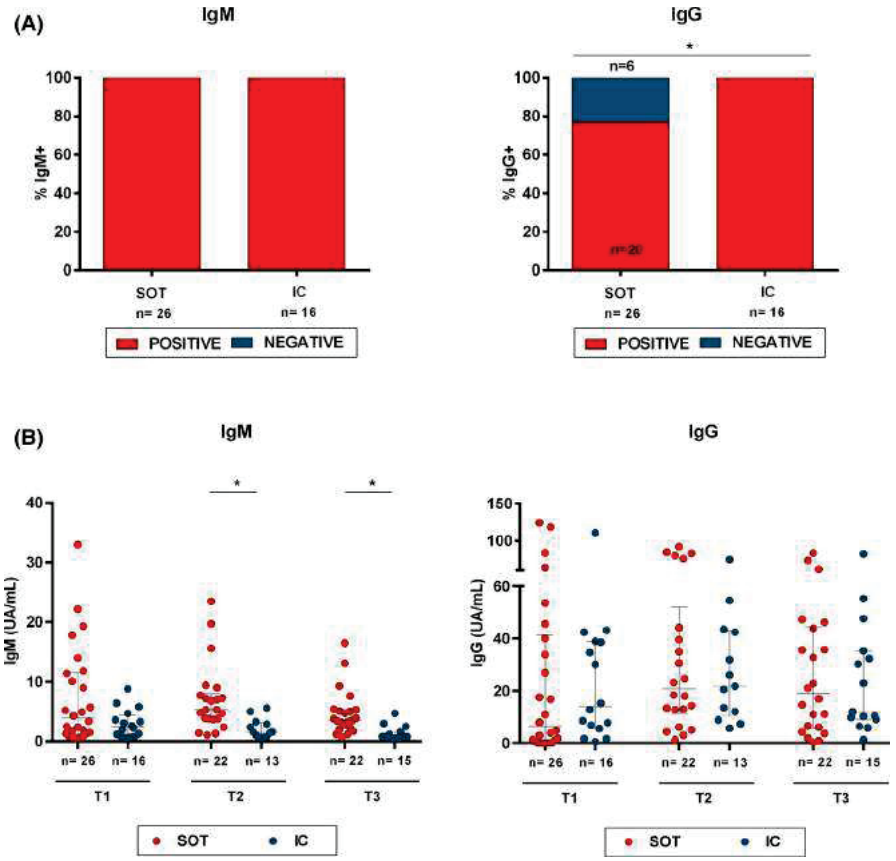

FIGURE 5 IgM and IgG antibody responses to SARS-CoV-2. (A) Percentage at T1 of SOT and IC patients with detectable SARS-CoV-2-specific IgM and IgG class-switching. *$p < .05$ (Chi-square test). (5B) IgM and IgG titers for every time point and study group (SOT and IC). *$p < .05$ (Mann-Whitney test analysis) [Color figure can be viewed at wileyonlinelibrary.com]

initial adaptive immune response than IC patients, most likely due to their chronic immunosuppressive therapy. Nonetheless, a rapid increase of such adaptive T cell immunity, similar to that of IC, is achieved by SOT during early COVID-19 convalescence.

Interestingly, a progressive emergence of both IL-5- and IL-21-producing T cells was detected during the convalescent period in both groups. Although we did not phenotypically characterize these immune cells due to the lack of viable cell samples, these data suggest the fact that for an optimal B-cell activation, cognate T cell help, most likely through antigen-specific follicular helper T cells, is needed.[21]

As similarly described in a recent published report,[28] we did not find any specific clinical, demographic, or immunological factors influencing worse clinical outcomes within the whole study group. Nonetheless, among the SOT group, significantly lower IL-2-producing T cell frequencies were observed in patients with the poorest clinical evolution. Conversely, the sole IC patient also needing MV support exhibited significantly more robust IL-2-specific T cell responses than SOT with the same severe outcome, a finding in line with a recent report [14] suggesting that patients with advanced age and higher comorbidity index showed higher IL-2 but decreasing portions of IFN-γ-secreting cells, in particular against antigen N. This different biological observation between SOT and IC may most likely rely in the chronic immunosuppressive effect of transplant immunotherapies, which abrogate IL-2 production on T cells.[29]

Importantly, SARS-CoV-2-reactive T cell responses and antibody titers progressively increased over time, during the convalescent period. Interestingly, this enhancement was more pronounced among SOT, who reached similar or even higher functional T cell and serological immune responses than IC patients. Interestingly, longer SARS-CoV-2 viral shedding has been reported among immunosuppressed patients,[30,31] which might account to some extent for a longer persistence of antigen stimulation ultimately leading to higher SARS-CoV-2-reactive T cell frequencies among SOT at later time points. This is of importance, since these data show that SOT patients may develop an optimal and sustained adaptive immune response, despite receiving chronic immunosuppressive therapy. Thus, vaccination against SARS-CoV-2 should be highly encouraged also among this prevalent high-risk population.[32]

In line with previous works,[33,34] non-specific T cell immune assessment did also reveal a severe global immune impairment of moderate/severe COVID-19, which was similarly depressed both in SOT and IC patients. Indeed, influenza and PWM-derived T cell responses were significantly abrogated at the acute phase of the infection, displaying a progressive restoration over time. In fact, influenza-specific memory T cell responses did not reach the same frequencies as those observed among healthy controls at the end of the follow-up, thus highlighting that recovery of adaptive immunity in some individuals was not fully achieved yet. These results underscore the difference between inflammation and adaptive immunity, which may raise concern about the hypothesis of potential therapeutic effects of some immunosuppressive agents, such as cyclosporine, aiming at reducing systemic inflammatory state in these patients.[35,36]

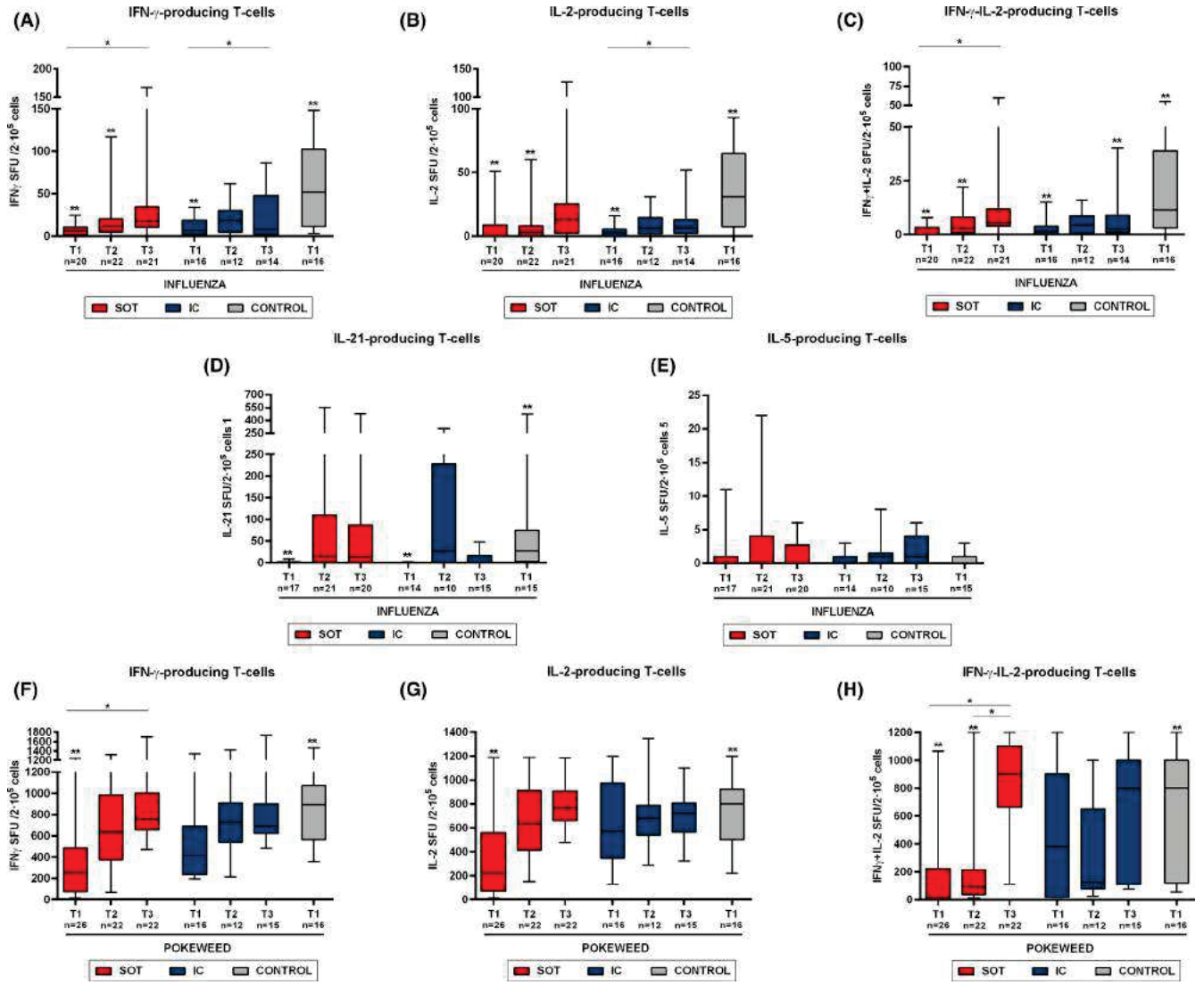

FIGURE 6 T cell responses against non-specific SARS-CoV-2 antigens (influenza and PWM) at the different time points of study. Percentile 5–95 represented by whiskers; median and IQR inside the boxes. Intragroup paired analysis; $^{*}p < .05$ evaluated with Friedman's test. Significant differences with healthy controls are shown by $^{**}p < .05$ (analyzed by Mann-Whitney U test). No differences were found between IC and SOT [Color figure can be viewed at wileyonlinelibrary.com]

Finally, we did not find SARS-CoV-2-reactive T cell responses against any of the four viral antigens in any HC thus, no evidence for T cell immune cross-reactivity was observed in out cohort, at least in vitro. Despite the presence of IL-6-producing T cell responses against SARS-CoV-2 in HC suggesting unspecific T cell stimulation, the assessment of SARS-CoV-2-reactive IL-6-producing T cell frequencies over time showed a similar pattern than that also observed in other T cell compartments.

There are some limitations in this study such as the small sample size evaluated, which was directly influenced by the difficulty in obtaining biological samples during acute COVID-19 infection. While our FluoroSpot assay allowed us to investigate in a functional manner the frequencies of different cytokine-producing T cells reactive to distinct SARS-CoV-2 antigens at single cell level, we could not describe the predominant T cell subset compartment, either CD4+ or CD8+ T cells, responsible of these SARS-CoV-2-reactive T cells. Although previous reports have shown a predominant role of SARS-CoV-2-reactive CD4+ T cells, CD8+ T cells do also account for a robust anti-viral T cell immunity.[11]

In summary, this study describes that despite the strong general immune impairment occurring in patients with severe acute COVID-19 infection, SARS-CoV-2 elicits robust adaptive immune responses also in SOT recipients, both at the cellular and humoral level, although with a certain functional immune delay as compared to IC individuals. Notably, the robust immune response against the virus during convalescence strongly supports the need of active immunization with the up-coming vaccines also in SOT patients.

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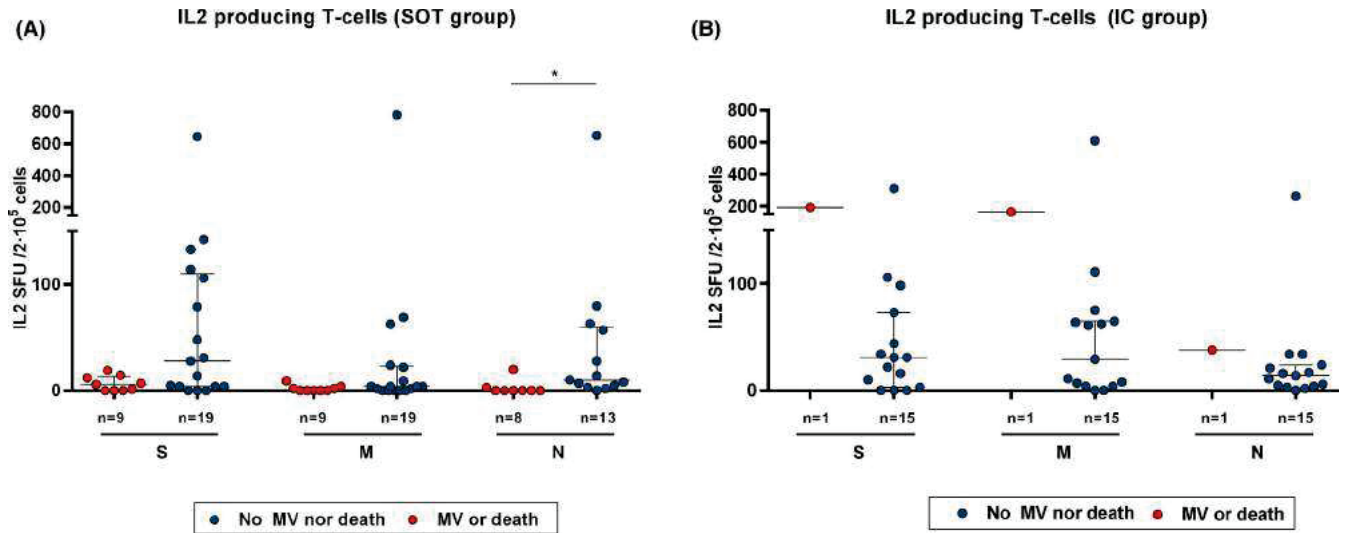

FIGURE 7 Baseline SARS-CoV-2-specific IL-2-producing T cell frequencies and clinical outcomes in SOT and IC patients with severe COVID-19 infection. IL-2-producing frequencies between patients with a poor outcome (VM or death) and those with a favorable clinical evolution. (A) SOT patients $^{**}p < .05$ (analyzed by Mann-Whitney U test). (B) IC patients. Only one IC patient required mechanical ventilation [Color figure can be viewed at wileyonlinelibrary.com]

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

AUTHOR CONTRIBUTIONS

A.F.: Designed the study, collected the data, performed experiments, analyzed the data, drafted the article, and revised the article critically. L.D.: Designed the study, collected the data, performed experiments, analyzed the data, drafted the article, and revised the article critically. I.T.: Performed experiments. N.S.: Collected the data and revised the article critically. V.P.: Collected the data and revised the article critically. J.C.: Revised the article critically. L.L.L.: Revised the article critically. M.M.: Collected the data and revised the article critically. J.J.L.: Performed statistical analyses, J.A.S.: Performed statistical analyses. E.R: Revised the article critically. A.C.: Revised the article critically. A.T.: Performed research and revised the article critically. R.U.: Collected the data and revised the article critically. E.C.: Designed the study, performed research, collected the data, analyzed the data and revised the article critically. E.M.: Revised the article critically. N.M.: Revised the article critically. A.M.: Revised the article critically. A.O.: Revised the article critically. J.M.C.: Revised the article critically. M.L.Q.: Revised the article critically. O.T.: Revised the article critically. O.B.: Conceived and designed the study, analyzed the data, drafted the article, and revised the article critically.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Guan W-J, Ni Z-Y, Hu YU, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382(18):1708-1720.
2. He XI, Lau EHY, Wu P, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nat Med*. 2020. https://doi.org/10.1038/s41591-020-0869-5
3. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the chinese center for disease control and prevention. *JAMA - J Am Med Assoc*. 2020;323(13):1239-1242.
4. Williamson EJ, Walker AJ, Bhaskaran K, et al. Factors associated with COVID-19-related death using OpenSAFELY. *Nature*. 2020;584(7821):430-436.
5. Hotez PJ, Corry DB, Strych U, Bottazzi ME. COVID-19 vaccines: neutralizing antibodies and the alum advantage. *Nat Rev Immunol*. 2020;20(7):399-400.
6. Robbiani DF, Gaebler C, Muecksch F, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature*. 2020;584(7821):437-442.

7. Seydoux E, Homad LJ, MacCamy AJ, et al. Analysis of a SARS-CoV-2-infected individual reveals development of potent neutralizing antibodies with limited somatic mutation. *Immunity*. 2020;53(1):98-105.e5.
8. Iyer AS, Jones FK, Nodoushani A, et al. Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. *Sci Immunol*. 2020;5(52):1-13.
9. Long Q-X, Tang X-J, Shi Q-L, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med*. 2020;26(8):1200-1204.
10. Chen X, Pan Z, Yue S, et al. Disease severity dictates SARS-CoV-2-specific neutralizing antibody responses in COVID-19. *Sig Transduct Target Ther*. 2020;5(1):180.
11. 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.
12. Zhang F, Gan R, Zhen Z, et al. Adaptive immune responses to SARS-CoV-2 infection in severe versus mild individuals. *Sig Transduct Target Ther*. 2020;5(1):156.
13. Sekine T, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. *Cell*. 2020;183(1):158-168.e14.
14. Sattler A, Angermair S, Stockmann H, et al. SARS-CoV-2 specific T-cell responses and correlations with COVID-19 patient predisposition. *J Clin Invest*. 2020;130(12):6477-6489. https://doi.org/10.1172/JCI140965. Published August 24, 2020.
15. Clinical management of COVID-19. WHO Information Web Site. https://www.who.int/publications/i/item/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected. Updated May 27, 2020. Accessed November 3, 2020.
16. Kolde R. Pheatmap: Pretty Heatmaps [Software]. 2019.
17. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;395(10229):1054-1062.
18. 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.
19. Mazzoni A, Salvati L, Maggi L, et al. Impaired immune cell cytotoxicity in severe COVID-19 is IL-6 dependent. *J Clin Invest*. 2020;130(9):4694-4703.
20. Le Bert N, Tan AT, Kunasegaran K, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature*. 2020;584(7821):457-462.
21. Ni L, Ye F, Cheng M-L, et al. Report detection of SARS-CoV-2-specific humoral and cellular immunity in COVID-19 convalescent individuals ll report detection of SARS-CoV-2-specific humoral and cellular immunity in COVID-19 convalescent individuals. *Immunity*. 2020;52(6):971-977.e3.
22. Poland GA, Ovsyannikova IG, Kennedy RB. Review SARS-CoV-2 immunity: review and applications to phase 3 vaccine candidates. *Lancet*. 2020;6736(20).
23. Babel N, Anft M, Blazquez-Navarro A, et al. Immune monitoring facilitates the clinical decision in multifocal COVID-19 of a pancreas-kidney transplant patient. *Am J Transplant*. 2020;20(11):3210-3215.
24. Candon S, et al. T cell and antibody responses to SARS-CoV-2: experience from a French transplantation and hemodialysis center during the COVID-19 pandemic. *Am J Transplant*. 2020;00:1-10.
25. Favà A, Cucchiari D, Montero N, et al. Clinical characteristics and risk factors for severe COVID-19 in hospitalized kidney transplant recipients: a multicentric cohort study. *Am J Transplant*. 2020;20(11).
26. Akalin E, Azzi Y, Bartash R, et al. Covid-19 and kidney transplantation. *N Engl J Med*. 2020;382:2475-2477.
27. Alberici F, Delbarba E, Manenti C, et al. A single center observational study of the clinical characteristics and short-term outcome of 20 kidney transplant patients admitted for SARS-CoV2 pneumonia. *Kidney Int*. 2020;97(6):1083-1088.
28. Thieme JC, Anft M, Paniskaki K, et al. Robust T cell response toward spike, membrane, and nucleocapsid SARS-CoV-2 proteins is nor associated with recovery in critical COVID-19 patients. *Cell Reports Medicine*. 2020;1(6):100092.
29. Halloran PF. Immunosuppressive drugs for kidney transplantation. *N Engl J Med*. 2004;351(26):2715-2729.
30. Caillard S, Benotmane I, Gautier Vargas G, Perrin P, Fafi-Kremer S. SARS-CoV-2 viral dynamics in immunocompromised patients. *Am J Transplant*. 2020;ajt.16353.
31. Aydillo T, Gonzalez-Reiche AS, Aslam S, et al. Shedding of viable SARS-CoV-2 after immunosuppressive therapy for cancer. *N Engl J Med*. 2020;383(26):2586-2588.
32. Peiris M, Leung GM. What can we expect from first-generation COVID-19 vaccines? *Lancet*. 2020.
33. Remy KE, Mazer M, Striker DA, et al. Severe immunosuppression and not a cytokine storm characterizes COVID-19 infections. *JCI Insight*. 2020;5(17):e140329.
34. 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.
35. Willicombe M, Thomas D, McAdoo S. COVID-19 and calcineurin inhibitors: should they get left out in the storm? *J Am Soc Nephrol*. 2020;31(6):1145-1146.
36. Schoot TS, Kerckhoffs APM, Hilbrands LB, van Marum RJ. Immunosuppressive drugs and COVID-19: a review. *Front Pharmacol*. 2020;11:1333.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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SUPPLEMENTARY MATERIAL

1 - Supplementary methods

1.1- Collection and management of Serum and PBMC samples

PBMCs were isolated from patient's peripheral blood by Ficoll density gradient centrifugation and subsequently frozen in liquid nitrogen until their use in functional analyses. Serum was isolated by centrifugation and samples were stored at -20ºC.

1.2 - Assessment of SARS-CoV-2-specific antibodies

Briefly, 10µL of serum were automatically diluted at 1:19 and incubated in the appropriate buffer with magnetic microbeads covered with anti-human IgM or recombinant 2019 nCov antigens, respectively, in order to form immune complexes. After precipitation in a magnetic field and washing, ABEI-stained recombinant 2019 nCov antigen or anti-human IgG were added to the samples, respectively. After a second magnetic separation and washing steps, the appropriate reagents were added to initiate a chemiluminescence reaction. At baseline (T1), 26 SOT and all 16 IC were assessed. At the second time point (T2), 22 SOT and 12 IC could be evaluated and at the last time point (T3), 22 SOT and 15 IC were assessed.

1.3- Assessment of polyfunctional SARS-CoV-2-specific T-cell responses

At baseline (T1), 26 SOT and all 16 IC were assessed. At the second time point (T2), 22 SOT and 12 IC were evaluated and at the last time point (T3), 22 SOT and 15 IC were assessed. 16 HC were evaluated for all the different stimuli.

Briefly, $2x10^5$ PBMCs (in 100 µl) were stimulated with the peptides for 24 hours for IFN-γ, IL-2, IFN-γ/IL-2, IL-6 and 48 hours for IL-5 and IL-21. After washing steps, the different cytokine fluorospots were detected using primary and secondary antibodies against each

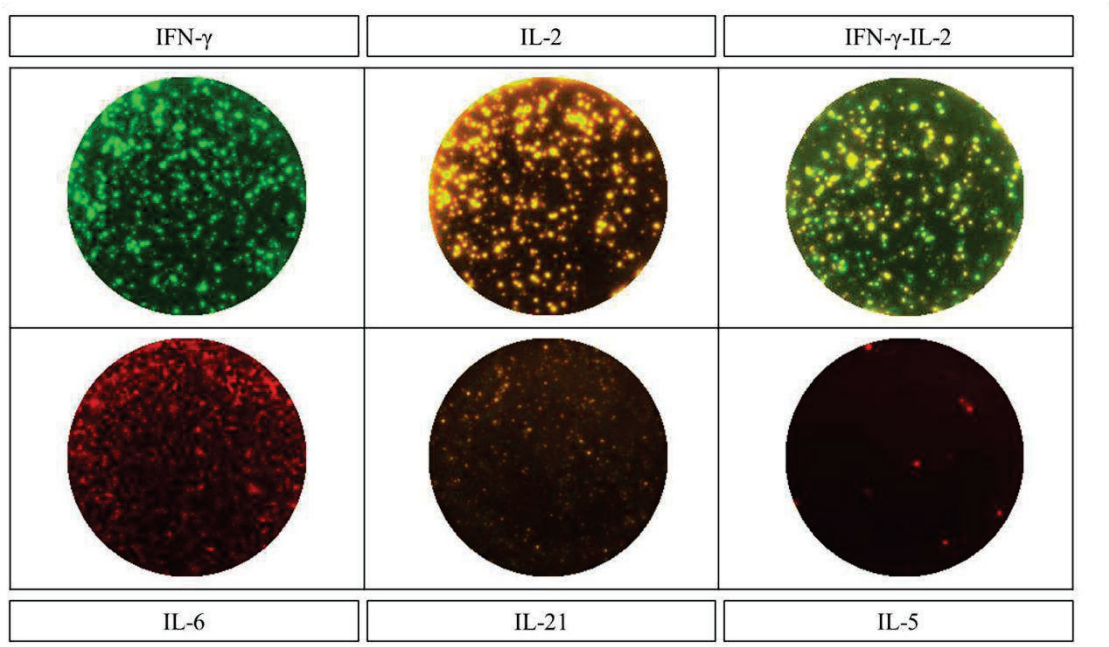
cytokine plus the addition of enhancer. The spots obtained were automatically counted with the Fluorspot Reader version 8 (AID® Gmbh, Strassberg, Germany). The results were considered after subtracting to each well the responses obtained in the respective negative control wells. **Supplemental figure 1** shows representative FluoroSpot wells for each cytokine-producing T cell against antigen S.

SARS-CoV-2 antigens

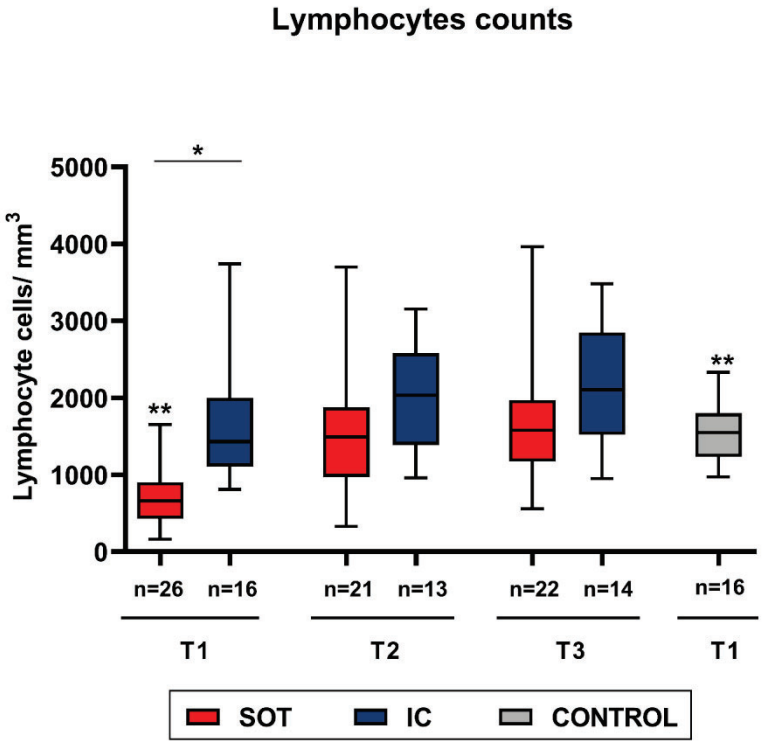
The Spike Glycoprotein (S) peptide (P0DTC2 protein, S gene) contained 158 + 157 peptides of >70% purity, the Membrane Protein (M) peptide (P0DTC5 protein, VME-1 gene) contained 16 peptides of >70% purity, the Nucleoprotein (N) peptide (P0DTC9 protein, NCAP gene) contained 102 peptides of >70% purity and the Envelope small membrane Protein (E) peptide (P0DTC4 protein, VEMP gene) contained 16 peptides of >70% purity. S, M, N and E peptides were reconstituted in DMSO and PBS and used at a final concentration of 2µg/mL. All SARS-CoV-2 peptides were designed by and purchased from JPT Innovative Peptide Solutions (JPT®, Berlin, Germany). Influenza-virus peptide pool was designed by and purchased from AID (AID® Gmbh, Strassberg, Germany).

2 - Supplementary Figures and Tables

Supplemental Figure 1. Representative images of polyfunctional T-cell responses to Spike SARS-CoV-2 structural protein.



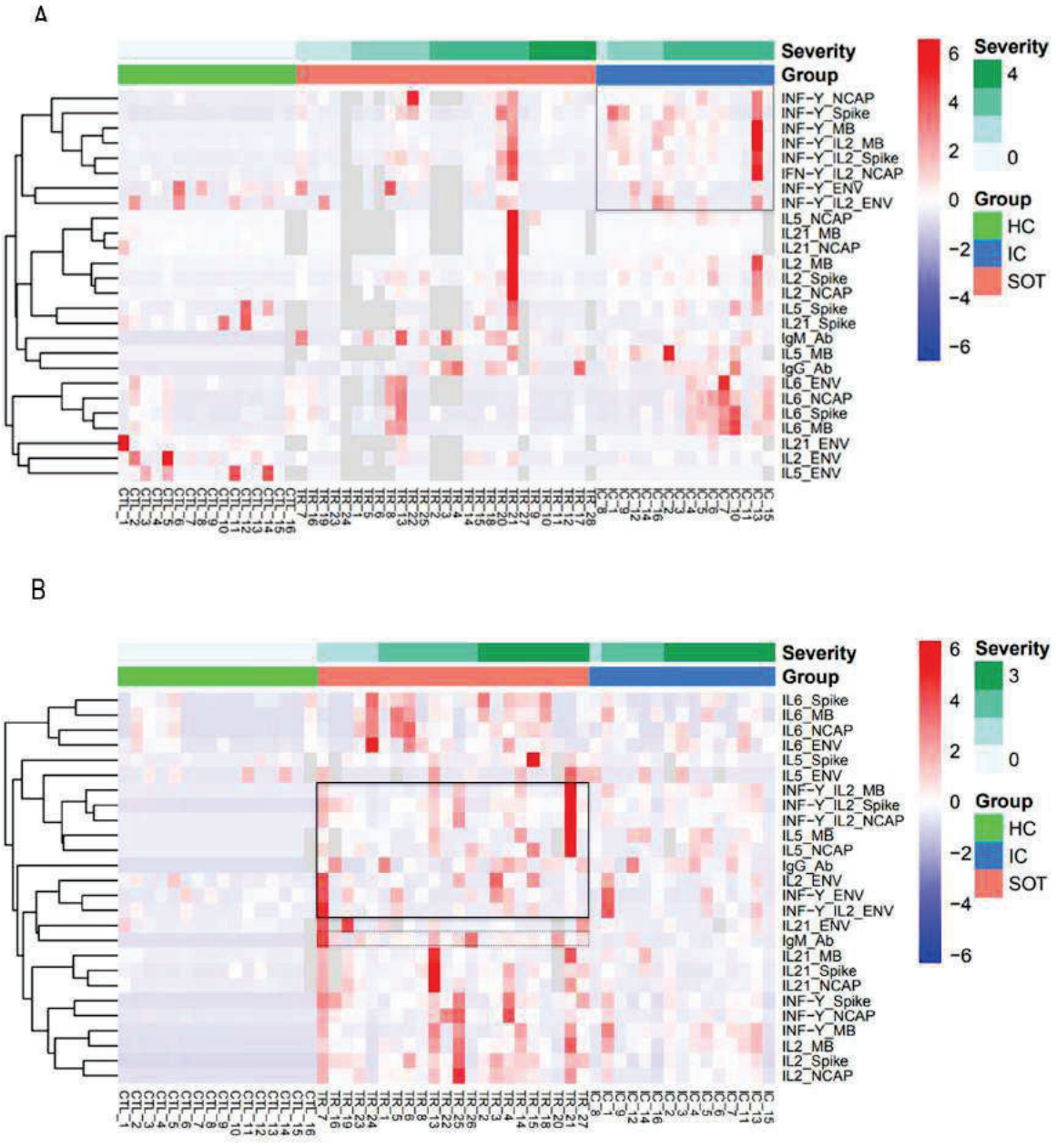
Supplemental Figure 2. Means for total lymphocyte counts between groups at the 3 different time points. Total lymphocyte counts in SOT and IC were 866 ± 427 and 1531 ± 490, 1594 ± 653 and 1975 ± 670, 1583 ± 760 and 2106 ± 729, at T1 ($p<0.001$), T2 ($p=0.076$) and T3 ($p=0.066$), respectively. Total lymphocyte counts in HC were 1564 ± 427 and were significantly higher than SOT (**$p<0.001$) but not for IC ($p=1$) at T1.



Supplemental Figure 3. Heatmaps generated by hierarchical clustering of SARS-CoV-2-specific immune responses for SOT, IC patients and HC, according to the COVID-19 disease severity (0 = no oxygen need; 1 = oxygen need; 2 = acute respiratory distress syndrome, 3 = death). Immune responses used for clustering were differentially expressed (fold change >2, false discovery rate P<0.05). Grey fields indicate missing values.

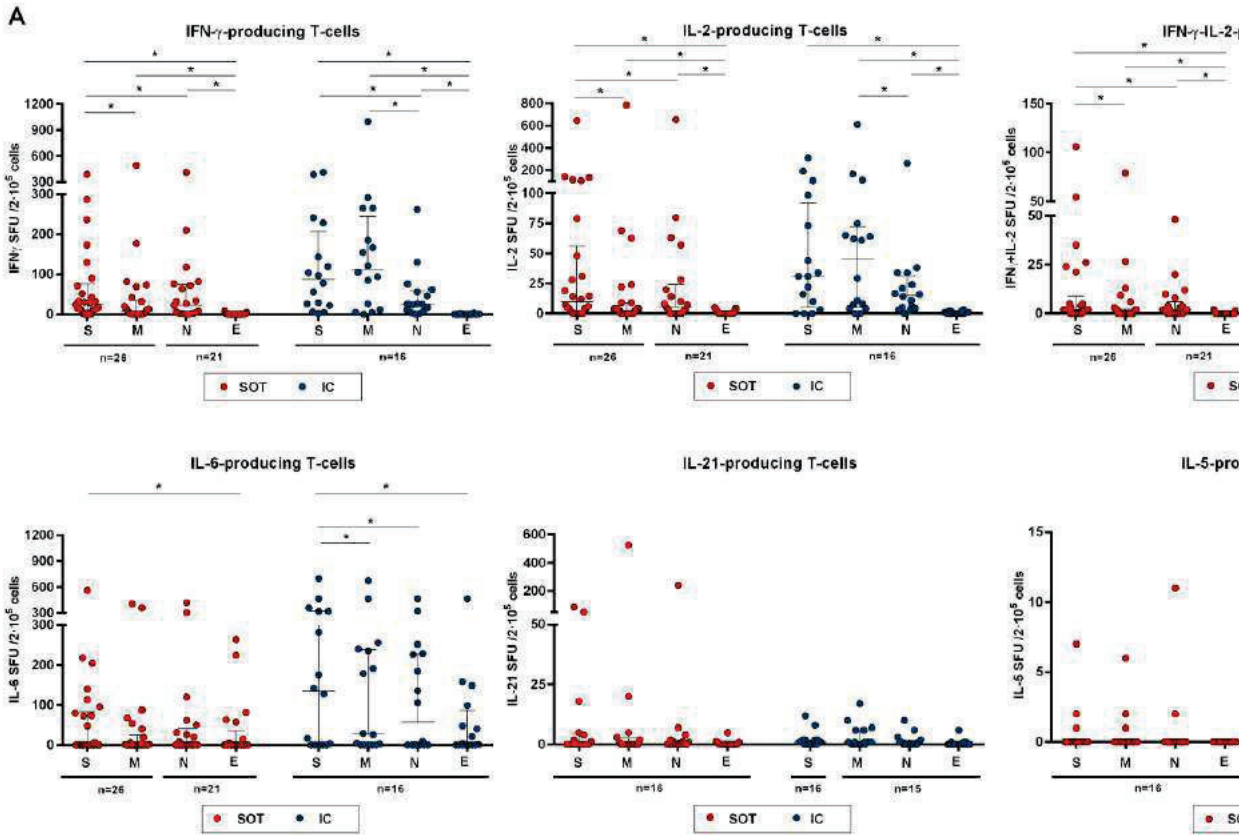
Figure 3A. Heatmap performed at first time point during acute COVID-19 infection (7; 4-11 days after the diagnosis) among 26 SOT, 16 IC and 16 HC**.**

Figure 3B. Heatmap performed during the early convalescent period (40; 37-44 days after the diagnosis) of COVID-19 disease in 22 SOT, 15 IC and 16 HC.

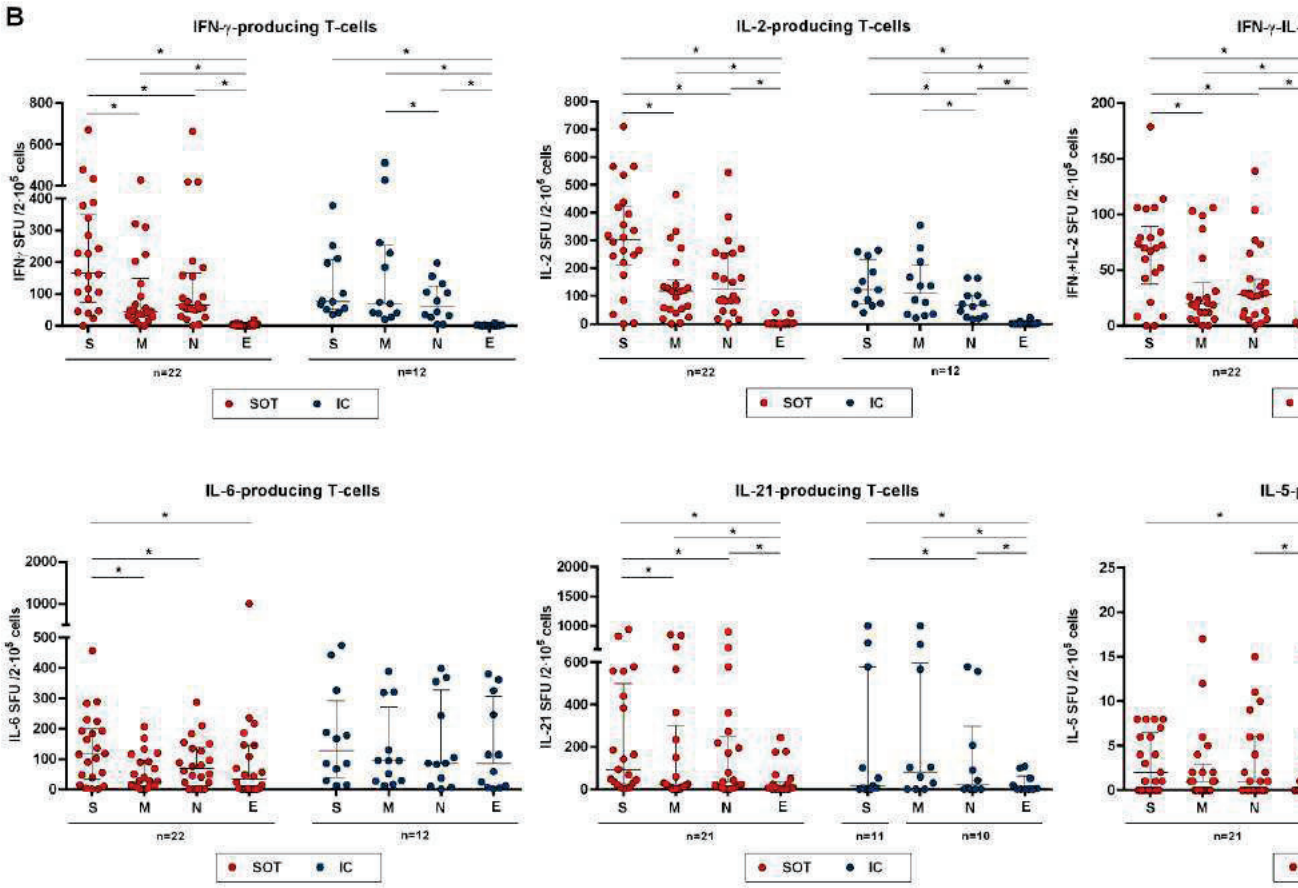


Supplemental Figure 4. Hierarchy of the polyfunctional T-cell responses for main structural SAR patients. *p<0.05, calculated with Friedman's test.

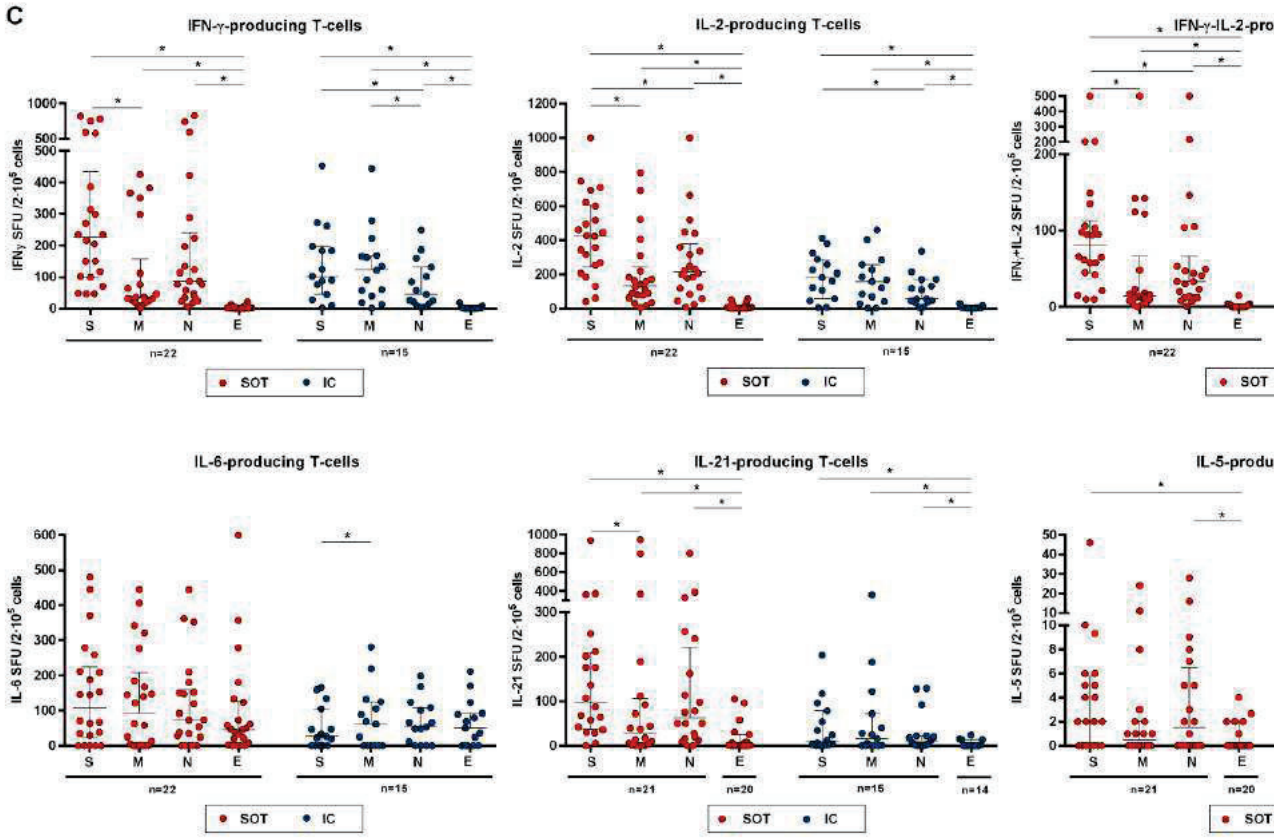
Supplemental figure 4A. T1=16; 12-19 days.



Supplemental figure 4B. T2=32; 25-37 days.



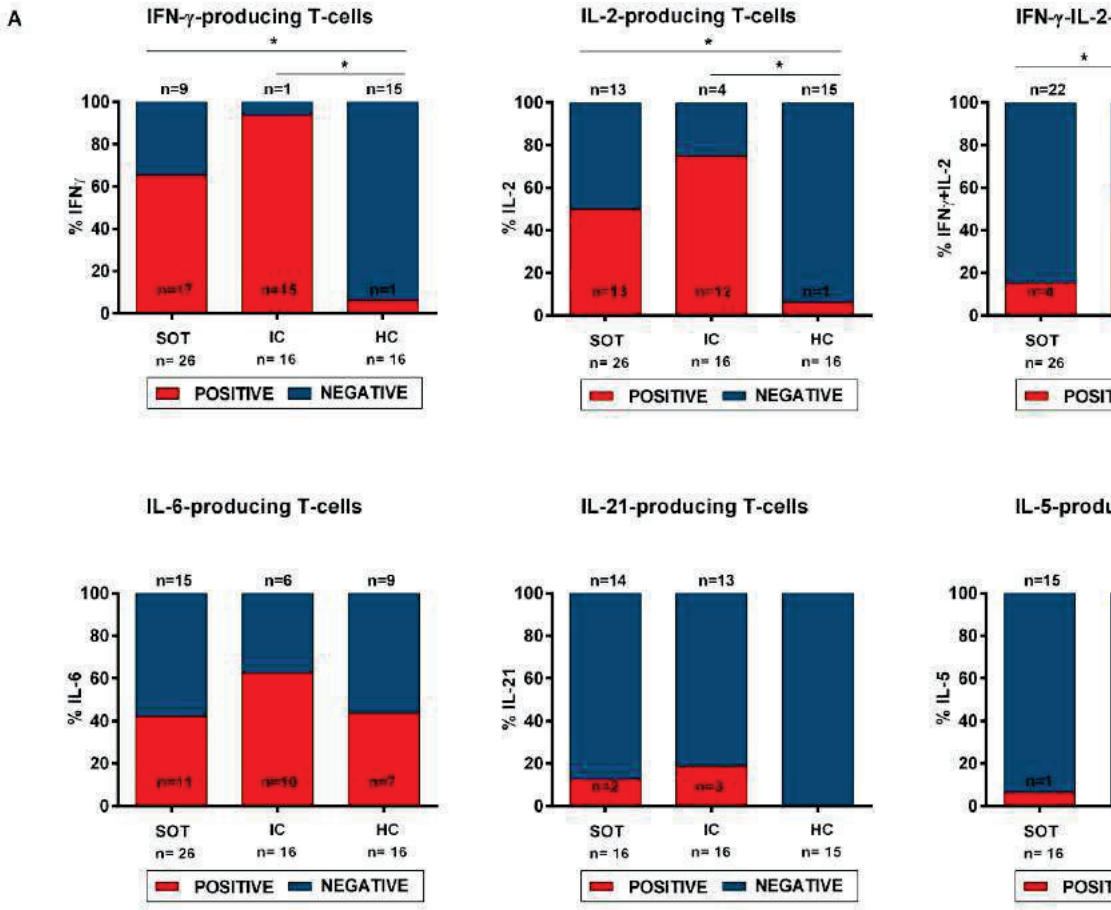
Supplemental figure 4C. T3=49; 43-53 days after symptoms onset.



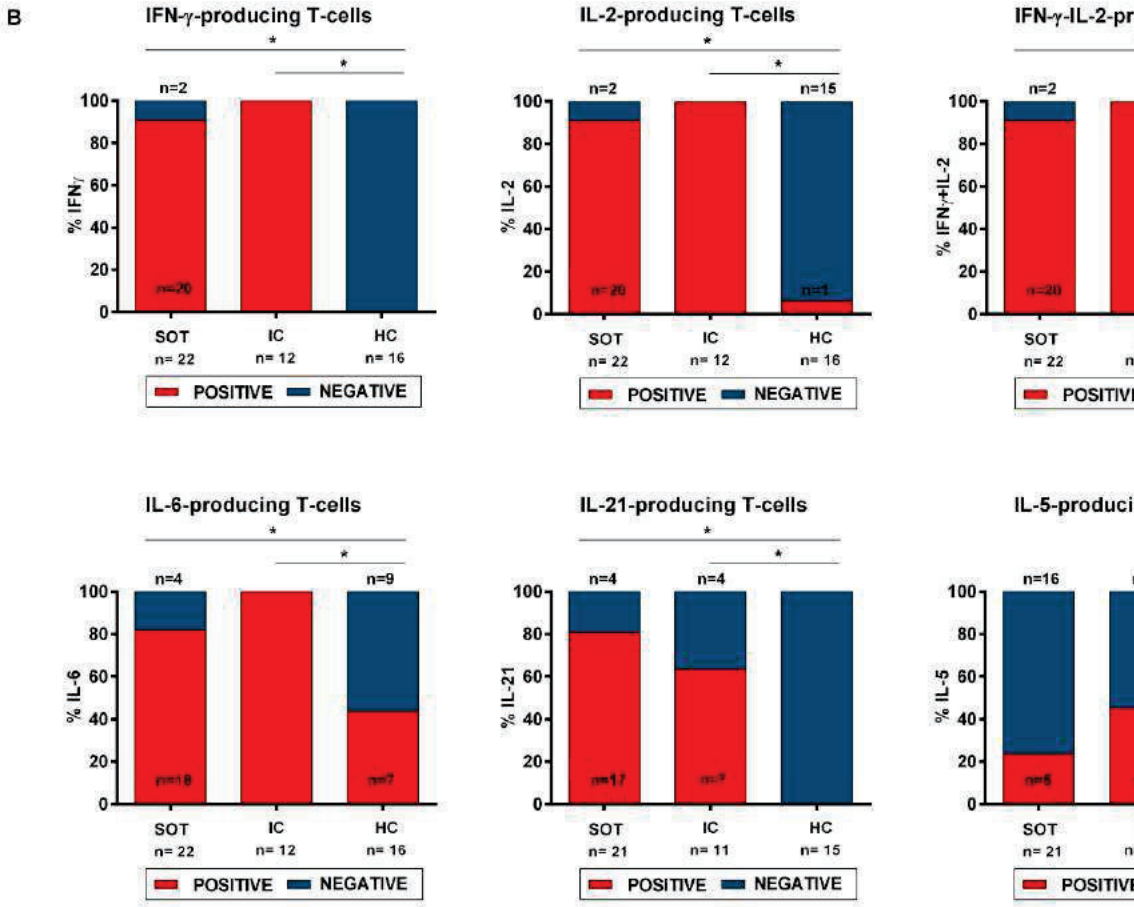
Supplemental Figure 5. Qualitative analysis of global T-cell reactivity to SARS-CoV-2 at different

T-cell reactivity defined in Material and Methods. *p<0.05 (Chi-Square Test).

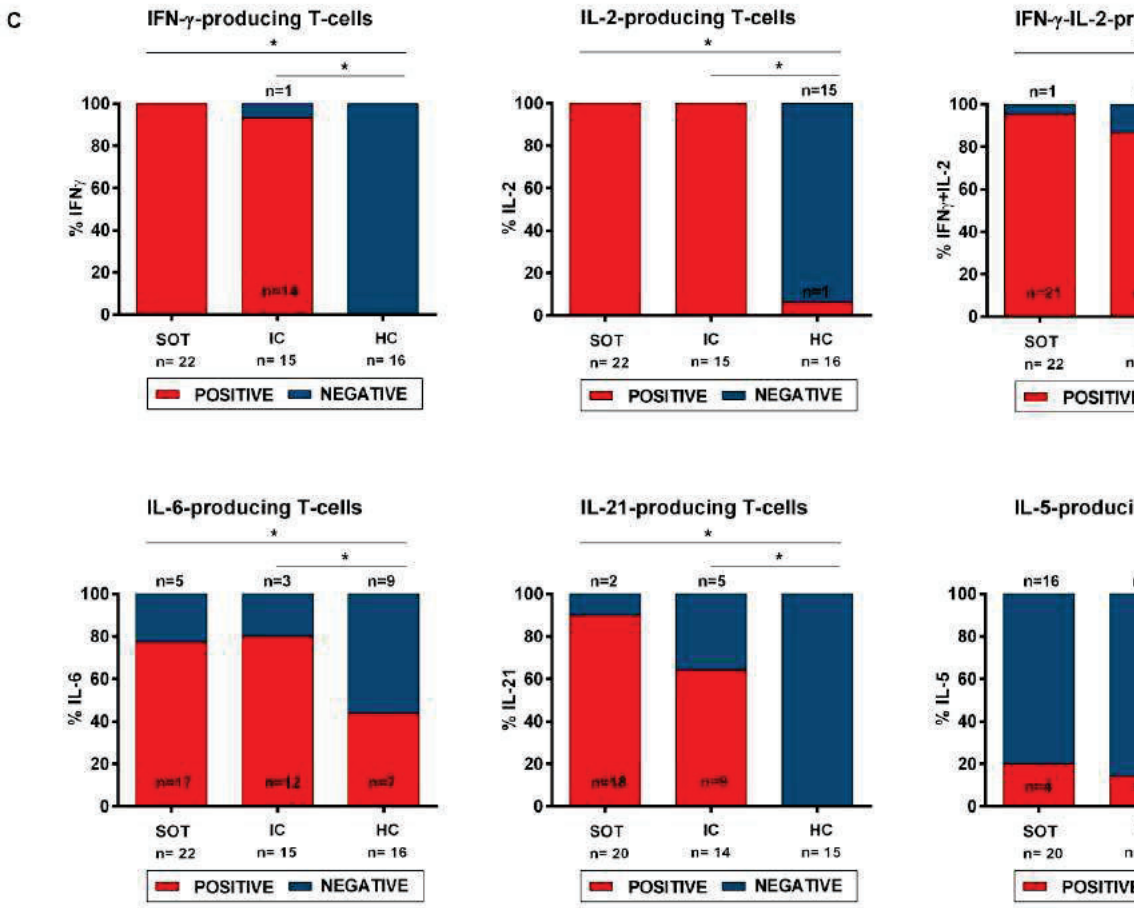
Supplemental figure 5A. T1=16; 12-19 days



Supplemental figure 5B. T2=32; 25-37 days.



Supplemental figure 5C. T3=49; 43-53 days after symptoms onset.



Supplemental Table 1. Correlations between SARS-CoV-2-specific T-cell responses and lymphocyte count

IFN-γ-producing T-cell frequencies					IL-2-producing T-cell frequencies				
	S	**M**	**N**	**E**	**S**	**M**	**N**	**E**	
Lymphocyte counts	R= 0.088 p= 0.363	R= 0.140 p= 0.148	R= 0.054 p= 0.585	R= 0.044 p= 0.660	R= 0.086 p= 0.375	R= 0.184 p= 0.056	R= 0.061 p= 0.539	R= 0.035 p= 0.721	R= p=
IL-6-producing T-cell frequencies					**IL-21-producing T-cell frequencies**				
	S	**M**	**N**	**E**	**S**	**M**	**N**	**E**	
Lymphocyte counts	R= 0.240 p= 0.012	R= 0.251 p= 0.008	R= 0.052 p= 0.603	R= 0.095 p= 0.335	R= 0.044 p= 0.671	R= 0.081 p= 0.438	R= 0.095 p= 0.365	R= 0.000 p= 0.997	R= p=

Supplemental Table 2. Correlations between SARS-CoV-2-specific T-cell responses at first time point of th

IFN-γ-producing T-cell frequencies					IL-2-producing T-cell frequencies				
	S	**M**	**N**	**E**	**S**	**M**	**N**	**E**	
S		R= 0.839 p <0.001	R= 0.584 p <0.001	R= 0.435 p=0.007		R=0.866 p <0.001	R= 0.882 p <0.001	R= 0.350 p= 0.034	
M			R= 0.583 p <0.001	R= 0.349 p= 0.034			R= 0.857 p <0.001	R= 0.394 p= 0.016	
N				R= 0.448 p= 0.005				R= 0.316 p= 0.056	
IL-6-producing T-cell frequencies					**IL-21-producing T-cell frequencies**				
	S	**M**	**N**	**E**	**S**	**M**	**N**	**E**	
S		R= 0.891 p <0.001	R= 0.858 p <0.001	R= 0.828 p <0.001		R= 0.453 p= 0.010	R= 0.490 p= 0.005	R= -0.001 p= 0.994	
M			R= 0.885 p <0.001	R= 0.790 p <0.001			R= 0.563 p= 0.001	R= 0.060 p= 0.747	
N				R= 0.861 p <0.001				R= -0.178 p= 0.339	

Supplemental Table 3. Statistical differences between patients of each group for all T-cell immune responses at T1.

T1	SOT median (IR)	IC median (IR)	HC median (IR)	SOT vs IC (p value)	SOT vs HC (p value)	IC vs HC (p value)
INF-ɣ Spike	26 (11-76)	88 (23-207)	3 (1-6)	0.286	**0.005**	**0.000**
INF-ɣ Membrane	7 (0-34)	113 (15-45)	0 (0-2)	**0.011**	**0.024**	**0.000**
INF-ɣ Nucleocapsid	23 (3-74)	26 (8-61)	1 (0-3)	1	**0.001**	**0.000**
INF-ɣ Envelope	0 (0-1)	0 (0-1)	1 (0-2)	0.473	0.473	0.473
IL-2 Spike	10 (1-56)	31 (5-92)	1 (0-4)	0.958	**0.015**	**0.002**
IL-2 Membrane	2 (0-9)	45 (5-74)	1 (0-4)	**0.009**	0.853	**0.001**
IL-2 Nucleocapsid	5 (0-24)	15 (4-31)	0 (0-3)	0.367	**0.032**	**0.000**
IL-2 Envelope	0 (0-1)	0 (0-1)	1 (0-4)	0.260	0.260	0.260
INF-ɣ-IL-2 Spike	2 (0-9)	8 (2-21)	1 (0-1)	0.388	0.135	**0.005**
INF-ɣ-IL-2 Membrane	0 (0-2)	13 (1-24)	0 (0-1)	**0.020**	0.791	**0.002**
INF-ɣ-IL-2 Nucleocapsid	2 (0-6)	4 (1-8)	0 (0-1)	0.832	0.072	**0.005**
INF-ɣ-IL-2 Envelope	0 (0-1)	0 (0-0)	0 (0-1)	0.471	0.471	0.471
IL-21 Spike	0 (0-5)	1 (0-2)	1 (0-8)	0.481	0.481	0.481
IL-21 Membrane	0 (0-3)	0 (0-6)	2 (0-8)	0.358	0.358	0.358
IL-21 Nucleocapsid	0 (0-2)	0 (0-2)	1 (0-7)	0.378	0.378	0.378
IL-21 Envelope	0 (0-1)	0 (0-1)	1 (0-2)	0.059	0.059	0.059
IL-5 Spike	0 (0-0)	0 (0-1)	0 (0-0)	0.205	0.205	0.205
IL-5 Membrane	0 (0-0)	0 (0-3)	0 (0-0)	0.176	1	**0.022**
IL-5 Nucleocapsid	0 (0-0)	0 (0-1)	0 (0-0)	0.120	0.120	0.120
IL-5 Envelope	0 (0-0)	0 (0-0)	0 (0-0)	0.121	0.121	0.121
IL-6 Spike	1 (0-84)	135 (0-319)	9 (0-59)	0.112	0.112	0.112
IL-6 Membrane	0 (0-0)	28 (0-239)	5 (0-83)	0.085	0.085	0.085
IL-6 Nucleocapsid	0 (0-41)	58 (0-228)	1 (0-68)	0.332	0.332	0.332
IL-6 Envelope	0 (0-37)	3 (0-86)	8 (0-55)	0.305	0.305	0.305

Supplemental Table 4. Correlations between SARS-CoV-2-specific T-cell responses at first time point of th

IFN-γ-producing T-cell frequencies					IL-2-producing T-cell frequencies				IFN-γ/I
	S	M	N	E	S	M	N	E	S
S		R= 0.581 p <0.001	R= 0.716 p <0.001	R= 0.556 p=0.001		R= 0.591 p <0.001	R= 0.799 p <0.001	R= 0.442 p= 0.009	
M			R= 0.495 p= 0.003	R= 0.162 p= 0.359			R= 0.777 p <0.001	R= 0.291 p= 0.094	
N				R= 0.440 p= 0.009				R= 0.471 p= 0.005	
IL-6-producing T-cell frequencies					IL-21-producing T-cell frequencies				IL-5
	S	M	N	E	S	M	N	E	S
S		R= 0.612 p <0.001	R= 0.645 p <0.001	R= 0.294 p= 0.091		R= 0.705 p <0.001	R= 0.893 p <0.001	R= 0.655 p <0.001	
M			R= 0.805 p <0.001	R= 0.480 p= 0.004			R= 0.788 p <0.001	R= 0.720 p <0.001	
N				R= 0.546 p= 0.001				R=0.707 p <0.001	

Supplemental Table 5. Correlations between SARS-CoV-2-specific T-cell responses at first time point of the

IFN-γ-producing T-cell frequencies					IL-2-producing T-cell frequencies				IFN-γ/IL
	S	M	N	E	S	M	N	E	S
S		R= 0.597 p <0.001	R= 0.782 p <0.001	R= 0.177 p=0.294		R= 0.690 p <0.001	R= 0.876 p <0.001	R= 0.399 p= 0.014	
M			R= 0.561 p <0.001	R= 0.210 p= 0.211			R= 0.752 p <0.001	R= 0.388 p= 0.017	
N				R= 0.220 p= 0.190				R= 0.506 P= 0.001	
IL-6-producing T-cell frequencies					**IL-21-producing T-cell frequencies**				**IL-5**
	S	M	N	E	S	M	N	E	S
S		R= 0.808 p <0.001	R= 0.580 p <0.001	R= 0.291 p= 0.080		R= 0.729 p <0.001	R= 0.712 p <0.001	R= 0.494 p= 0.004	
M			R= 0.710 p <0.001	R= 0.310 p= 0.062			R= 0.721 p <0.001	R= 0.586 p <0.001	
N				R= 0.480 p= 0.003				R= 0.616 p <0.001	

Supplemental Table 6. Statistical differences between patients of each group for all T-cell immune responses at T2.

T2	SOT median (IR)	IC median (IR)	HC median (IR)	SOT vs IC (p value)	SOT vs HC (p value)	IC vs HC (p value)
INF-ɣ Spike	165 (75-298)	77 (52-208)	3 (1-6)	1	**0.000**	**0.001**
INF-ɣ Membrane	45 (26-124)	71 (40-253)	0 (0-2)	0.776	**0.000**	**0.000**
INF-ɣ Nucleocapsid	65 (188-43)	61 (28-125)	1 (0-3)	1	**0.000**	**0.000**
INF-ɣ Envelope	2 (0-5)	1 (0-2)	1 (0-2)	0.239	0.239	0.239
IL-2 Spike	315 (209-449)	122 (71-231)	1 (0-4)	0.258	**0.000**	**0.004**
IL-2 Membrane	117 (50-162)	112 (35-210)	1 (0-4)	1	**0.000**	**0.000**
IL-2 Nucleocapsid	126 (73-260)	67 (24-102)	0 (0-3)	0.590	**0.000**	**0.001**
IL-2 Envelope	2 (0-4)	0 (0-3)	1 (0-4)	0.260	0.260	0.260
INF-ɣ-IL-2 Spike	71 (37-105)	40 (30-65)	1 (0-1)	1	**0.000**	**0.001**
INF-ɣ-IL-2 Membrane	20 (11-39)	43 (13-81)	0 (0-1)	1	**0.000**	**0.000**
INF-ɣ-IL-2 Nucleocapsid	28 (10-48)	17 (10-30)	0 (0-1)	1	**0.000**	**0.000**
INF-ɣ-IL-2 Envelope	1 (0-2)	0 (0-1)	0 (0-1)	0.233	0.233	0.233
IL-21 Spike	94 (27-412)	35 (0-612)	1 (0-8)	0.399	**0.000**	0.113
IL-21 Membrane	25 (7-192)	81 (0-595)	2 (0-8)	1	**0.047**	**0.039**
IL-21 Nucleocapsid	36 (8-207)	24 (0-300)	1 (0-7)	0.774	**0.005**	0.284
IL-21 Envelope	4 (0-25)	2 (0-64)	1 (0-2)	0.463	0.463	0.463
IL-5 Spike	1 (0-6)	3 (0-8)	0 (0-0)	1	**0.018**	**0.025**
IL-5 Membrane	1 (0-2)	6 (1-12)	0 (0-0)	0.119	**0.041**	**0.000**
IL-5 Nucleocapsid	1 (0-5)	1 (0-5)	0 (0-0)	1	**0.002**	**0.018**
IL-5 Envelope	0 (0-0)	0 (0-1)	0 (0-0)	0.267	0.267	0.267
IL-6 Spike	117 (45-200)	126 (38-291)	9 (0-59)	1	**0.009**	**0.012**
IL-6 Membrane	28 (8-97)	95 (28-271)	5 (0-83)	0.229	0.273	0.005
IL-6 Nucleocapsid	68 (22-138)	86 (17-327)	1 (0-68)	1	0.062	**0.016**
IL-6 Envelope	36 (0-145)	86 (8-305)	8 (0-55)	0.097	0.097	0.097

Supplemental Table 7. Statistical differences between patients of each group for all T-cell immune responses at T3.

T3	SOT median (IR)	IC median (IR)	HC median (IR)	SOT vs IC (p value)	SOT vs HC (p value)	IC vs HC (p value)
INF-ɣ Spike	225 (101-434)	102 (45-197)	3 (1-6)	0.383	**0.000**	**0.000**
INF-ɣ Membrane	34 (21-159)	122 (40-169)	0 (0-2)	1	**0.000**	**0.000**
INF-ɣ Nucleocapsid	87 (37-240)	45 (17-131)	1 (0-3)	0.633	**0.000**	**0.000**
INF-ɣ Envelope	1 (0-7)	0 (0-2)	1 (0-2)	0.541	0.541	0.541
IL-2 Spike	425 (242-606)	181 (58-289)	1 (0-4)	0.07	**0.000**	**0.002**
IL-2 Membrane	133 (74-241)	158 (41-257)	1 (0-4)	1	**0.000**	**0.000**
IL-2 Nucleocapsid	215 (110-379)	56 (38-171)	0 (0-3)	0.127	**0.000**	**0.001**
IL-2 Envelope	2 (0-10)	3 (0-5)	1 (0-2)	0.273	0.273	0.273
INF-ɣ-IL-2 Spike	80 (44-113)	57 (20-90)	1 (0-1)	0.482	**0.000**	**0.000**
INF-ɣ-IL-2 Membrane	15 (10-67)	46 (10-81)	0 (0-1)	1	**0.000**	**0.000**
INF-ɣ-IL-2 Nucleocapsid	33 (12-66)	13 (9-48)	0 (0-1)	0.931	**0.000**	**0.000**
INF-ɣ-IL-2 Envelope	0 (0-3)	0 (0-2)	0 (0-1)	0.830	0.830	0.830
IL-21 Spike	107 (36-212)	10 (2-83)	2 (0-8)	**0.025**	**0.000**	0.294
IL-21 Membrane	37 (1-112)	11 (0-85)	2 (0-8)	1	**0.034**	0.368
IL-21 Nucleocapsid	75 (16-241)	13 (1-39)	1 (0-7)	0.084	**0.000**	0.189
IL-21 Envelope	1 (0-25)	0 (0-14)	1 (0-2)	0.424	0.424	0.424
IL-5 Spike	2 (0-6)	0 (0-4)	0 (0-0)	0.178	**0.001**	**0.342**
IL-5 Membrane	1 (0-2)	2 (0-7)	0 (0-0)	1	**0.015**	**0.004**
IL-5 Nucleocapsid	2 (0-7)	0 (0-5)	0 (0-0)	1	**0.016**	0.232
IL-5 Envelope	0 (0-2)	0 (0-1)	0 (0-0)	0.065	0.065	0.065
IL-6 Spike	108 (22-224)	27 (0-105)	1 (0-83)	0.181	**0.016**	1
IL-6 Membrane	92 (0-207)	61 (0-125)	1 (0-69)	0.079	0.079	0.079

IL-6 Nucleocapsid	74 (18-159)	54 (0-108)	8 (0-56)	0.079	0.079	0.079
IL-6 Envelope	46 (7-126)	50 (0-92)	2 (0-68)	0.191	0.191	0.191

Supplemental Table 8. Clinical characteristics of COVID-19 patients with severe outcomes

	MV or death	No MV nor death	P-Value
All patients	**(N=10)**	**(N=34)**	
Age (years, mean±SD)	65.5 ±10.9	57.6 ± 12.7	0.102
Sex: Male/Female (n, %)	7/3 (70/30)	23/11 (67.6/32.4)	0.606
Comorbidities (n, %)			
· Diabetes	5 (50%)	7 (20.6%)	0.105
· Arterial Hypertension	7 (70%)	18 (52.9%)	0.279
· Obesity	2 (20%)	7 (20.6%)	0.909
· Pulmonary disease	1 (10%)	3 (8.8%)	0.317
· Heart Disease	2 (20%)	6 (17.6%)	0.594
Laboratory findings (n, %)			
· CRP (mg/L, mean ± SD)	103.7±70.5	91.6±75.5	0.561
· LDH (IU/ml, mean ± SD)	298.9±92.8	281.7±102.8	0.499
· Lymphocytes (cells/ mm^3, mean ± SD)	719±439	839±564	0.738
SOT	**(N=9)**	**(N=19)**	
Age (years, mean±SD)	65.6±11.5	56.5±13.8	0.101
Sex: Male/Female (n, %)	6/3 (66.7/33.3)	13/4 (76.5/23.5)	0.398
Comorbidities (n, %)			
· Diabetes	5 (55.5%)	6 (35.3%)	0.212
· Arterial Hypertension	7 (77.8%)	12 (70.6%)	0.374
· Obesity	2 (22.2%)	4 (23.5%)	0.844
· Pulmonary disease	1 (11.1%)	1 (5.9%)	0.548
· Heart Disease	2 (22.2%)	4 (23.5%)	0.650
Laboratory findings (n, %)			
· CRP (mg/L, mean ± SD)	119.2±130.1	87±77.3	0.562
· LDH (IU/ml, mean ± SD)	298.9±92.8	277.3±121	0.428
· Lymphocytes (cells/ mm^3, mean ± SD)	701±463	517±321	0.339

Abbreviations: MV, mechanical ventilation (invasive or non-invasive); CRP, C-reactive protein; LDH, lactate dehydrogenase; SOT, solid organ transplant.

Supplemental Table 9. Baseline immunosuppression regimes and modifications during COVID-19 infection

	Baseline Immunosupression	CNI withdrawal during COVID19	MMF withdrawal during COVID19	
CNI based = 19 (67.9%)	• TAC + MMF + ST = 14 (50%) • TAC + iMtor + ST = 1 (3.6%) • CsA + MMF + ST = 2 (7.1%) • TAC + ST = 1 (3.6%) • CsA + MMF = 1 (3.6%)	• 7/19 = 36.9%	• 16/17 = 94.1%	•
CNI free = 9 (32,1%)	• MMF + iMtor + ST = 2 (7.1%) • MMF + iMtor = 2 (7.1%) • MMF + ST = 2 (7.1%) • MMF = 3 (10.7%)		• 6/9 = 66.7%	•

Abbreviations: MV, mechanical ventilation (invasive or non-invasive); SOT, solid organ transplant; MMF, mycophenol
ST, steroids.

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IV. MATHERIALS, METHODS AND RESULTS

Article 3.

A comprehensive assessment of long-term SARS-CoV-2-specific adaptive immune memory in convalescent COVID-19 Solid Organ Transplant recipients

Kidney Int. 2022 May;101(5):1027-1038. doi: 10.1016/j.kint.2021.12.029

Objectives:

To investigate the relative persistence of peripheral adaptive immune memory specific to SARS-CoV-2 up to six months after COVID19 by evaluating both serological and T and B cell memory/effector immune compartments in SOT recipients, compared to a matched cohort of immunocompetent convalescents patients

To comprehensively characterize the strength and durability of SARS-CoV-2-specific adaptive immunity across distinct clinical presentations of COVID-19, from severe to asymptomatic infections, in convalescent SOT and immunocompetent patients.

A comprehensive assessment of long-term SARS-CoV-2–specific adaptive immune memory in convalescent COVID-19 Solid Organ Transplant recipients

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Long-term adaptive immune memory has been reported among immunocompetent individuals up to eight months following SARS-CoV-2 infection. However, limited data is available in convalescent patients with a solid organ transplant. To investigate this, we performed a thorough evaluation of adaptive immune memory at different compartments (serological, memory B cells and cytokine [IFN-γ, IL-2, IFN-γ/IL12 and IL-21] producing T cells) specific to SARS-CoV-2 by ELISA and FluoroSpot-based assays in 102 convalescent patients (53 with a solid organ transplants (38 kidney, 5 liver, 5 lung and 5 heart transplant) and 49 immunocompetent controls) with different clinical COVID-19 severity (severe, mild and asymptomatic) beyond six months after infection. While similar detectable memory responses at different immune compartments were detected between those with a solid organ transplant and immunocompetent individuals, these responses were predominantly driven by distinct COVID-19 clinical severities (97.6%, 80.5% and 42.1%, all significantly different, were seropositive; 84% vs 75% vs 35.7%, all significantly different, showed IgG-producing memory B cells and 82.5%, 86.9% and 31.6%, displayed IFN-γ producing T cells; in severe, mild and asymptomatic convalescent patients, respectively). Notably, patients with a solid organ transplant with longer time after transplantation did more likely show detectable long-lasting immune memory, regardless of COVID-19 severity. Thus, our study shows that patients with a solid organ transplant are capable of maintaining long-lasting peripheral immune memory after COVID-19 infection; mainly determined by the degree of infection severity.

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KEYWORDS: adaptive immunity; COVID-19 infection; solid organ transplantation

The coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has challenged global health in an unprecedented manner, resulting in a widespread morbidity and mortality. Even though most people develop mild symptoms or remain asymptomatic after SARS-CoV-2 infection,[1,2] some patients develop a severe respiratory syndrome that associates with an excessive systemic inflammatory process, ultimately leading to respiratory failure and death.[3,4] Notably, some specific group of patients seem to be at significantly higher risk of fatal outcomes such as recipients of solid organ transplants (SOT), most likely due to their chronic immunosuppressive therapy that broadly targets adaptive T-cell immunity.[5,6]

Recent important studies have shown that during acute COVID-19 and early convalescence, infected patients develop robust adaptive immune responses by means of high SARS-CoV-2–specific IgG antibody titers and T-cell frequencies, both CD4 and CD8 T cells, in peripheral blood.[7] Remarkably, the strength of these adaptive immune responses seems to also vary according to distinct COVID-19 disease severity,[8-12] thus, suggesting a key role of SARS-CoV-2–specific immunity controlling and limiting primary viral replication.[13-16] On the other hand, a long-lasting protective immunity, both serological and cellular, has also been reported among convalescent COVID-19 patients from the general population between 5 and 8 months after infection.[17]

In the setting of SOT, however, scarce information has been reported regarding the degree, durability, and biological interplay between different adaptive immune mechanisms in response to SARS-CoV-2. In this regard, our group recently showed that SOT patients developing a moderate or severe COVID-19 infection are able to generate, albeit with a notable initial delay, similarly strong SARS-CoV-2–specific serological and T-cell immune responses during early convalescence as compared with immunocompetent (IC) patients with the similar severe infection.[18,19] Nevertheless, unlike SARS-CoV-2 convalescent immunity, weak adaptive immune responses have been reported in SOT recipients after 2 doses of messenger RNA-based vaccination.[20,21] Importantly, understanding whether memory immune responses specific to SARS-CoV-2 last for long convalescent periods and how the serological and B and T cellular compartments behave over time is key to establish guided preventing strategies among this high-risk patient population.

Herein, we performed a thorough evaluation of both serological and functional T- and B-cell immune memory against main immunogenic SARS-CoV-2 antigens using functional cell-based immune assays, in a cross-sectional cohort of 102 convalescent SOT (n = 53) and IC healthy individuals (n = 49) with distinct disease severity, beyond 6 months after COVID-19 infection.

METHODS

Patients of the study and clinical definitions

One-hundred and two COVID-19 convalescent patients from different European transplant centers were evaluated in this study (Bellvitge University Hospital [N = 67]; Vall d'Hebron University Hospital [N = 13]; Montpellier University Hospital [N = 4]; Fundació Puig-Vert [N = 3]; Lyon University Hospital [N = 2]; Hospital del Mar [N = 2]; Hospital Clinic [N = 1]) and a general medical assistance center (N = 10). A total of 53 SOT recipients (38 kidney, 5 liver, 5 lung, and 5 heart transplants) and 49 IC healthy individuals, in whom peripheral blood mononuclear cells and serum samples could be obtained, with a median follow-up after COVID-19 infection beyond 6 months (199 days; interquartile range [IQR], 170-215), were included in this study. In addition, 35 subjects (21 SOT and 14 IC) having developed a severe COVID-19 were compared with their 1-month postinfection immune memory status. None of the participants had been vaccinated, before or during the study follow-up.

All patients had been tested positive for SARS-CoV-2 infection by a real-time reverse transcription–polymerase chain reaction analysis on nasopharyngeal swab samples and diagnosed for COVID-19 between March and October 2020. Samples from 16 prepandemic uninfected individuals were used as negative controls for T-cell assays, as described elsewhere.[18] Additional 10 historic biological samples were employed as controls for the B-cell functional assays.

As shown in the flowchart of the study (Figure 1), both SOT recipients and IC patients included in the study were classified according to 3 distinct COVID-19 clinical presentations: 41 had been hospitalized for a severe COVID-19 (SEV) requiring oxygen supply (22 SOT and 19 IC), 42 presented with mild symptoms (MILD) and were not hospitalized (22 SOT, 20 IC), and 19 were asymptomatic (ASYMP) and found positive for SARS-CoV-2 by a real-time reverse transcription–polymerase chain reaction on nasopharyngeal swab in routine screening or contact tracking tests (9 SOT and 10 IC). Main clinical, demographic, and immunologic patient characteristics were recorded.

The study was approved by the ethical review boards (PR115/20) at each center, and patients were recruited in the study after providing a signed informed consent.

Collection and management of serum and peripheral blood mononuclear cell samples

Detailed description is depicted in the Supplementary Methods.

Assessment of SARS-CoV-2–specific humoral immunity

SARS-CoV-2–specific serological memory. Serum IgG antibodies were assessed against 2 main SARS-CoV-2 antigens: the nucleoprotein and spike glycoprotein in 101 of 102 (99%) study patients using 2 distinct enzyme-linked immunosorbent assay platforms. Detailed information of the methodology and interpretation is provided as Supplementary Material.

SARS-CoV-2–specific IgG-producing memory B cells. Circulating SARS-CoV-2–specific IgG-producing memory B-cell (mBC) frequencies was assessed against the receptor binding domain of SARS-CoV-2 spike protein in 71 of 102 (69.6%) individuals of the study using a colorimetric B-cell enzyme-linked immunosorbent spot assay. A thorough description of the method and interpretation (Supplementary Figure S1) of this assay is depicted in the Supplementary Material.

SARS-CoV-2–reactive cytokine-producing memory T cells. Circulating SARS-CoV-2–reactive cytokine-producing memory T-cell frequencies could be assessed in 97 of 101 (95.1%) patients of the study using a multicolor FluoroSpot Immune assay (AID Gmbh), in which 4 distinct cytokine-producing T-cell frequencies were

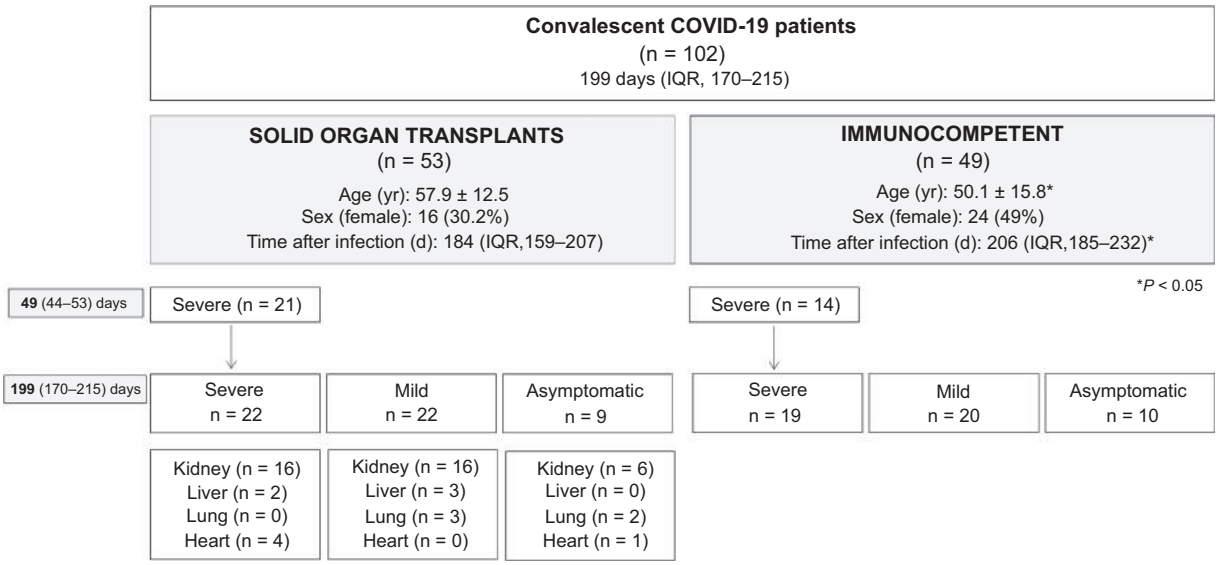

Figure 1 | Flowchart of the study. *$P < 0.05$ (χ^2 test and *t* test). COVID-19, coronavirus disease 2019; IQR, interquartile range.

simultaneously assessed: effector (interferon-γ [IFN-γ]), proliferative (interleukin-2 [IL-2]), central (IFN-γ/IL-2) T helper cell 1 and IL-21 T helper cell 1 memory responses. These responses were evaluated against the 4 main structural SARS-CoV-2 proteins: spike glycoprotein (S), membrane protein (M), nucleoprotein (N), and envelope small membrane protein (E) (JPT). A strong positive correlation of T-cell immune responses between all viral antigens was observed but for antigen E, which were barely detectable (Supplementary Table S1). Thus, all the analyses were focused against antigens S, M, and N. Global SARS-CoV-2–reactive T-cell immune responses were calculated by means of the median T-cell frequencies against the 3 main immunogenic antigens (S, M, and N) in each patient. Furthermore, because a hierarchical T-cell immune response was observed and was predominantly driven by IFN-γ-producing T cells against antigen S (Supplementary Figure S2), the qualitative assessment of T-cell immune memory was based on this response. A detailed description of the methodology and interpretation (Supplementary Figure S3) is provided as Supplementary Material.

Statistics

Continuous variables were expressed as mean ± SD or median and IQR, and categorical variables as number of total (n) and percentage (%). A comparison between groups was performed using Pearson's χ^2 test for categorical data. Continuous measurements were compared among groups using the Kruskal-Wallis and Mann-Whitney *U* test for non-normally distributed data, whereas analysis of variance and *t* tests were used when data were normally distributed. *P* values of <0.05 were considered statistically significant. Univariate logistic regression models were used to investigate the influence of clinical covariates (age, gender, symptomatic/asymptomatic infection, and years after transplant) by means of odds ratio (OR) with 95% confidence interval (CI) for humoral and cellular responses. Those covariates that were associated with a *P* value of <0.1 were introduced into a multivariate binary logistic regression model. SARS-CoV-2–reactive cellular and humoral responses were centered and scaled, and a heatmap was built by means of the pheatmap R package 16 using Euclidean distance and complete method as agglomeration method. R package version 1.0.12 was used (https://CRAN.R-project.org/package=pheatmap). All other analyses were performed using SPSS version 26 software, and graphs were generated using GraphPad Prism version 8.0 software (Graphpad Software).

RESULTS

Patients of the study

As shown in the study flowchart (Figure 1) and Table 1, 102 COVID-19 convalescent patients after a median time of 199 days (IQR, 170–215 days) after infection were investigated (53 SOT and 49 IC). SOT had a median time after transplantation of 5 years (IQR, 1–12 years), and most of them were receiving a calcineurin-inhibitor-based immunosuppression (83%). All patients were classified and matched according to the clinical severity of COVID-19 infection: 41 SEV (22 SOT, 19 IC), 42 MILD (22 SOT, 20 IC), and 19 ASYM (9 SOT, 10 IC).

In general, IC patients were slightly younger (50.1 ± 15.8 vs. 57.9 ± 12.5 mean age, $P = 0.017$) and their convalescence period was longer (206 [185–232] vs. 184 [159–207] median days, $P = 0.005$) than SOT (Figure 1), and these differences were mainly driven by the MILD IC group, which was composed of health care workers (Table 1). Among the remaining groups, IC and SOT were matched for age and time after infection. There were no differences regarding the type of immunosuppression, SOT, or time after transplantation between the 3 distinct clinical groups.

None of the included patients was diagnosed of transplant rejection during the acute SARS-CoV-2 infection or the follow-up, but 1 kidney transplant individual who presented a subclinical antibody-mediated rejection in a 12-month protocol biopsy.

Disease severity but not patient condition drives long-lasting immune memory

As illustrated in an unsupervised heatmap in Figure 2, SARS-CoV-2–specific immune memory responses, both at the serological and functional B- and T-cell compartments, were

Table 1 | Demographic and clinical characteristics of patients infected by SARS-CoV-2

	Severe (n = 41)		Mild (n = 42)		Asymptomatic (n = 19)		
COVID-19 patients (n = 102)	SOT (n = 22)	IC (n = 19)	SOT (n = 22)	IC (n = 20)	SOT (n = 9)	IC (n = 10)	*P* value
Age, yr, mean ± SD	56.7 ± 13.7	60.4 ± 9.2	60.6 ± 9.6	35.2 ± 10.6[a]	54.3 ± 15.3	60.5 ± 8.9	<0.001
Sex (female), n (%)	4 (18.2)	7 (36.8)	8 (40)	11 (55)	4 (44.4)	6 (60)	0.145
Time after infection, d, median (IQR)	196 (181–213)	201 (185–206)	177 (132–203)	231[a] (213–252)	161 (121–168)	163 (139–185)	<0.001
Transplant organ, n (%)							
Kidney	16 (72.7)	NA	16 (80)	NA	6 (66.7)	NA	
Liver	2 (10)	NA	3 (15)	NA	0	NA	0.161
Heart	0 (0)	NA	3 (15)	NA	2 (22.2)	NA	
Lung	4 (18.2)	NA	0	NA	1 (11.1)	NA	
Type of immunosuppression							
Calcineurin inhibitors	16 (72.7)	NA	20 (100)	NA	8 (88.9)	NA	0.241
Mycophenolate mofetil	21 (94.5)	NA	19 (95)	NA	6 (66.7)	NA	0.099
mTor inhibitors	4 (18.2)	NA	2 (10)	NA	2 (22.2)	NA	0.566
Steroids	18 (81.8)	NA	17 (85)	NA	9 (100)	NA	0.304
Time after transplant, yr, mean ± SD	8.05 ± 7.45	NA	5.82 ± 6.79	NA	7.56 ± 7.09	NA	0.571

COVID-19, coronavirus disease 2019; IC, immunocompetent; IQR, interquartile range; mTOR, mammalian target of rapamycin; NA, not applicable; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SOT, solid organ transplant.
[a]Statistical differences were only observed between mild SOT and IC patients.

predominantly explained by the clinical severity of COVID-19 infection rather than by the patient condition, either SOT or IC. As shown, all differences were fundamentally driven by the severe clinical groups but not for IL-21-producing T cells, which did not significantly differ across different clinical severities (Supplementary Table S2).

Long-term SARS-CoV-2–specific humoral memory serological memory

Beyond 6 months after infection, 81 of 101 (80.2%) and 78 of 101 (77.2%) patients showed detectable SARS-CoV-2 IgG antibody levels against antigens spike (S) and nucleoprotein (N), respectively. As illustrated in Figure 3a and b, there were no differences regarding both seroconversion rates (81.13% vs. 79.17%; $P = 0.805$) and IgG titters (108 [28.85–396.5] vs. 85.8 [16.5–398.5] UA/ml; $P = 0.58$) against antigen S between SOT and IC, respectively. Conversely, although similar seroconversion against antigen N was observed between SOT and IC, N-specific IgG titers were lower among SOT patients (6.7 [0.67–33] vs. 34.3 [4.43–75.63] UA/ml; $P = 0.027$).

A clear seroconversion gradation according to the 3 distinct clinical severities was observed, regardless of patient condition, either SOT or IC (40 of 41 [97.56%] vs. 33 of 41 [80.48%] vs. 8 of 19 [42.1%]; $P < 0.001$; and 38 of 41 [92.68%] vs. 31 of 41 [75.6%] vs. 9 of 19 [47.36%]; $P < 0.001$) for SEV, MILD, and ASYMP against antigens S and N, respectively (Figure 3c and d; Supplementary Table S3). The same observation was found for IgG titers (435 [189–775.5] vs. 39.4 [15.85–113] vs. 4.94 [0–68]; $P < 0.001$; and 35.7 [8.63–81.25] vs. 9.39 [0.89–50.6] vs. 0.08 [0.08–13.7]; $P < 0.001$) in SEV, MILD, and ASYMP patients against antigens S and N, respectively (Figure 3e and f).

Nonetheless, despite that higher IgG titers against antigen S were observed among MILD-SOT than MILD-IC patients (76.7 [30.4–209.8] vs. 20.9 [15.5–45.2] UA/ml; $P = 0.034$), most likely due to the later time of analysis of MILD-IC subjects (Table 1), SEV-IC patients displayed numerically higher IgG titers against antigen N than SEV-SOT patients (61.8 [36.2–92.1] vs. 15.7 [4–33.4] UA/ml; $P < 0.001$).

B-cell memory

A total of 49 of 71 (69%) patients displayed detectable circulating receptor binding domain of SARS-CoV-2 spike–specific IgG-producing mBC, with similar proportion (22 of 31 vs. 28 of 40; $P = 0.929$) and frequencies (0.0134 [0–0.0557] vs. 0.0116 [0–0.054]; $P = 0.883$) between SOT and IC patients, respectively (Figure 4a and b). Likewise to serology, detection of mBC was highly influenced by the 3 different clinical presentations (84% vs. 75% vs. 35.7%; $P = 0.004$, in SEV, MILD, and ASYMP, respectively), regardless of patient condition (Figure 4c and d; Supplementary Table S4). Even though no statistical differences were observed regarding IgG-producing mBC frequencies between groups, SEV patients showed numerically higher frequencies, this difference being especially evident between SEV-IC versus ASYMP-IC patients (0.059 [0.013–0.189] vs. 0 [0–0.031]; $P = 0.024$) (Figure 4d).

Long-term SARS-CoV-2–specific T-cell memory

Overall, there were no differences regarding the proportion of patients with detectable SARS-CoV-2–reactive T cells or their frequencies for any of the evaluated cytokine-producing T cells between SOT and IC patients against the different viral antigens (Supplementary Figures S4 and S5; Supplementary Table S5).

A hierarchical T-cell immune response was observed that was mainly dominated by antigen S (Supplementary Figure S2; Supplementary Table S6). Similar to humoral immunity, the proportion of T-cell responders significantly decreased along with the different clinical presentations (Figure 5), and these differences were independent of the patient condition (Supplementary Figure S6). As described in Figure 6, a clear decrease in all SARS-CoV-2–reactive cytokine-producing T-cell frequencies but not for IL-21-

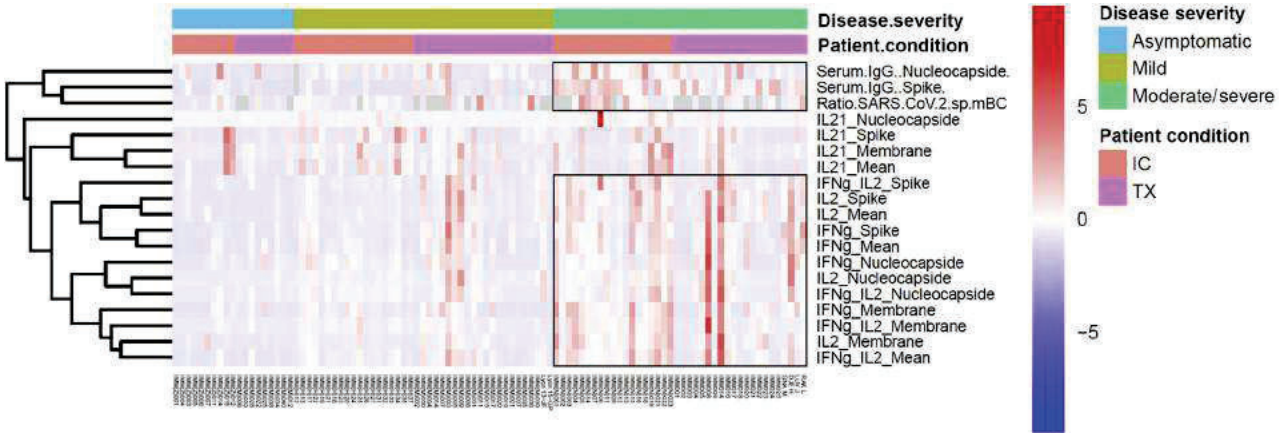

Figure 2 | Heatmaps generated by hierarchical clustering of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–specific immune responses for solid organ transplant (SOT) and immunocompetent (IC) patients, according to the coronavirus disease 2019 (COVID-19) disease severity (moderate/severe, mild, or asymptomatic). Immune responses used for clustering were differentially expressed (fold change >2, false discovery rate $P < 0.05$). Gray fields indicate missing values. IFN, interferon; IL, interleukin.

producing T cells was observed in line with the less severe clinical presentation. Of note, a less pronounced SARS-CoV-2–specific T-cell gradient was observed among SOT as compared with IC patients, especially between severe and mild convalescent COVID-19 patients.

Relationship between serological and cellular SARS-CoV-2–specific immunity

A significant positive correlation between serum IgG titers and frequencies of IgG- and cytokine-producing memory B and T cells, respectively, against protein S was observed, which was more robustly observed within the IC group (Figure 7). Conversely, no correlation was found between serologic and cellular responses against protein N and between frequencies of IgG-producing mBC and cytokine-producing T cells.

Next, we compared all memory immune compartments in each individual according to the different clinical presentations in all patients in whom the humoral (either mBC or antibodies) and cellular immune responses could be investigated (97 of 102 [95.1%] patients; 40 SEV, 38 MILD, and 19 ASYMP). As shown in Figure 8, although all SEV

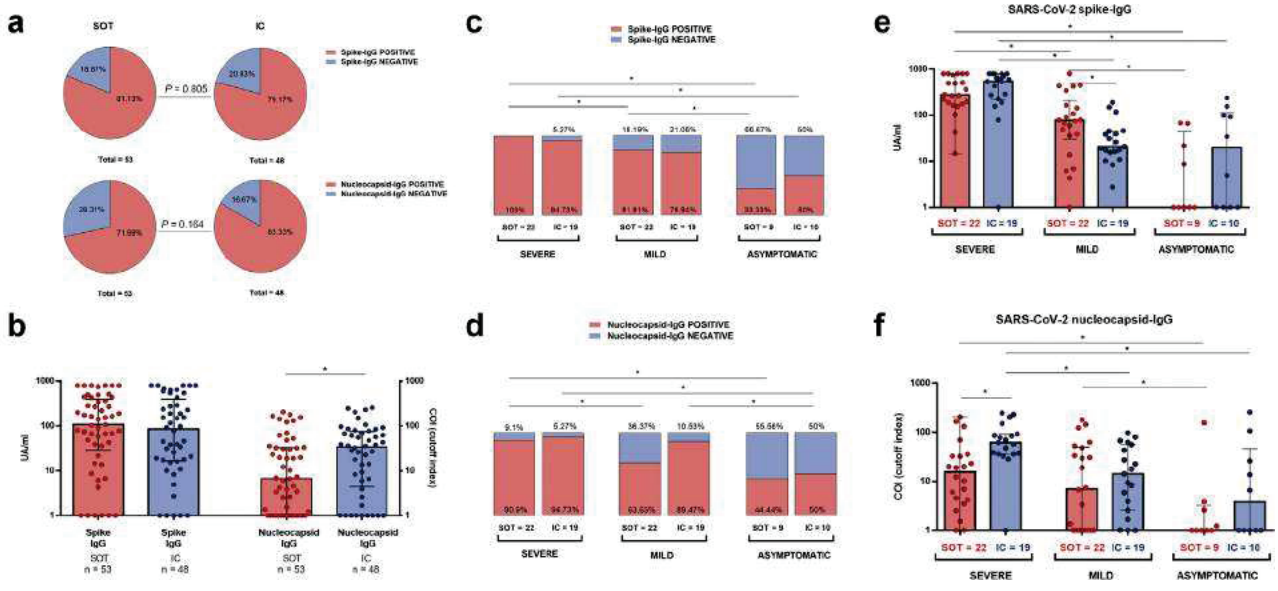

Figure 3 | IgG antibody responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike and nucleocapsid proteins. (**a**) Proportion of solid organ transplants (SOT) and immunocompetent (IC) individuals with detectable IgG antibodies. (**b**) IgG antibody titters against antigens Spike and nucleoprotein among SOT and IC; *$P < 0.05$. (**c,d**) Seropositive proportion of patients for spike (**c**) and nucleoprotein (**d**), according to infection severity at the onset. In columns, immunosuppression status for every cluster of severity. (**e,f**) IgG-spike (**e**) and IgG-nucleoprotein (**f**) titters according to severity and immunosuppression group; *$P < 0.05$. Detailed data on antibody titters are available in Supplementary Table S3.

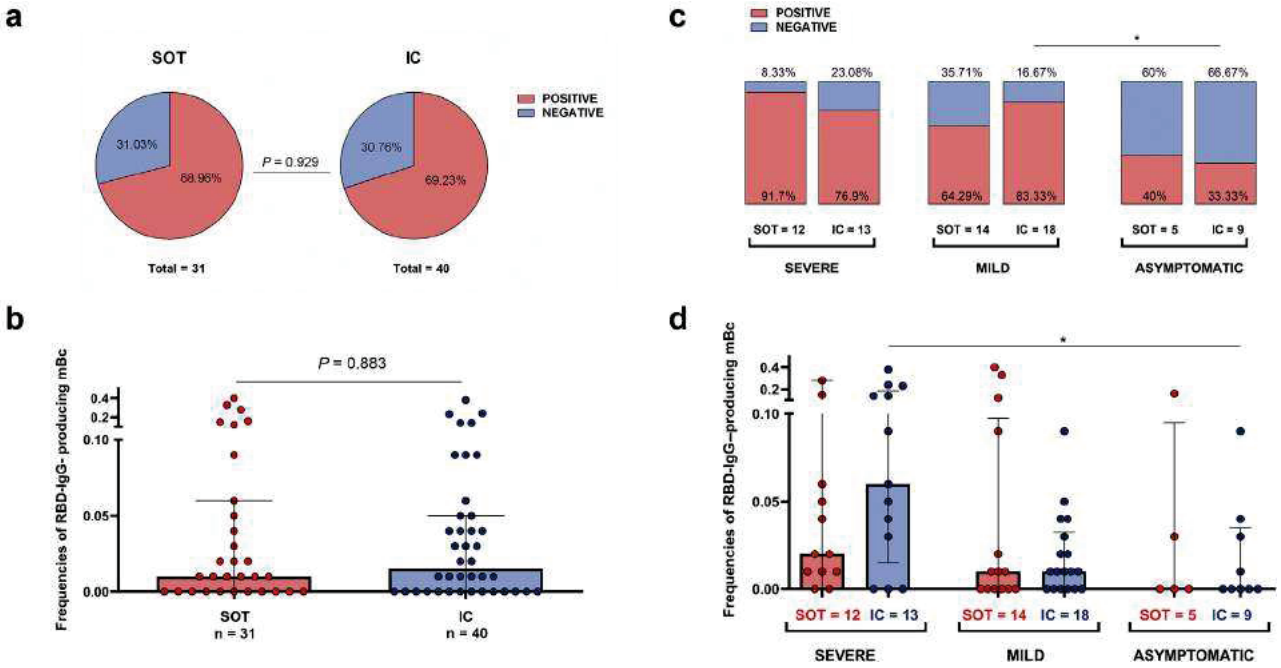

Figure 4| Frequencies of receptor binding domain (RBD)–specific IgG-producing memory B cells (mBCs). (**a**) Proportion of solid organ transplant (SOT) and immunocompetent (IC) individuals with detectable RBD-IgG–producing mBCs. (**b**) Frequencies of RBD-IgG–producing mBCs between SOT and IC. (**c**) Proportion of individuals with detectable RBD-IgG–producing mBCs according to infection severity at the onset. In columns, immunosuppression status for each severity group. (**d**) Frequencies of RBD-IgG–producing mBCs according to severity and immunosuppression group; *$P < 0.05$. Detailed data on ratio of RBD-IgG–producing mBC are provided in Supplementary Table S4.

patients showed some detectable SARS-CoV-2–specific immune memory, 5.3% (2 of 38) of MILD and up to 42.1% (8 of 19) of ASYMP patients did not show detectable antiviral immunity in any of the 3 immune compartments ($P < 0.001$). No differences were found between SOT and IC (data not shown).

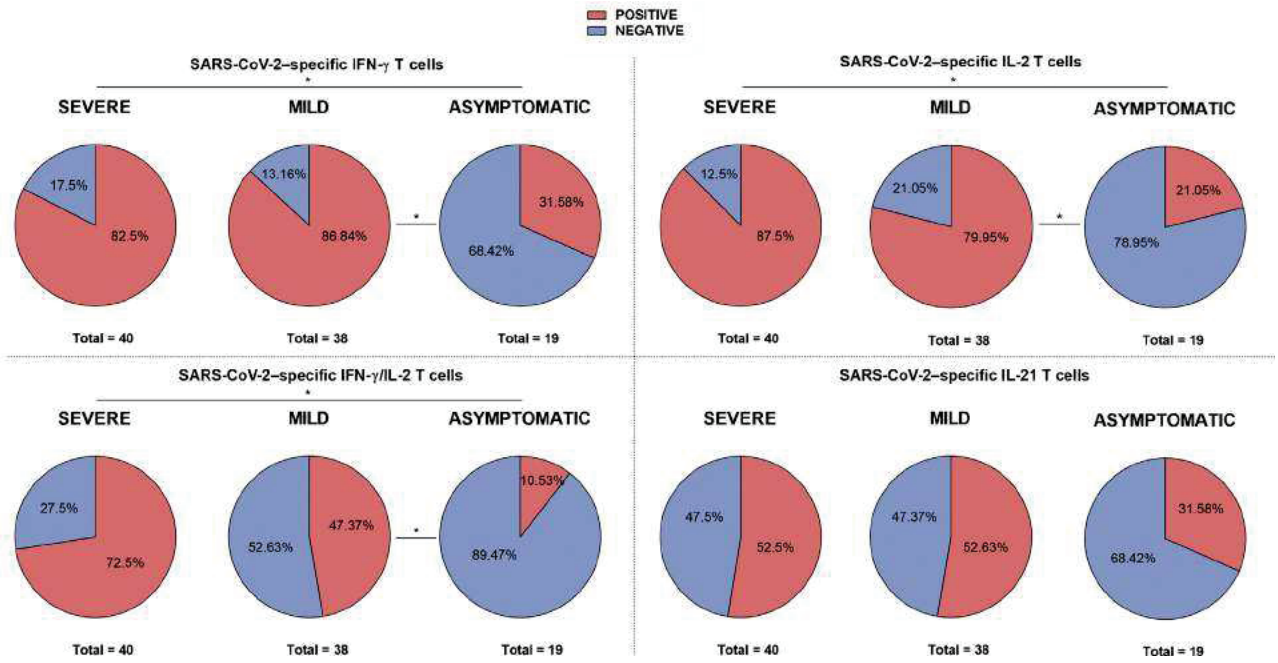

Figure 5| Proportion of patients with detectable severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–reactive cytokine-producing T-cell responses according to infection severity. Interferon-γ (IFN-γ), interleukin-2 (IL-2), IFN-γ/IL-2, and IL-21 were assessed. *$P < 0.05$.

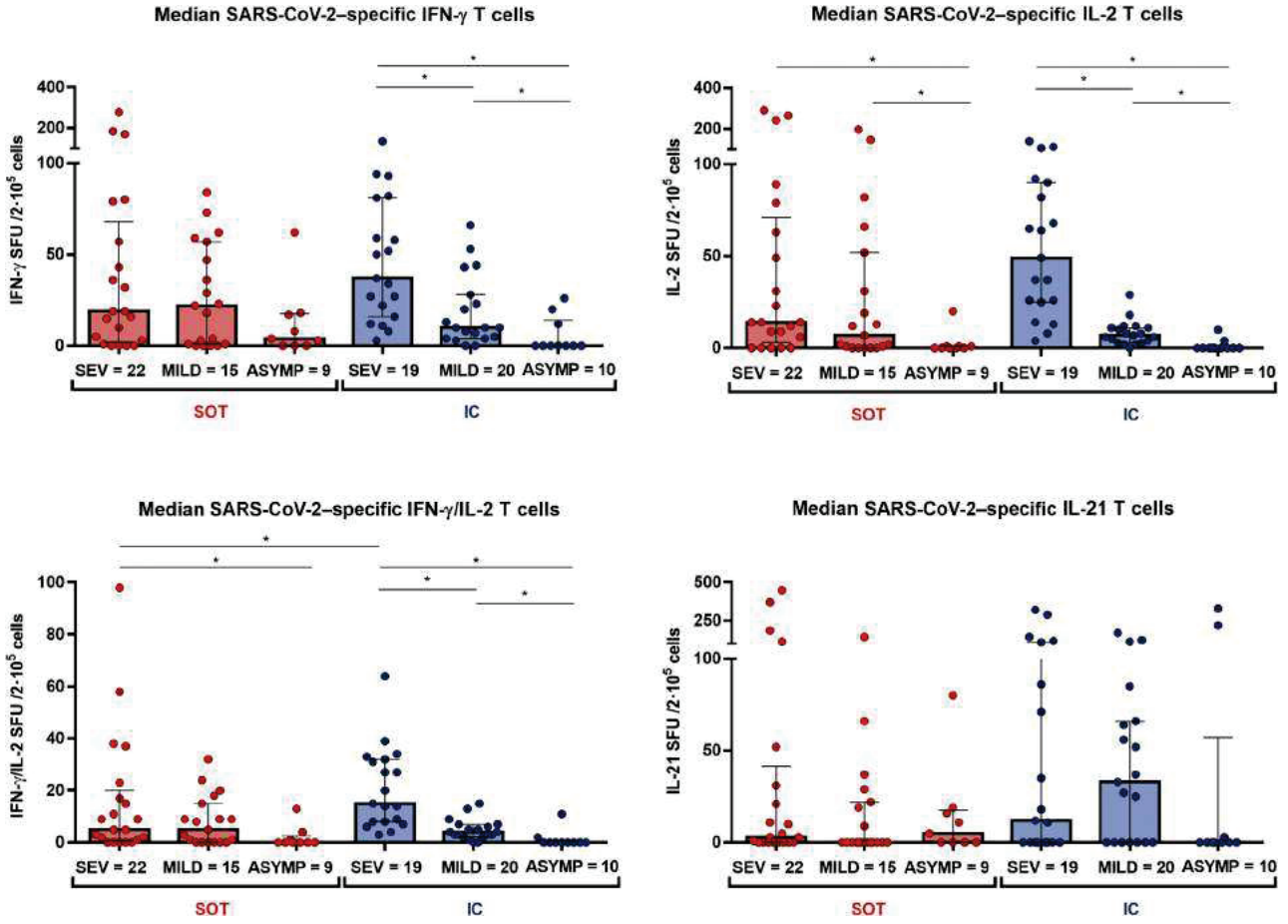

Figure 6 | Global T-cell responses specific to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; median T-cell frequencies against the 3 SARS-CoV-2 immunogenic antigens: S, M, and N). Significant intra- and intergroup differences (solid organ transplant [SOT] severe symptoms [SEV], SOT mild symptoms [MILD], SOT asymptomatic [ASYMP], immunocompetent [IC] SEV, IC MILD, and IC ASYMP) are shown; *$P < 0.05$. IFN-γ, interferon-γ; IL-2, interleukin-2; SFU, spot forming unit.

Longitudinal analysis of SARS-CoV-2 immune memory in severe convalescent COVID-19 patients

In a subgroup of 35 severe convalescent patients (21 SOT and 14 IC), SARS-CoV-2 immune memory could be compared with a previous initial time point after COVID-19 infection (49 days; IQR, 43–53). As illustrated in Figure 9a, although no differences were observed regarding percentages of seropositivity against antigens S and N as well as in IgG titers against antigen N between the 2 time points in the 2 groups, anti-S IgG titers significantly dropped in both groups (800 [285–800] vs. 277.5 [186.5–800]; $P = 0.029$ for SOT; 800 [524–800] vs. 571 [263–713]; $P = 0.002$ for IC).

Notably, a significant decline was observed in all cytokine-producing SARS-CoV-2–reactive T-cell frequencies but not in IL-21, in both groups (Figure 9b), with a higher proportion of SOT becoming non–T-cell responders than IC (Figure 9c).

Main clinical determinants influencing long-term immune memory

We then investigated whether main demographic characteristics such as age, gender, or time after transplant influenced long-term immune memory at the distinct compartments, adjusting for the type of COVID-19 clinical severity, given its preponderance leading to distinct long-lasting SARS-CoV-2–specific immune responses.

Contrary to infection severity, age and gender did not impact on long-term immunity of IC individuals (data not shown).

Among SOT, however, in addition to COVID-19 disease severity, time (years) since transplantation was also revealed as an independent factor modulating the maintenance of long-term peripheral immune memory (Supplementary Table S7), specifically for anti-N IgG antibodies (OR, 1.2; 95% CI, 1.02–1.40; $P = 0.02$), IFN-γ, and IFN-γ/IL-2-reactive T cells (OR, 1.4; 95% CI, 1.08–1.83; $P = 0.013$; and OR, 1.14; 95% CI, 1.01–1.28; $P = 0.028$, respectively).

DISCUSSION

In this study, we investigated the persistence and magnitude of adaptive immune memory specific to SARS-CoV-2 beyond 6 months after infection in a large cohort of convalescent SOT recipients and IC individuals having experienced 3 distinct clinical presentations, severe, mild, or asymptomatic COVID-19. Herein, we show that SOT patients are capable of

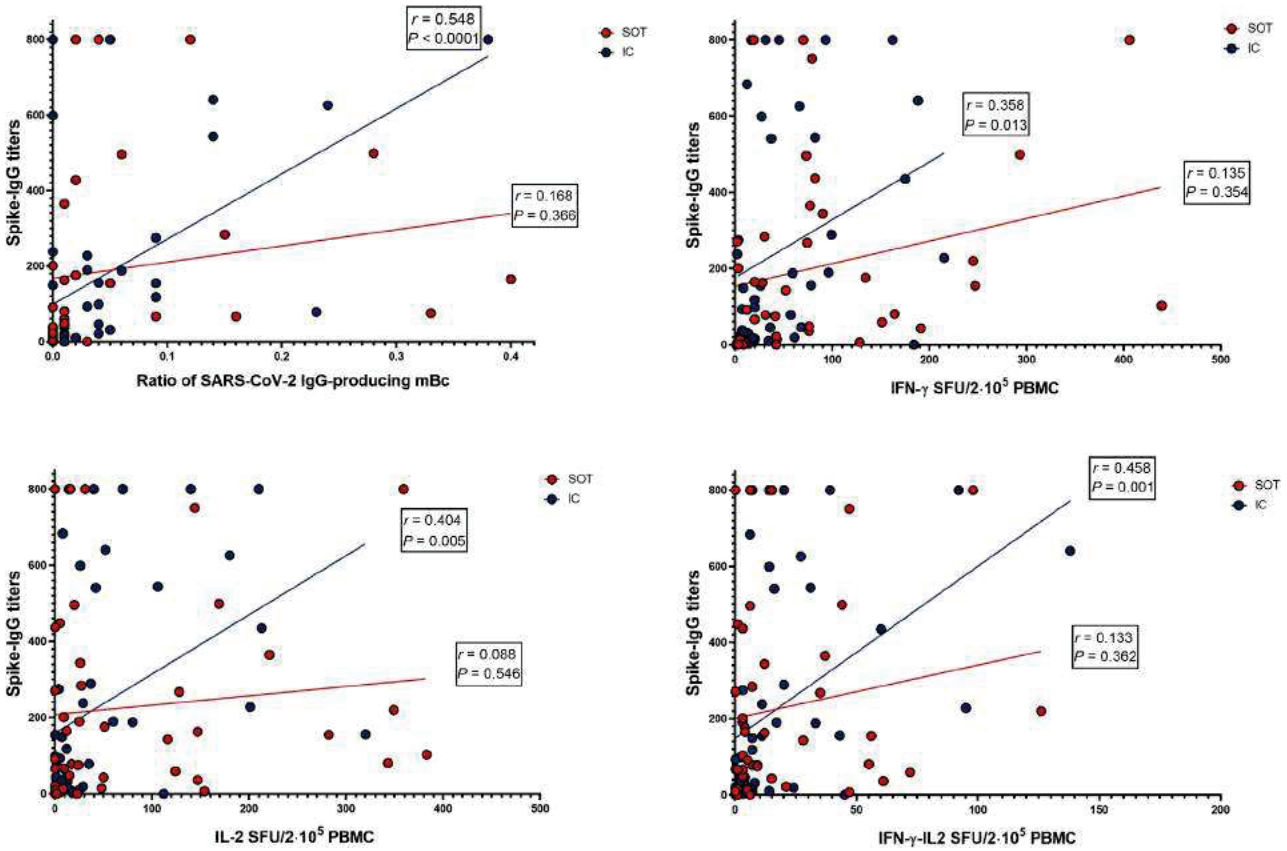

Figure 7 | Correlations between serologic and cellular immune compartments against (spike) severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigen. IgG titers against antigen S and circulating (receptor binding domain [RBD]–spike)-specific memory B cell (mBc) frequencies exhibited a significant positive correlation ($r = 0.355$, $P = 0.003$), which was fundamentally driven by immunocompetent (IC) subjects ($r = 0.548$, $P < 0.001$). A similar pattern was observed between spike-specific IgG titers and the different (spike)SARS-CoV-2–reactive cytokine-producing T-cell frequencies but for IL-21 (data not shown) was mainly observed within IC individuals (interferon-γ [IFN-γ]: $r = 0.358$, $P = 0.013$; interleukin-2 [IL-2]: $r = 0.404$, $P = 0.005$; and IFN-γ/IL-2: $r = 0.458$, $P = 0.001$). PBMC, peripheral blood mononuclear cell; SFU, spot forming unit; SOT, solid organ transplant.

maintaining a long-lasting functional immune response specific to SARS-CoV-2 similar to IC individuals both at the serological and T- and B-cell memory immune compartments. Most importantly, we found that the persistence and magnitude of this response is mainly influenced by the degree of COVID-19 clinical severity; thus a high proportion of asymptomatic and some mild convalescent patients did not display any detectable adaptive immune memory in any biological compartment. To note, even though no major differences were generally observed between SOT and IC, SOT

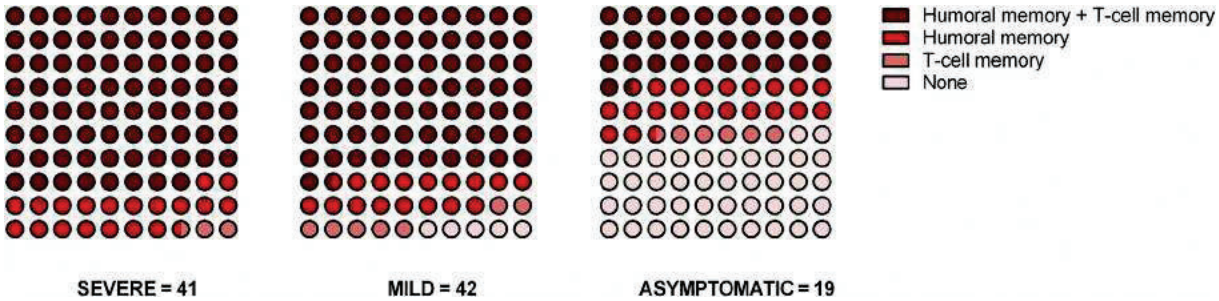

Figure 8 | Dot plots showing the proportion of subjects with detectable responses at the different immune compartments according to disease severity. Humoral memory (H) + T-cell memory (T) = detectable (receptor binding domain [RBD]–spike)-specific memory B cell (mBC) and/or anti-spike IgG and spike-specific interferon-γ (IFN-γ)–producing T cells. Humoral memory = detectable (RBD-spike)-specific mBC or anti-spike IgG. T-cell memory = detectable spike-specific IFN-γ-producing T cells. None (N): no detectable humoral or cellular immunity. SEVERE group: 80% (H+T), 17.5% (H), 2.5% (T), 0% (N); MILD group: 78.9% (H+T), 7.9% (H), 7.9% (T), 5.3% (N); asymptomatic (ASYMP) group: 26.3% (H+T), 26.3% (H), 5.3% (T), 42.1% (N); $P < 0.001$.

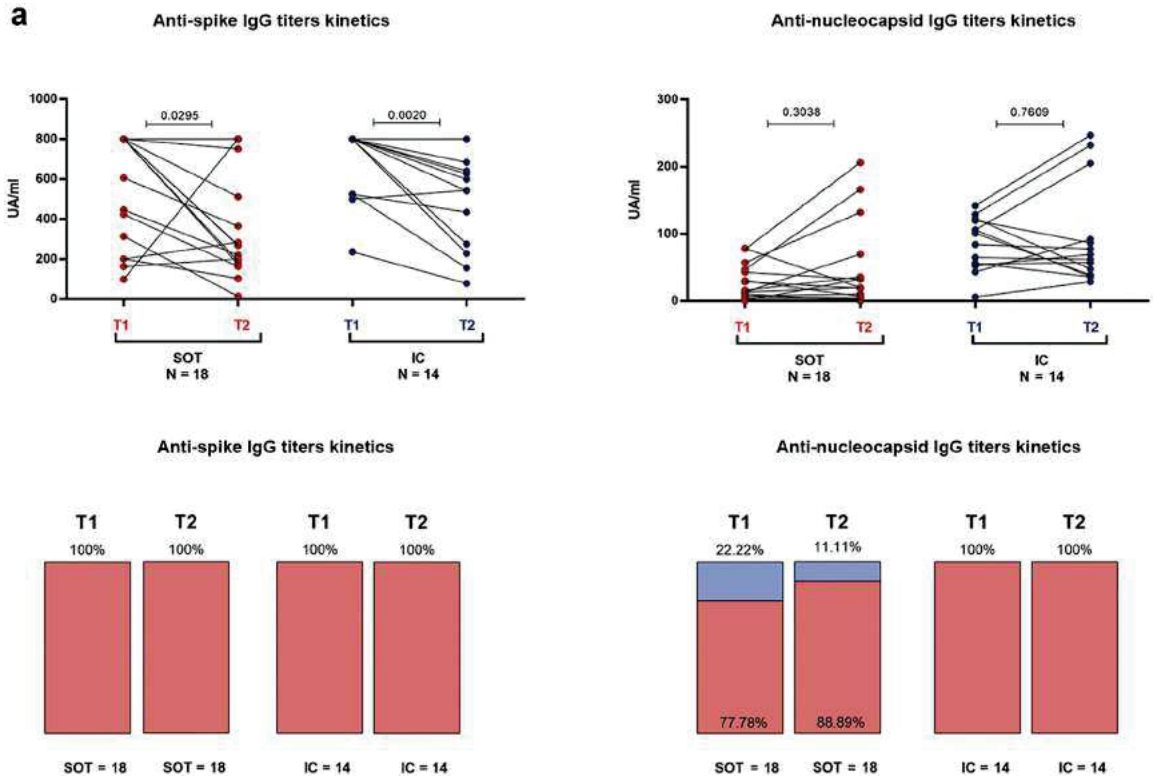

Figure 9| Kinetics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) IgG antibodies and T-cell responses in severe coronavirus disease 2019 individuals between months 1 and 6 after infection. A total of 35 convalescent patients (21 solid organ transplants [SOTs], 14 immunocompetent [IC]) were longitudinally assessed at 2 time points: T1 = 49 (interquartile range [IQR], 44–53) days and T2 = 201 (IQR, 185–208) days after infection. (**a**) Quantitative and qualitative antibody (spike and nucleoprotein) responses; IgG titters (UA/ml). (Continued)

individuals displayed weaker humoral responses to SARS-CoV-2 antigen N, a weaker correlation between serologic and cellular responses than convalescent IC patients with the same clinical disease severity, and a more pronounced decline of SARS-CoV-2–reactive cytokine-producing T-cell frequencies over time. Furthermore, our data highlight a more impaired long-term immune preservation among most recently transplanted SOT individuals.

Recent studies have shown that for seasonal coronaviruses, protective immunity seems to be predominantly short-lived.[22] However, detectable long-term immune memory against SARS-CoV-2 within 3 main compartments (serological and B- and T-cell memory) has been described in convalescent IC individuals beyond 6 months after COVID-19.[8,17]

In our study, although we confirm that COVID-19 provides detectable peripheral immunity at the 3 main immune compartments (serological and functional B- and T-cell immune responses) beyond 6 months after infection in convalescent IC individuals, we show for the first time that SOT patients are similarly capable of maintaining a long-lasting immune memory response at the serological and functional memory B- and T-cell level. In fact, the robust immune responses detected at the different immune compartments in many SOT with the longest follow-up (up to 355 days after infection) strongly suggest that memory immune responses in this patient population may last even further despite receiving chronic immunosuppression. Rather, the most relevant feature determining the persistence and magnitude of protective immunity was the degree of COVID-19 clinical severity. Notably, a clear gradient of immune responses from the more severe to the mild and asymptomatic groups was clearly delineated in our patients.[8-12] In fact, whereas more than 80% of severe convalescent COVID-19 subjects were seropositive and displayed robust SARS-CoV-2–specific IgG- and cytokine-producing memory B and T cells, respectively, only in 40% of the asymptomatic group were these detectable. A potential explanation for these findings may rely in recent observations showing that severe hospitalized cases, both IC and SOT, display higher viral loads, viremia, and longer viral shedding as compared with milder COVID-19 cases,[23-26] which may lead to higher antigen exposure ultimately triggering stronger and long-lasting immune responses.

In line with previous studies,[8,27] we also found a high correlation between the different immune compartments specific to SARS-CoV-2. However, these differences were predominantly driven by the IC group, suggesting a more impaired functional immune response of SOT related to chronic immunosuppression. Indeed, the gradient of strength and detection of immune responses at the T-cell level between the distinct clinical COVID-19 presentations within SOT was

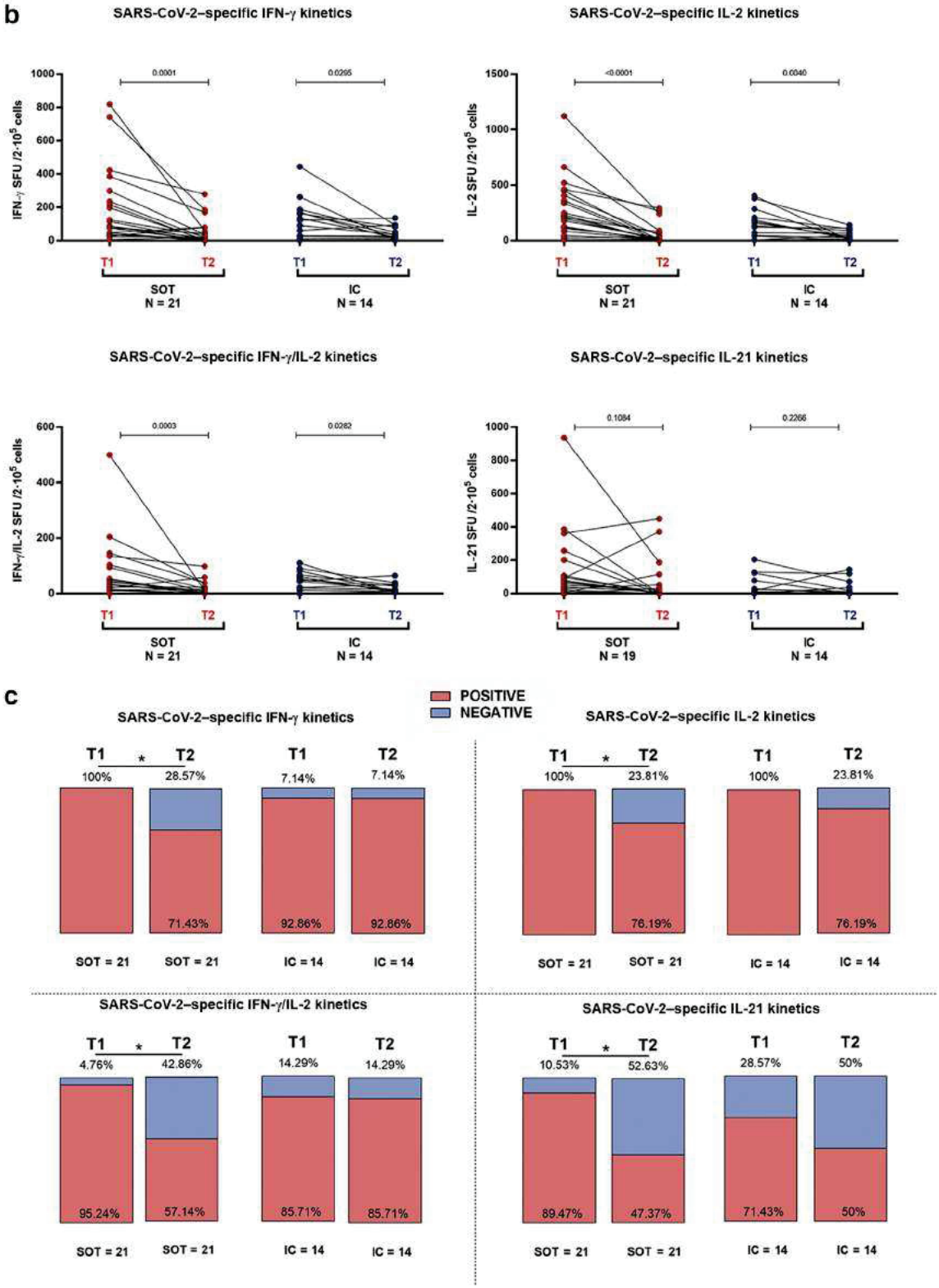

Figure 9| (Continued) (**b**) T-cell frequencies for interferon-γ (IFN-γ), interleukin-2 (IL-2), IFN-γ/IL-2, and IL-21; T-cell frequencies (spot forming unit [SFU]/2 × 10^5 peripheral blood mononuclear cell [PBMC]). (**c**) Proportion of patients with detectable T-cell responses for IFN-γ, IL-2, IFN-γ/IL-2, and IL-21. *$P < 0.05$.

not pronounced as compared with IC patients, especially between severe and mild patients. Moreover, in a longitudinal analysis of immune response progression within severe convalescent COVID-19 patients, SOT displayed a clearer decline of functional T-cell immune memory than IC patients, again illustrating a certain deleterious effect of chronic immunosuppression on antiviral immunity over time. In addition, despite similar seropositivity rates for both antigens S and N between IC and SOT, the latter displayed significantly lower anti-N IgG titers than IC patients. Unlike in the general population,[17] it has recently been described that SOT patients seem to show lower anti-N IgG titers,[28] especially those with higher immunosuppressive burden,[29] suggesting higher susceptibility of anti-N seroconversion to chronic immunosuppression.

Finally, when we investigated major determinants influencing the presence of SARS-CoV-2–specific immune memory within SOT patients, besides COVID-19 clinical severity, we found that more recently transplanted patients exhibited an independent higher risk of not maintaining detectable serological and T-cell immunity than those with a longer functioning graft. These data highlight the negative effect of the initial immunosuppressive burden challenging adaptive anti-viral immune responses.

Our study has some limitations. First, we have to consider an inherent selection bias in our cohort, because all the included individuals had successfully recovered from SARS-CoV-2 infection, which is not the general COVID-19 outcome among this at-risk patient population. On the other hand, our immune evaluation was restricted to the original SARS-CoV-2 strain, due to the infection time period (March to October 2020), so we are not able to fully ensure whether these data would replicate with the more virulent viral strains. Finally, this study was performed before the successful vaccination campaigns,[30] so we cannot completely extrapolate these findings to breakthrough infections in patients after unsuccessful vaccination.

Also, the mild infection group of the study, which was fundamentally based on health care workers, was a bit younger and were analyzed at a later time. However, in general, these differences did not impact on the immune responses compared with the same mild SOT group. Although the number of asymptomatic patients was lower than the other 2 groups, the consistency of the results observed within this group counterbalances this constraint. Finally, we could not describe the predominant T- or B-cell subsets, responsible of these SARS-CoV-2–reactive T and B cells. However, our FluoroSpot assay allowed us to functionally assess the frequencies of different IgG- and cytokine-producing B and T cells specific to SARS-CoV-2 at the single cell level.

In conclusion, our findings show that robust humoral and cellular immune memory persists among IC and SOT convalescent COVID-19 patients for more than 6 months after infection, and these responses are highly dependent on the clinical degree of COVID-19 severity, which might ultimately illustrate a distinct level of viral antigen exposure. However, long-lasting adaptive immunity seems to be challenged to some extent by chronic immunosuppression, especially among those more recently transplanted. Our data may have some relevant implications regarding the long-lasting immune response achieved after vaccination, highlighting the need of an accurate and broader assessment of SARS-CoV-2 immune response to establish guided preventive strategies.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

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SUPPLEMENTARY MATERIAL

Supplementary File (Word)

Supplementary Methods.

Figure S1. Representative images of receptor binding domain (RBD)–specific and polyclonal IgG detection from memory B-cells (mBCs), prior differentiation to antibody secreting cells. No RBD-specific IgG detection was found among healthy donors.

Figure S2. Hierarchical cytokine profile of T-cell responses against main structural severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) proteins: spike (S), membrane (M), and nucleoprotein (N).

Figure S3. Representative images of a convalescent solid organ transplant (SOT) from severe coronavirus disease 2019 (COVID-19) and an unexposed individual, including specific interferon-γ (IFN-γ) responses against the spike overlapping peptide pool; pokeweed mitogen (PWM), as internal positive control; isolated medium, as internal negative control; and the readouts after subtraction.

Figure S4. Percentage of solid organ transplant (SOT) and immunocompetent (IC) patients with detectable (spike) severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) specific for different cytokine-producing T cells.

Figure S5. Global T-cell responses specific to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (median [interquartile range] T-cell frequencies against the 3 main SARS-CoV-2 immunogenic antigens: spike [S], membrane [M], and nucleoprotein [N]) for solid organ transplant (SOT) and immunocompetent (IC) patients and for each cytokine assessed (interferon-γ [IFN-γ], interleukin-2 [IL-2], IFN-γ/IL-2, and IL-21).

Figure S6. Proportion of patients with detectable cytokine-producing T-cell responses against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), according to the immunosuppression status and infection severity.

Table S1. Correlations between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–specific T-cell and B-cell responses.

Table S2. Statistical differences and false discovery rate (fdr) for all the immune responses clustered in Figure 2 heatmap.

Table S3. IgG titers against spike and nucleoprotein (median UA/ml [interquartile range]), according to immunosuppression status and infection severity.

Table S4. Ratio of IgG-producing memory B cells (median [interquartile range]) against receptor binding domain (RBD), according to immunosuppression status and infection severity.
Table S5. Specific T-cell responses (median spots [interquartile range]) against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; median of SMN antigens), according to immunosuppression status and infection severity.
Table S6. Hierarchical cytokine profile of T-cell responses against main structural severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) proteins: spike (S), membrane (M), and nucleoprotein (N). Frequencies of interferon-γ (IFN-γ)–, interleukin-2 (IL-2)–, IFN-γ/IL-2–, and IL-21–producing T cells were assessed among the 6 groups of study.
Table S7. Univariate and multivariate analyses based on a binary logistic regression model for major determinants influencing persistence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody and cellular responses among solid organ transplants (SOT) after 6 months (odds ratio [95% confidence interval]).
Supplementary References.

REFERENCES

1. Oran DP, Topol EJ. Prevalence of asymptomatic SARS-CoV-2 infection: a narrative review. *Ann Intern Med*. 2020;173:362–367.
2. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in china: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. *JAMA*. 2020;2019:3–6.
3. Guan W, Ni Z, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382:1708–1720.
4. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 2020;323:1061–1069.
5. Domínguez-Gil B, Coll E, Fernández-Ruiz M, et al. COVID-19 in Spain: transplantation in the midst of the pandemic. *Am J Transplant*. 2020;20:2593–2598.
6. Elias M, Pievani D, Randoux C, et al. COVID-19 infection in kidney transplant recipients: disease incidence and clinical outcomes. *J Am Soc Nephrol*. 2020;31:2413–2423.
7. 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;181:1489–1501.e15.
8. Zuo J, Dowell AC, Pearce H, et al. Robust SARS-CoV-2-specific T cell immunity is maintained at 6 months following primary infection. *Nat Immunol*. 2021;22:620–626.
9. Vanshylla K, Di Cristanziano V, Kleipass F, et al. Kinetics and correlates of the neutralizing antibody response to SARS-CoV-2 infection in humans. *Cell Host Microbe*. 2021;29:917–929.e4.
10. Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med*. 2020;26:1200–1204.
11. Sekine T, Perez-Potti A, Rivera-Ballesteros O, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. *Cell*. 2020;183:158–168.e14.
12. Piccoli L, Park YJ, Tortorici MA, et al. Mapping neutralizing and immunodominant sites on the SARS-CoV-2 spike receptor-binding domain by structure-guided high-resolution serology. *Cell*. 2020;183:1024–1042.e21.
13. Ren L, Zhang L, Chang D, et al. The kinetics of humoral response and its relationship with the disease severity in COVID-19. *Commun Biol*. 2020;3:780.
14. Lucas C, Klein J, Sundaram ME, et al. Delayed production of neutralizing antibodies correlates with fatal COVID-19. *Nat Med*. 2021;27:1178–1186.
15. Rydyznski Moderbacher C, Ramirez SI, Dan JM, et al. Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. *Cell*. 2020;183:996–1012.e19.
16. Blanco-Melo D, Nilsson-Payant BE, Liu WC, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell*. 2020;181:1036–1045.e9.
17. Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science*. 2021;371(6529):eabf4063.
18. 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*. 2021;21:2749–2761.
19. Thieme CJ, Anft M, Paniskaki K, et al. The magnitude and functionality of SARS-CoV-2 reactive cellular and humoral immunity in transplant population is similar to the general population despite immunosuppression. *Transplantation*. 2021;105:2156–2164.
20. Boyarsky BJ, Werbel WA, Avery RK, et al. Antibody response to 2-dose SARS-CoV-2 mRNA vaccine series in solid organ transplant recipients. *JAMA*. 2021;325:2204–2206.
21. Cucchiari D, Egri N, Bodro M, et al. Cellular and humoral response after MRNA-1273 SARS-CoV-2 vaccine in kidney transplant recipients. *Am J Transplant*. 2021;21:2727–2739.
22. Edridge AWD, Kaczorowska J, Hoste ACR, et al. Seasonal coronavirus protective immunity is short-lasting. *Nat Med*. 2020;26:1691–1693.
23. Fajnzylber J, Regan J, Coxen K, et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. *Nat Commun*. 2020;11:5493.
24. Liu Y, Yan L, Wan L, et al. Viral dynamics in mild and severe cases of COVID-19: the first COVID-19 case in Afghanistan acquired from Iran. *Lancet Infect Dis*. 2020;20:656–657.
25. Tan AT, Linster M, Tan CW, et al. Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. *CellReports*. 2021;34:108728.
26. Benotmane I, Wendling GGM, Perrin P, et al. In-depth virological assessment of kidney transplant recipients with COVID-19. *Am J Transplant*. 2020;20:3162–3172.
27. Jiang X, Wang G, Zhao X, et al. Lasting antibody and T cell responses to SARS-CoV-2 in COVID-19 patients three months after infection. *Nat Commun*. 2021;12:897.
28. Chang CC, Vlad G, Vasilescu ER, et al. Disparity between levels of anti-RBD IgG and anti-nucleocapsid protein IgG antibodies in COVID-19–recovered patients who received a kidney transplant. *Kidney Int*. 2021;100:240–241.
29. Burack D, Pereira MR, Tsapepas DS, et al. Prevalence and predictors of SARS-2 antibodies among solid organ transplant recipients with confirmed infection. *Am J Transplant*. 2021;21:2254–2261.
30. Bestard O, Jouve T, Castells L, et al. Reconciling short-term clinical and immunological outcomes of SARS-CoV-2 vaccination in solid organ transplant recipients. *Am J Transplant*. 2022;22:673–675.

SUPPLEMENTARY MATERIAL

1 - Supplementary methods

1.1- Collection and management of serum and peripheral blood mononuclear cells (PBMC) samples

PBMCs were isolated from patient's peripheral blood by Ficoll density gradient centrifugation and subsequently frozen in liquid nitrogen until their use in functional analyses. Serum was isolated by centrifugation and samples were stored at -20ºC.

1.2 - Assessment of SARS-CoV-2-specific Serological Memory

Briefly, serological response to SARS-CoV-2 was determined by the detection of specific antibodies against both nucleocapsid and spike SARS-CoV-2 antigens. Two commercial chemiluminescence immunoassays (CLIA) were used, according to the manufacture instructions:

1) Elecsys® Anti-SARS-CoV-2 (Roche Diagnostics, Mannheim, Germany) performed on the Cobas 8800 system (Roche Diagnostics, Switzerland) for the determination of total antibodies (including IgG, IgM and IgA) against nucleocapsid SARS-CoV-2 proteins (cut-off ≥ 1.0 index). Human serum reactive (ACOV2 Cal2) and non-reactive (ACOV1 Cal1) for SARS-CoV-2 antibodies were used as negative and positive calibrators (https://www.fda.gov/media/137605/download).
2) LIAISON® SARS-CoV-2 TrimericS IgG (DiaSorin, Stillwater, MN, USA) performed on the LIAISON® XL Analyzer (DiaSorin, Italy) for the determination of IgG antibodies spike glycoprotein of SARS-CoV-2 (cut-

off ≥ 13.0 AU/mL; measures up to 800 AU/ml). Human serum reactive and non-reactive for SARS-CoV-2 IgG antibodies 0.2% ProClin 300 were used as internal negative and positive controls, as provided by the manufacturer (https://www.fda.gov/media/149059/download).

1.3- Assessment of RBD-specific B-cell Memory

Briefly, to differentiate circulating mBCs to antibody-secreting cells (ASCs), peripheral blood mononuclear cells were cultured (1.5×10^6 cells per ml at 37 °C in 5% CO2) for 6 days in Iscove Modified Dulbecco Media (IMDM) enriched medium, 500 ng/ml Human CD40/TNFRSF5 Antibody (Bio-Techne R&D Systems, S.L.U., USA), 600 IU/ml human interleukin-2 (Sigma Aldrich, USA), 100 ng/ml human interleukin-21 (Peprotech, UK), 25 ng/ml human interleukin-10 (Peprotech, UK), 2.5 ug/ml CpG-B DNA (ODN 2006) (HycultBiotech, The Netherlands) and 10 ul/ml ITS Liquid Media Supplement (Sigma Aldrich, USA), as previously described by our group [S1].

After 6-day stimulation, 4.5×10^5 stimulated cells were seeded in each well to assess SARS-CoV-2 specific IgG spots, whereas 4.5×10^4 and 4.5×10^3 stimulated cells were seeded to assess the polyclonal IgG spot detection.

For the detection of specific SARS-CoV-2 mBCs, we used RBD-WASP (recombinant SARS-CoV-2 Receptor Binding Domain (RBD) of the Spike protein (aa 319-541) with a C-terminal WASP peptide tag [PDYRPYDWASPDYRD]) at 1:20 dilution; followed by anti-WASP-HRP (horseradish peroxidase) at 1:1000 dilution.

For the polyclonal IgG detection, mAbs MT78/145-Biotin (1 ug/ml) and Streptavidin-HRP (1:1000) were used, respectively (anti-human IgG MT78/145 is a mouse monoclonal antibody to IGHG1, IGHG2, IGHG4, and immunoglobulin heavy constant gamma 1 [G1m marker]; this biotinylated antibody interacts and binds to the streptavidin-HRP complex to ultimately generate a detectable signal). Next, 100 uL of ready-to-use TMB (3,3' , 5,5'-tetramethylbenzidine) solution was used as substrate for HRP, in order to develop the reaction until distinct spots emerge. After the plate was dried, spots were count in the Fluorspot Reader version 8 (AID® Gmbh, Strassberg, Germany). The ratio between RBD-specific mBCs over the total polyclonal IgG mBCs in each patient was used as a reliable approach to characterize the proportion or enhancement of a given RBD-specific IgG-antibody secreting cell (ASC) within the global IgG-ASC population. This approach method allows for qualitative and quantitative easy comparisons between sample stimulations [S1,S2].

PBMC's from 10 non-immunosuppressed individuals on the waiting list for kidney transplantation that were obtained 2 years before the pandemic (2018) were used as healthy controls, and all showed absence of detectable IgG RBD-specific spots (**Supplementary Figure S1**).

Any ELISPOT test with non-detectable RBD-specific spots were considered as negative when assessed in a qualitative manner. Patients showing suboptimal proliferation results were excluded from the analysis (**Supplementary Figure S1**).

1.4- Assessment of polyfunctional SARS-CoV-2-specific T-cell responses

Briefly, $2x10^5$ PBMCs (in 100 µl) were stimulated with the peptides for 24 hours for IFN-γ, IL-2, IFN-γ/IL-2 and 48 hours for IL-21. After washing steps, the different cytokine fluorospots were detected using primary and secondary antibodies against each cytokine plus the addition of enhancer. The spots obtained were automatically counted with the Fluorospot Reader version 8 (AID® Gmbh, Strassberg, Germany).

In each test, complete medium alone (20% Fetal Bovine Serum (FBS) and 80% RPMI solution) and Pokeweed (PWM) mitogen were used as negative and positive controls, respectively. The results were considered after subtracting to each well the responses obtained in the respective internal negative control well (**Supplementary Figure S3**).

As external negative controls, we previously showed[18] that pre-pandemic unexposed individuals to SARS-CoV-2 did not respond to any of the used overlapping peptide pools of SARS-CoV-2 proteins (never exceeded 5 spots/$2x10^5$ stimulated PBMCs).

Any antigen-specific ELISPOT test with less than 5 spots/$2x10^5$ PBMC was considered as negative when assessed in a qualitative manner.

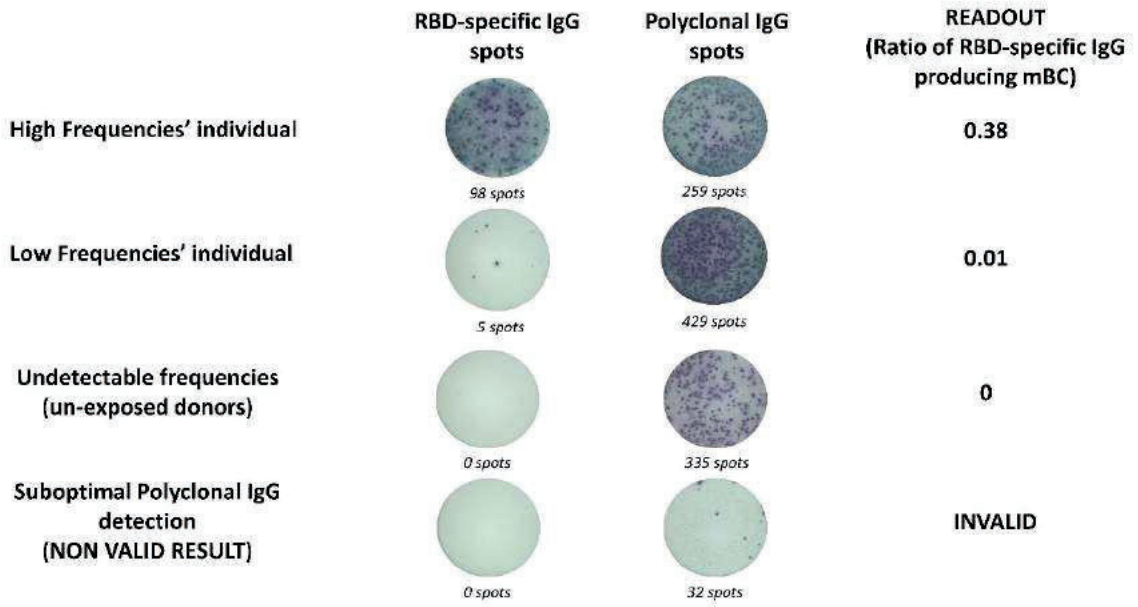
SARS-CoV-2 antigens

The Spike Glycoprotein (S) overlapping peptide pool (P0DTC2 protein, S gene) contained 158 + 157 peptides of >70% purity, the Membrane Protein (M) overlapping peptide pool (P0DTC5 protein, VME-1 gene) contained 16 peptides of >70% purity, the Nucleoprotein (N) peptide overlapping pool (P0DTC9 protein, NCAP gene) contained 102 peptides of >70% purity and the Envelope small

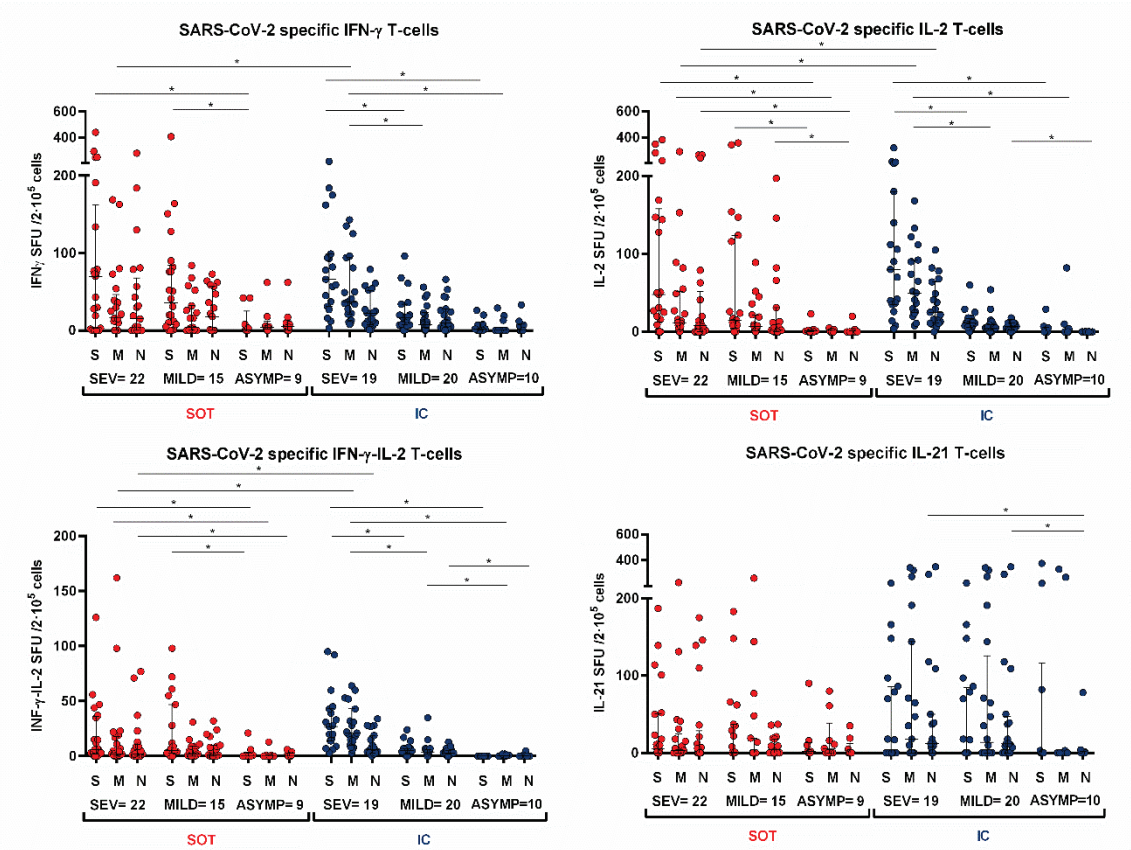
membrane Protein (E) overlapping peptide pool (P0DTC4 protein, VEMP gene) contained 16 peptides of >70% purity. S, M, N and E peptides were reconstituted in DMSO and PBS and used at a final concentration of 2µg/mL. All SARS-CoV-2 peptides were designed by and purchased from JPT Innovative Peptide Solutions (JPT®, Berlin, Germany).

2 - Supplementary Figures

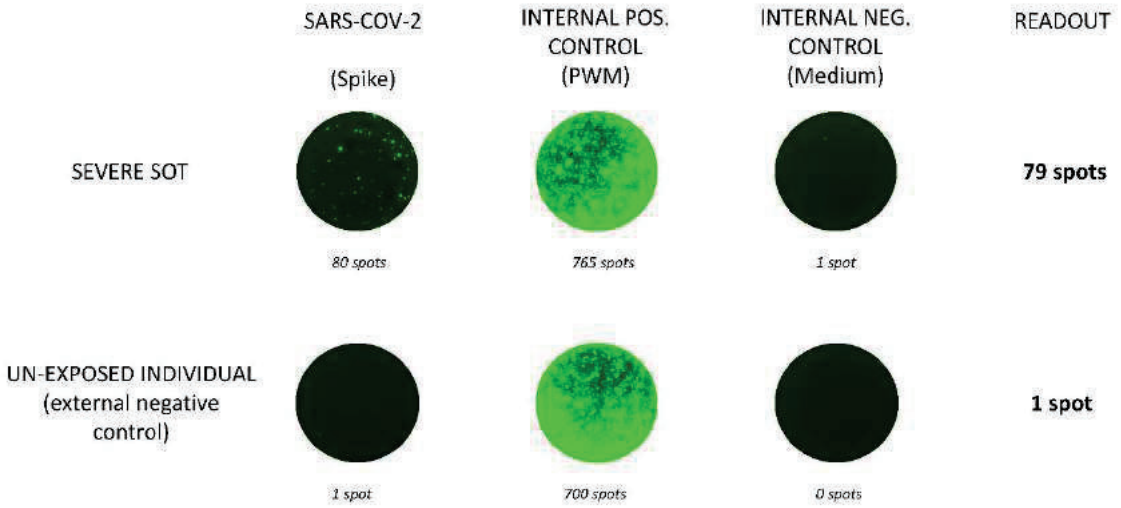
2.1- Supplementary Figure S1. Representative images of RBD-specific and Polyclonal IgG detection from mBC, prior differentiation to antibody secreting cells (ASC). No RBD-specific IgG detection was found among healthy donors.



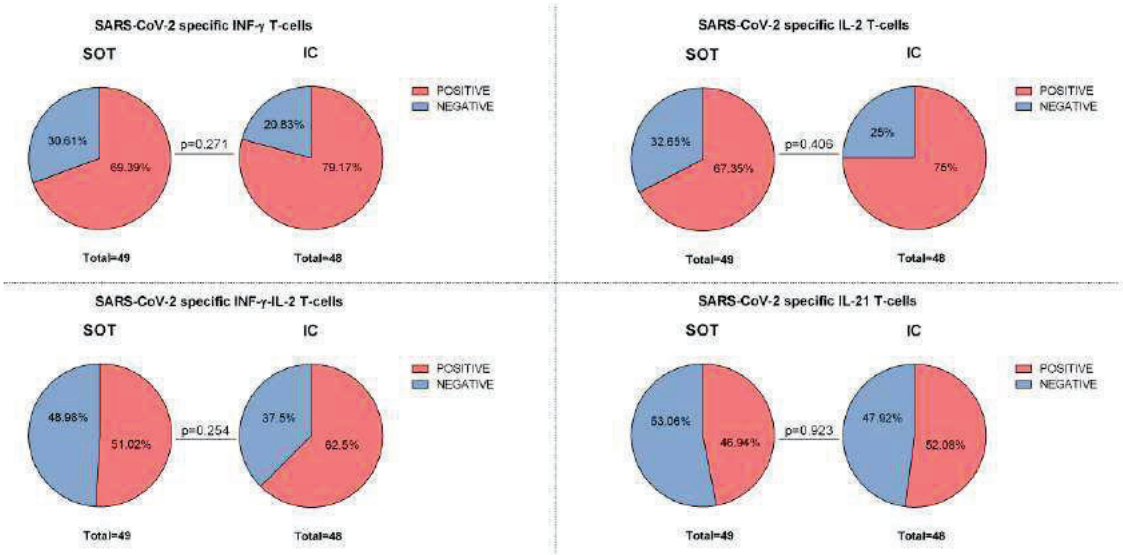
2.2- Supplementary Figure S2. Hierarchical cytokine profile of T-cell responses against main structural SARS-CoV-2 proteins Spike (S), Membrane (M) and Nucleoprotein (N). Frequencies of IFN-γ, IL-2, IFN-γ/IL-2 and IL-21-producing T cells were assessed among the six groups of study (median spots [IQR]).*p<0.05. Detailed data on antigen specific T cell responses is provided in Supplementary Table 6.



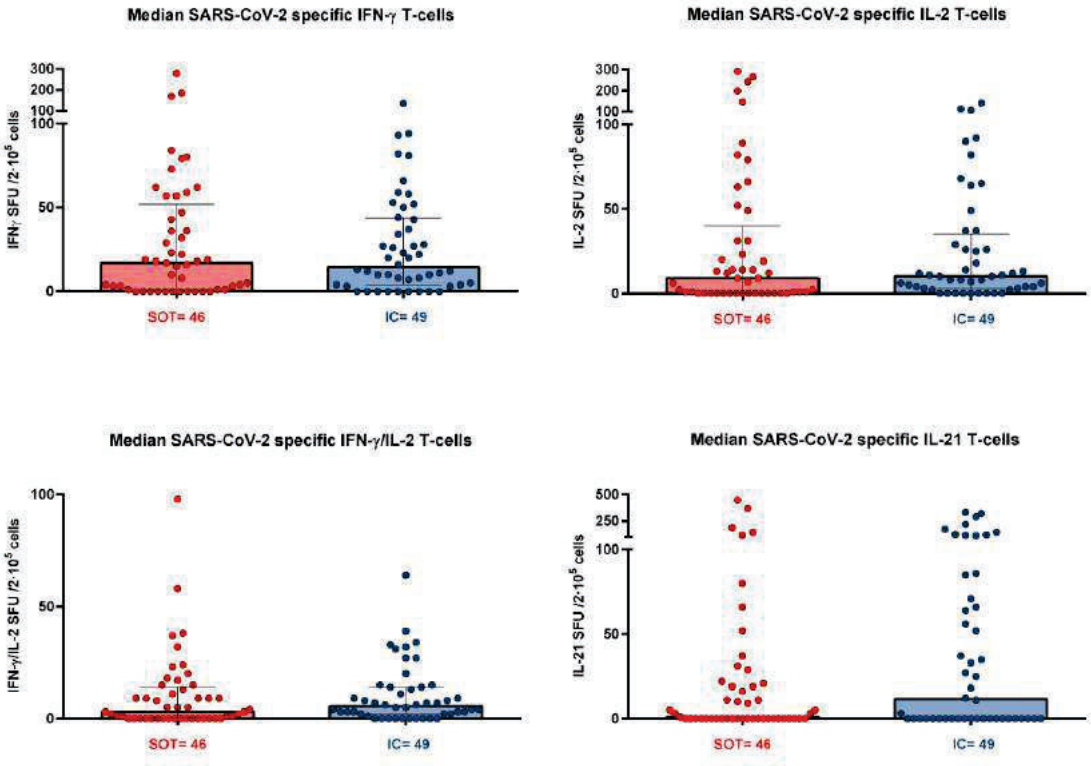
2.3- Supplementary Figure S3. Representative images of a convalescent SOT from severe COVID-19 and an un-exposed individual, including specific INF-γ responses against the Spike overlapping peptide pool; Pokeweed mitogen (PWM), as internal positive control; isolated medium, as internal negative control; and the readouts after subtraction.



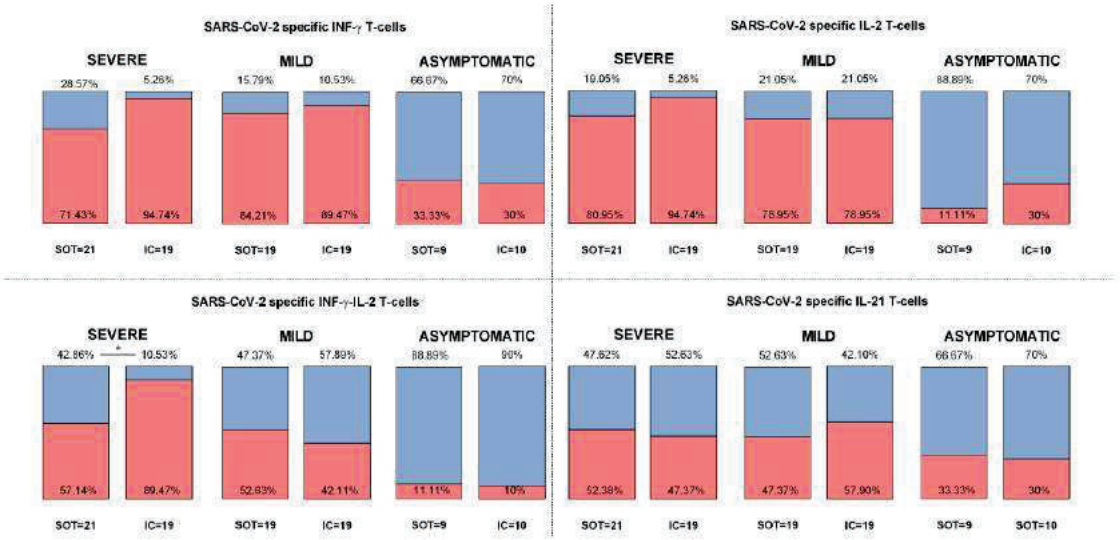
2.4- Supplementary Figure S4. **Percentage of SOT and IC patients with detectable (Spike)SARS-CoV-2-specific for different cytokine-producing T cells** (IFN-γ, IL-2, IFN-γ/IL-2 and IL-21).



2.5- Supplementary Figure S5. Global T-cell responses specific to SARS-CoV-2 (median [IQR] T-cell frequencies against the three main SARS-CoV-2 immunogenic antigens: Spike(S), Membrane (M) and Nucleoprotein (N)) for SOT and IC patients and for each cytokine assessed (IFN-γ, IL-2, IFN-γ/IL-2 and IL-21).*p<0-05. Detailed data on global T-cell responses is available in Supplementary Table 5.



2.6- Supplementary Figure S6. Proportion of patients with detectable cytokine producing T-cell responses against SARS-CoV-2, according to the immunosuppression status and infection severity. IFN-γ, IL-2, IFN-γ/IL-2 and IL-21 were assessed; *p<0.05



3 - Supplementary Tables. Supplementary Table S1. Correlations between SARS-CoV-2-specific T

IFN-γ-producing T-cell frequencies							IL-2-producing T-cell frequencies						I	
	S	M	N	E	SMN	(S)IgG -mBc	S	M	N	E	SMN	(S)IgG-mBc	S	
S		R= 0.585 p <0.001	R= 0.748 p <0.001	R= 0.085 p=0.438	R= 0.775 p<0.001	R=0.172 p=0.134		R=0.685 p <0.001	R= 0.840 p <0.001	R= -0.002 p= 0.982	R=0.884 p<0.001	R=0.097 p=0.400		
M			R= 0.692 p <0.001	R= 0.187 p= 0.084	R=0.825 p<0.001	R=0.055 p=0.632			R= 0.719 p <0.001	R= 0.012 p= 0.914	R=0.831 p<0.001	R=0.116 p=0.317		
N				R= 0.314 p= 0.003	R=0.937 p<0.001	R=-0.009 P=0.941				R= -0.021 p= 0.849	R=0.955 p<0.001	R=0.049 p=0.672		
E					R=0.261 p=0.016	R=0.015 p=0.899					R=-0.047 p=0.672	R=0.142 p=0.232		
SMN						R=0.053 p=0.643						R=0.090 p=0.438		

IL-21-producing T-cell frequencies							IL-5-producing T-cell frequencies					
	S	M	N	E	SMN	(S)IgG -mBc	S	M	N	E	SMN	(S)IgG-mBc
S		R= 0.551 p< 0.001	R= 0.044 p= 0.671	R= 0.546 p< 0.001	R=0.555 P<0.001	R=-0.126 p=0.274		R= 0.831 p< 0.001	R= 0.345 p< 0.001	R= -0.020 p= 0.858	R=0.411 p<0.001	R=-0.106 p=0.359
M			R= 0.132 p= 0.199	R= 0.490 p< 0.001	R=0.724 P<0.001	R=-0.123 p=0.288			R= 0.356 p< 0.001	R= -0.006 p= 0.959	R=0.457 p<0.001	R=-0.116 p=0.315
N				R= 0.064 p= 0.561	R=0.148 p=0.148	R=0.091 p=0.431				R= -0.090 p= 0.412	R=0.170 p=0.097	R=0.042 p=0.717
E					R=0.665 P<0.001	R=-0.091 p=0.447					R=0.181 p=0.100	R=0.133 p=0.264
SMN						R=-0.124 p=0.282						R=0.051 p=0.661

Supplementary Table S2. Statistical differences and false discovery rate (fdr) for all the immune resp

Heatmap.

	OR	upper	lower	p	fdr
Serum.IgG..Spike.	7.39E+94	6.67E+12	1632.243	4.99E-11	9.49E-10
Serum.IgG..Nucleocapside.	43684815	3159.71	8.874408	0.031436	0.039818
Ratio.SARS.CoV.2.sp.mBC	1.110838	1.03992	14.6629	0.008939	0.012132
INFg_Spike	2.66E+18	31853.23	59.8249	8.81E-05	0.000209
INFg_Membrane	3.41E+08	98.6596	72.15894	4.38E-05	0.000119
INFg_Nucleocapside	1621058	180.151	15.68984	0.007037	0.010286
INFg_Mean	1.46E+11	374.6006	76.56793	3.49E-05	0.000111
IL2_Spike	3.17E+21	86520.29	77.87009	3.27E-05	0.000111
IL2_Membrane	8.71E+10	256.8614	93.68414	1.6E-05	0.000111
IL2_Nucleocapside	2.17E+09	841.1752	24.3306	0.001901	0.00301
IL2_Mean	5.36E+13	1330.919	80.99657	2.82E-05	0.000111
INFg_IL2_Spike	437657.6	31.81584	42.69751	0.000295	0.000561
INFg_IL2_Membrane	78895.12	16.4289	56.17528	0.000111	0.000234
INFg_IL2_Nucleocapside	217.8021	5.180204	26.39057	0.00147	0.00254
INFg_IL2_Mean	11310.33	8.312855	82.03516	2.68E-05	0.000111
IL21_Spike	0.194398	13665.76	0.841984	0.863796	0.863796
IL21_Membrane	248.8279	76080.15	1.63368	0.62464	0.659343
IL21_Nucleocapside	1.62E+20	2.07E+12	5.16087	0.103981	0.123478
IL21_Mean	1018.098	3058.281	2.37014	0.390272	0.436187

Supplementary Table S3. IgG titters against Spike and Nucleoprotein (median UA/ml [IQR]), according to immunosuppression status and infection severity.

		SOT	IC	P Value
IgG Spike titters (UA/ml)		108 [25.85-396.5]	85.8 [16.5-398.5]	p=0.581
IgG Nucleoprotein titters (UA/ml)		6.73 [0.665-33]	34.3 [4.425-75.625]	P=0.027
IgG Spike titters (UA/ml)	SEV	277.5 [172.75-763.25]	544 [228-800]	p=0.274
	MILD	76.7 [30.425-209.75]	20.9 [15.5-45.2]	p=0.034
	ASYMP	0 [0-44.1]	19.62 [0-112.775]	p=0.315
IgG Nucleoprotein titters (UA/ml)	SEV	15.62 [4.025-33.375	61.8 [36.2-92.1]	p<0.001
	MILD	7.055 [0.405-49.55]	14.2 [2.6-56.3]	p=0.403
	ASYMP	0.08 [0.08-3.24]	3.345 [0.08-46.4]	p=0.661

Supplementary Table S4. Ratio of IgG producing memory B cells (median [IQR]) against RBD, according to immunosuppression status and infection severity. RBD = receptor binding domain.

		SOT	IC	P Value
Ratio of SARS-CoV-2 IgG-producing mBC		0.0134 [0-0.0557]	0.0116 [0-0.054]	p=0.883
Ratio of SARS-CoV-2 IgG-producing mBC	SEV	0.0193 [0.006-0.0554]	0.0588 [0.0133-0.1889]	p=0.355
	MILD	0.0093 [0-0.988]	0.0136 [0.002-0.0351]	p=0.808
	ASYMP	0 [0-0.0636]	0 [0-0.0306]	p=0.898

Supplementary Table S5. Specific T cell responses (median spots [IQR]) against SARS-CoV-2 (median of SMN antigens). according to immunosuppression status and infection severity.

		SOT	IC	P Value
IFN-γ		17 [1-52]	18 [4-43.75]	p=0.742
IL2		9 [0-40]	9 [3-35]	p=0.575
IFN-γ/IL2		3 [0-14]	5.5 [2-14]	p=0.271
IL21		1 [0-21.5]	11.5 [0-81.5]	p=0.204
IFN-γ	**SEV**	19 [2-68]	37 [22-81]	p=0.124
	MILD	22 [1-57]	10 [4-28]	p=0.751
	ASYMP	4 [0-17.5]	0 [0-14]	p=0.315
IL2	**SEV**	14 [3-71]	49 [14-90]	p=0.111
	MILD	7 [0-52]	7 [3-11]	p=0.954
	ASYMP	0 [0-1]	0 [0-1]	p=0.497
IFN-γ/IL2	**SEV**	5 [0.5-20]	15 [8-32]	p=0.047
	MILD	5 [0-15	4 [2-7]	p=0.795
	ASYMP	0 [0-2.5]	0 [0-0.5]	p=0.661
IL21	**SEV**	3 [0-41.5]	12 [0-109]	p=0.688
	MILD	0 [0-22]	33 [0-66]	p=0.057
	ASYMP	5 [0-17.5]	0 [0-57.25]	p=0.497

Supplementary Table S6. Hierarchical cytokine profile of T cell responses against main structural SARS-CoV-2 proteins Spike (S), Membrane (M) and Nucleoprotein (N). Frequencies of IFN-γ, IL-2, IFN-γ/IL-2 and IL-21-producing T cells were assessed among the six groups of study.

			SOT	IC	P Value
IFN-γ	SEV	S	70 [2.5-162.5]	66 [31-162]	p=0.250
		M	16 [0-46.5]	37 [22-94]	p=0.013
		N	15 [0-68]	22 [8-52]	p=0.520
	MILD	S	41 [10-90]	16 [7-34]	p=0.128
		M	4 [0-32]	8 [4-23]	p=0.767
		N	18 [0-57]	10 [4-28]	p=0.795
	ASYMP	S	1 [0-25]	2 [0-11]	p=0.878
		M	4 [1.5-12]	0 [0-14.5]	p=0.377
		N	5 [0-13.5]	0 [0-8.25]	p=0.400
IL2	SEV	S	48 [12.5-158]	70 [35-180]	p=0.292
		M	12 [2-52]	50 [25-92]	p=0.009
		N	8 [0-52]	25 [11-64]	p=0.040
	MILD	S	15 [5-124]	11 [6-17]	p=0.305
		M	7 [1-22]	5 [2-9]	p=0.531
		N	2 [0-31]	7 [2-12]	p=0.544
	ASYMP	S	1 [0-2.5]	0 [0-5.5]	p=0.904
		M	0 [0-2]	0 [0-5.5]	p=0.573
		N	0 [0-1.5]	0 [0-0]	p=0.466
IFN-γ/IL2	SEV	S	6 [2-36]	27 [14-45]	p=0.011
		M	2 [0-18]	21 [14-43]	p=0.001
		N	2 [0-11]	6 [2-19]	p=0.161
	MILD	S	2 [3-47]	5 [2-7]	p=0.357
		M	2 [0-9]	4 [2-6]	p=0.343
		N	1 [0-10]	3 [2-5]	p=0.800
	ASYMP	S	0 [0-3.5]	0 [0-4.25]	p=0.987
		M	0 [0-1]	0 [0-1.5]	p=0.947
		N	0 [0-3]	0 [0-0.5]	p=0.537
IL21	SEV	S	6 [0-51]	0 [0-86]	p=0.915
		M	3 [0-24.5]	10 [0-144]	p=0.452
		N	0 [0-28.5]	12 [0-109]	p=0.704
	MILD	S	1 [0-37]	37 [0-114]	p=0.325
		M	0 [0-19]	27 [0-77]	p=0.163
		N	0 [0-18]	25 [0-116]	p=0.720
	ASYMP	S	2 [0-13]	0 [0-116.5]	p=0.968
		M	6 [0-38.5]	0 [0-69]	p=0.497
		N	0 [0-12]	0 [0-1.75]	p=0.831

Supplementary Table S7. Univariate and multivariate analyses based on binary logistic regression
influencing persistence of SARS-CoV-2 antibody and cellular responses among SOT after 6 month
Gender: female. * Reference symptoms: asymptomatic. OR = Odds Ratio; Uni.=Univariate analysis; M

	IgG-Spike		IgG-Nucleoprotein		IFN-γ		IL2		
	Univ. OR (95% CI)	**Multiv.**	**Univ. OR (95% CI)**	**Multiv. OR (95% CI)**	**Univ. OR (95% CI)**	**Multiv. OR (95% CI)**	**Univ. OR (95% CI)**	**Multiv. OR (95% CI)**	**U (9**
Time after Transplant (years)	1.12 (0.97-1.29); p=0.137	NA	**1.16 (1.01-1.35) P=0.034**	**1.20 (1.02-1.40) p=0.020**	**1.21 (1.03-1.43) p=0.021**	**1.4 (1.08-1.83) p=0.013**	1.05 (0.96-1.16) p=0.239	**NA**	**(1 p**
Age (years)	1.01 (0.95-1.06) p= 0.86	NA	0.98 (0.94-1.03) p= 0.642	NA	1.05 (0.99-1.10) p=0.078	1.07 (0.99-1.15) p=0.086	**1.08 (1.02-1.15) p=0.007**	**1.11 (1.02-1.21) p=0.008**	(1 p
Gender (M/F)	1.01 (0.22-4.53) p=0.98	NA	0.81 (0.22-2.93) p=0.75	NA	0.45 (0.10-0.92) p=0.291	NA	1.05 (0.29-3.8) p=0.946	NA	0.5 03
Symptoms (YES/NO)	**20 (3.56-112.29) p=0.001**	NA	**4.25 (0.95-18.84) p=0.057**	**7.17 (1.13-45.48) p=0.037**	**6.89 (1.43-33.18) p=0.016**	**39.57 (1.83-611.07) p=0.018**	**32 (3.48-294.2) p=0.002**	**79.2 (4.44-1412.39) p=0.003**	(0.8 p

4- Supplementary References

S1- Luque S, Lúcia M, Crespo E, Jarque M, et al. A multicolour HLA-specific B-cell FluoroSpot assay to functionally track circulating HLA-specific memory B cells. J Immunol Methods. 2018;462(July):23-33. doi:10.1016/j.jim.2018.07.011

S2- Lúcia M, Luque S, Crespo E, et al. Preformed circulating HLA-specific memory B cells predict high risk of humoral rejection in kidney transplantation. Kidney Int. 2015;88(4):874-887. doi:10.1038/ki.2015.205

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V. DISCUSSION

The coronavirus disease 2019 (COVID-19) pandemic posed a significant burden on global healthcare systems, collapsing due to the unprecedented viral spread and the lack of information about the new pathogen. Although immunosuppression has long been considered a risk factor for severe infections, early reports on cytokine-mediated inflammation as a feature of COVID-19 raised questions about its protective role in this setting[36–39] and the distinct expressions that the disease might adopt among the immunocompromised host[40].

In solid organ transplantation (SOT), defining the interplay between infection and adaptive immunity was important to weigh the risks and the benefits of immunosuppression adjustment during acute infection. The use of non-proven therapies and their potential interactions with anti-rejection drugs added further complexity to these patients' management. Given the lack of available data, a main goal was to define the clinical characteristics and the primary outcomes for COVID-19 in this particularly vulnerable patient population, aiming at identifying those at higher risk for progression. On the other hand, a better understanding of the precise kinetics of SARS-CoV-2 specific adaptive responses as well as their magnitude, immunodominance, and durability in SOT were also key issues to solve in order to ultimately improve our knowledge of immunosuppression's clinical impact and gain insight into immune protection.

Although the advent of SARS-CoV-2 vaccines provided robust protection against hospitalization, allowing large communities with high immunization rates to adopt less stringent public health measures, SOT remained at high risk for progression to severe disease, given the challenged immunity of these patients as compared with the general population. Understanding the distinct contribution of SARS-CoV-2 vaccines and infection to immune memory and their interaction with clinical and demographic factors has been essential to improve the current management of this vulnerable population.

Notwithstanding, this doctoral thesis was meant to provide some new insight into all these questions and addressed some of these concerns through three different studies by which we characterized the clinical course and the adaptive immune trajectories of

COVID-19 in the SOT population at the time of the Pandemic onset, yet, prior any active immunization scheme with novel vaccines were available. Here, characterization of main clinical and epidemiological factors rending higher susceptibility to worse clinical outcomes as well as defining de novo adaptive immunity to SARS-CoV-2 at the time of the first infection and during short and mid-term convalescence were the main goals.

Several single-center cohorts of COVID-19 among SOT were published early after the outbreak. However, many patients in these series remained hospitalized at the time of publication[179,180], which undermined the precise assessment of hard outcomes; this is important, given that >20% of SOT deaths occur after the first month of hospitalization[181]. Furthermore, severe COVID-19 cases were reported as ICU admissions; considering the limited capacity of the most fragile health systems[182], thus a more objective metric was necessary to define severity. Therefore, risk factors for severe COVID-19 remained unexplored for the SOT population. Hence, **our first work** aimed at comprehensively characterizing COVID-19 in a multicentric cohort of kidney transplant (KT) recipients while defining main risk factors for severe outcomes, on top of chronic immunosuppressive therapy[183]. We established the adult respiratory distress syndrome (ARDS) as a primary outcome, while reporting a complete follow-up (discharged/deceased) for all individuals.

In this first study, we showed that moderate-severe COVID-19 in SOT resembled that of the general population, with fever as a guiding symptom and significantly increased inflammatory markers among those with the poorest outcomes[17]. Hence, immunosuppression did not seem to modify the inflammatory clinical pattern of COVID-19, as previously suggested in some reports[40,179]. ARDS was the leading cause of in-hospital mortality, conferring a 11.4 fold mortality risk. In our patient cohort, 54% of the cohort met this diagnosis without significant age differences between groups, contrary to previous reports on the general population[184]. In addition, half of the patients developed acute kidney injury. Several studies reported a varying incidence of AKI among the general population, ranging from 30 to 40%, with increased proportions among those with preexisting chronic kidney disease or requiring intensive care[23], in line with our findings. Of note, supra-therapeutic CNI levels were significantly prevalent among individuals developing severe AKI (KDIGO II-III), collectively supporting that KT

patients might be at higher risk for this complication. Furthermore, these data underscored the harmful consequences of the indiscriminate use of some therapies, such as protease inhibitors, later failing to demonstrate their efficacy[185]. On the other hand, while we did not find any association between age and the risk of developing ARSD, we cannot exclude that the sample size of our study could have hampered this observation. This disease spectrum derived in a high mortality rate of 26.7%, in line with other hospitalized cohorts of SOT[186,187]. Now, the elderly were at the highest risk for mortality, suggesting that while all KT were susceptible to severe forms, fatal outcomes converged around the oldest.

Finally, in our patient cohort, a generalized immunosuppression reduction in most patients (>90%) was done, especially among those progressing to severe disease. Indeed, and akin to other viral infections, transient interruption of immunosuppression has been widespread during the pandemic, especially among this specific patient population, where high pharmacological interactions with adverse events were observed[186]. Nevertheless, the optimal management in this setting remains uncertain, given the lack of supportive evidence. In our work, we did not report any rejection episodes, while a more extensive study described 1.7% of allograft rejection after COVID-19, especially among the youngest and those recently transplanted[188]. The risk and benefit balance of immunosuppression withdrawal must be carefully considered in every case.

Our study has some limitations. Larger cohorts established additional risk factors in SOT, such as age, recent transplantation[189] or chronic mycophenolate use[190]; these factors did not show statistical significance in our study, which our limited sample might explain. Furthermore, we exclusively focused on hospitalized KT, underestimating the real burden of COVID-19 in this population. A massive antibody testing of 855 consecutive KT revealed a seroprevalence of 10.4%, while just 3.9% reported a previous diagnosis, thus highlighting the burden of undiagnosed infections[191]. Consequently, we acknowledge that our results on mortality are restricted to moderate forms, resembling the outcomes of severe COVID-19 in non-SOT populations during the first wave of the pandemic[33]. These figures lead some authors to question the effects of immunosuppression on the disease[34,35]; however, it is crucial to underscore that only

those studies comprising all outpatient diagnoses can provide a reliable comparison between SOT and the general population, showing in these cases an increased progression rate to hospitalization and death for SOT.[32,163,164]

In addition to investigating main clinical and epidemiological variables associated with distinct clinical outcomes during acute COVID-19, measuring adaptive immune responses to SARS-CoV-2 in SOT was an additional relevant objective in order to gain insight into each individuals' capability to respond during acute viral infection as well as to prevent future reinfections.

In **the second study** of this thesis[192], we investigated the magnitude and kinetics of both serological and specific T-cell responses to the four structural viral antigens (S,M,N,E) in SOT during the acute infection phase. Importantly, due to the absence of standardized cut-offs for humoral and cellular readouts, we included a control group of immunocompetent (IC) subjects admitted with similar COVID-19 severity at identical time points. Herein, we first showed that SOT are capable of developing a wide range of SARS-CoV-2 specific adaptive responses, comparable to IC patients, after COVID-19. Antiviral cytokine-producing T-cell responses were assessed with a multicolor FluoroSpot, showing a Th1 skew with high frequencies for SARS-CoV-2 specific IFN-γ, IL-2 and co-producing IFN-γ/IL-2 T cells. While IL-5 responses were barely identified, IL-6-producing T-cell frequencies were detected in all patients, including in healthy controls (HC), suggesting an antigen-independent production[193]. As described in the general population, Spike-specific T cells exhibited the greatest frequencies, whereas the Envelope was the less immunogenic of the viral structure. Likewise, all the participants showed detectable IgG antibodies at short-term convalescence, regardless of their immunosuppression state.

Nonetheless, at the disease onset, SOT showed lower T-cell frequencies (especially against M) and IgG seroconversion rates compared with IC individuals, displaying a more persistently detectable IgM in the circulation during the follow-up. Given the homogeneous disease severity of both groups at the enrollment, these data suggest a certain immune delay among SOT, as also reported by others[170,194]. In our work, SOT displayed higher rates of mechanical ventilation and death. Specifically, those SOT with

the poorest outcomes revealed even further immune abrogation at this phase, showing the lowest IgG seroconversion rates (62.5%) and IL-2-specific T-cell frequencies at the baseline. We believe that, although the number of patients evaluated is low, this may be a pretty good prognostic biomarker of disease outcomes among this patient population.

Longitudinal immune analyses in the general population support the critical role of early adaptive immunity during COVID-19. Indeed, prompt SARS-CoV-2 specific CD4+ and CD8+ responses have been associated with milder disease[98,117] and shorter viral clearance[116]. Of note, increasing SARS-CoV-2-specific T-cell frequencies and antibody titters negatively correlate to viral loads during the infection[193,195], indicating a dynamic interplay between de novo immune responses and viral shedding. Conversely, delayed humoral responses have also been associated with the poorest outcomes[105,114,115]. Hence, the available literature suggests an early critical time window to limit disease severity and improve viral control. In fact, interventional trials have demonstrated significant benefits from antibody-based therapies when administered shortly after the diagnosis[196,197], lacking efficacy in severely affected individuals[198]. In line with these data, our work suggests that chronic immunosuppression might influence the early immune trajectories of SOT, ultimately leading to a longer viral shedding and progression risk reported in the literature.

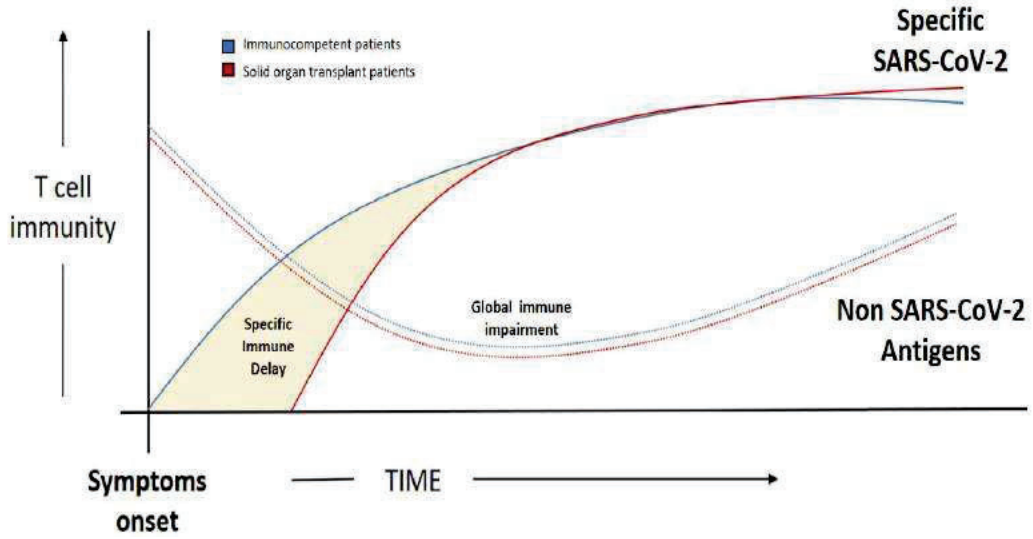
We did not find evidence for T-cell immune cross-reactivity in our study, as reported by others[106,199]. Importantly, we used the same stimuli and readout methods in a cohort of PBMC from waitlisted KT patients stored in our biobank from up to 2 years before the pandemic outbreak. However, cross-recognition has been described in a wide range of patients, in between 20-50% of pre-pandemic donors[127,130,200]. These discrepancies are likely to rely on distinct antigenic dominance and cell-stimulation methods. On the one hand, un-exposed donors show the highest reactivity against non-structural proteins (NSP), with marginal responses directed to structural (S,M,N) viral antigens[127,200]. On the other, the greatest proportions of cross-reactivity have been reported when employing prolonged times for in vitro PBMC stimulation (9-15 days)[130,140], leading to increased detection of low-frequency naive but not memory T-cell responses. The shorter

stimulation period and the restricted usage of structural proteins in our assays may explain these results.

Last, but not least, we further investigated the non-antigen-specific T-cell effector functions during COVID-19 by means of Influenza peptide and PWM polyclonal stimulation in order to understand the status of the global adaptive immune response in these patients. Remarkably, both groups displayed significantly reduced IFN-γ and IL-2 responses compared to the HC group, which progressively restored over time, indicating a global T-cell functional impairment as a hallmark of the acute disease. Other works have also reported a decreased cytokine-producing capacity after ex-vivo pan-T-cell stimulation (non-SARS-CoV-2 antigens) in COVID-19 patients[201–203]. These findings might explain, to some extent, the significant incidence of other viruses reactivation (mainly herpesviridae) reported in 20-30% of severe infections[204,205]. Accordingly, many studies suggested an exhausted CD8+ phenotype as a pathogenic immune mechanism based on the expression of inhibitory receptors such as PD-1[206]. Nonetheless, despite these features in the global T-cell compartment, specific SARS-CoV-2 clones show intact effector capacities[207], already in a very early phase of the disease.

In sum, our second study shows that SARS-CoV-2 elicits robust adaptive immune responses also among SOT, albeit with a notable delay in their kinetics (**Figure 6**). Moreover, we describe a functional abrogation of the non-specific T-cell compartment

Figure 6: De novo adaptive immune kinetics during COVID-19 in SOT



during infection, altogether challenging the hypothesis of the beneficial role of T-cell targeting immunosuppression in COVID-19[37,38].

Since immunological memory is the basis for durable protective immunity, exploring the maintenance and magnitude of the SARS-CoV-2 immunity elicited in SOT may have important implications for guiding preventive strategies. Our **third study** aimed to carefully evaluate both humoral and cellular immune memory in a cross-sectional comparison of 53 SOT and 49 IC individuals beyond six months after COVID-19. Given the broad spectrum of the disease with a significant proportion of asymptomatic infections, also among SOT[191], we included all forms of disease expression in our study to evaluate its influence and provide a more granular immune characterization.

While confirming that COVID-19 provides detectable peripheral immunity at the three main immune compartments (serological and memory B and T cells) in IC individuals, we showed that SOT are similarly capable of maintaining long-lasting immune memory responses after SARS-CoV-2 infection. Notably, robust immune responses were found among those SOT with the longest follow-up (up to 355 days after infection), thus suggesting that infection-derived immunity may last for long periods of time in this group of patients despite being on stable chronic immunosuppression.

In particular, 81.13% of SOT displayed detectable anti-Spike IgG antibodies, consistent with other published studies in SOT showing up to 82% of detection nine months after COVID-19[208]. Furthermore, we described for the first time the presence of long-lasting functional IgG-producing mBC in the circulation in 70% of convalescents SOT, demonstrating its capability to differentiate and produce specific antibodies upon antigen recall, which is of utmost importance considering the waning nature of circulating antibodies. Nonetheless, the longitudinal analysis conducted in a sub-group of severely affected patients confirmed a significant decline in anti-IgG titters.

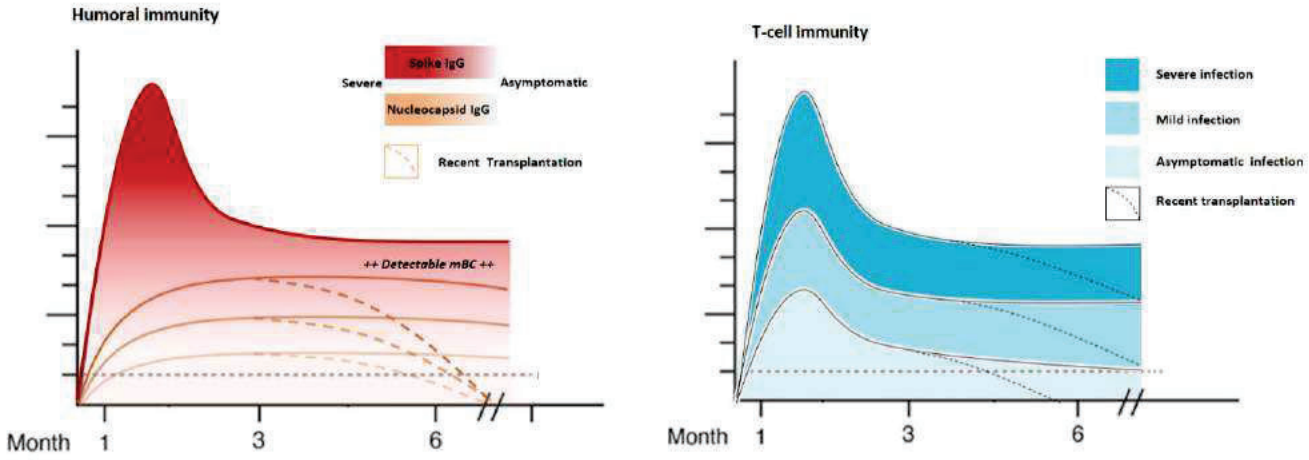
Comparable SARS-CoV-2 specific Th1 responses were also detected in both groups against all the structural (S,M,N) antigens, in accordance to previous reports[209]. On the other hand, a hierarchical reactivity was dominated by antigen S, as reported by others[106,127,209], including the second study of this thesis[192].

To note, although no major differences were generally observed between SOT and IC, the former group displayed relatively reduced anti-N antibody titers[210,211] and a clearer decline of SARS-CoV-2-reactive cytokine-producing T-cell frequencies over time. In addition, while the humoral and T-cell compartments positively correlated, this pattern was mainly driven by the IC group, which might also reflect, to some extent, the effects of chronic immunosuppression[209]. However, the most significant evidence of immunosuppression's influence was the faster immune waning associated with recent transplantation, which has also been reported in other infection settings[176,208] as well as in vaccine-derived[212–214] immunological studies.

Heterogeneity is a central feature of SARS-CoV-2-induced immunity. Notably, a gradient of immune responses from the more severe to the milder and asymptomatic groups was clearly delineated in both groups, with less than 40% of asymptomatic infections showing detectable responses after six months. Thus, our study describes that disease severity was the most important factor determining long-term immunity, in accordance with other works done within the general[103,104,107] and SOT[208] population. A potential explanation for these findings relies on the viral kinetics during infection. SARS-CoV-2 plasma viral loads have been associated with poorer outcomes in hospitalized individuals[170,215]. In addition to severely affected subjects, the elderly and the immunosuppressed show the longest virus persistence[157]. Notably, these groups of patients exhibit the greatest specific T-cell responses and IgG titers upon recovery[216,217]. Hence, the available data suggest that a higher antigen exposure during infection might trigger more robust and long-lasting immune responses.

Thus, in this third study, we show that patients with a SOT are capable of maintaining long-lasting humoral and cellular immune memory after COVID-19, mainly determined by the degree of infection severity. However, these immune responses might be challenged among those recently transplanted in whom residual effects of strong induction therapy may still be present (**Figure 7**).

Figure *7*: Immunological memory after COVID-19 in SOT



These two last works do also have some limitations. First, the small sample size, which was directly influenced by the difficulty in obtaining biological samples during the pandemic. Given the low number of asymptomatic diagnoses during 2020, these patients were underrepresented in our third study; however, the consistent results observed within this group significantly counterbalance this constraint. Second, we could not describe the predominant T or B-cell subset phenotypes in our cohorts. Nonetheless, our FluoroSpot assay allowed their functional assessment at the single cell level, which provides an accurate qualitative and quantitative measure of cytokine-producing cells detected in the circulation, which ultimately describes their immune effector function. Also, the inclusion of a non-immunocompromised control group provided a better understanding of the magnitude of these responses. Finally, our immune evaluation was restricted to the original SARS-CoV-2 strain due to the infection period (March to October 2020), so we cannot fully ensure whether these data would replicate with the current VOC. Of note, a recent humoral assessment in kidney transplants showed that COVID-19 triggered higher neutralizing antibodies against distinct VOC than vaccinated naïve individuals, which was also valid for those infected with the original strain[218]. On the other hand, it has been shown that viral T-cell epitopes of current VOC, such as Omicron, remain conserved[151]. Even though our results do not show evidence of immunogenicity or protection for current variants, they provide

insights into the potential contribution of infection to the SARS-CoV-2 immune memory in SOT.

- *Perspective of the results of this Thesis studies with vaccine-derived immune responses in SOT*

According to the literature, the immune phenotypes defined in our works entirely diverge from those elicited by SARS-CoV-2 vaccination in naïve SOT subjects, defined by greater proportions of primary non-responders and a rapid decline following the three mRNA doses.

Thus far, direct comparisons between infection and vaccine-derived immunity have been barely investigated, given the modest impact of previous pathogens compared with the SARS-CoV-2 outbreak. However, some works in healthy individuals revealed a faster humoral decline and diminished cellular responses after Influenza vaccination compared to the viral infection[219,220], which has also been documented among SOT[221]. In this line, two distinct publications comparing SARS-CoV-2 infected and vaccinated SOT individuals revealed stronger serological responses in the former group[222,223], which is consistent with our findings.

The enhanced responses in previously infected SOT individuals after vaccination further support the role of convalescent immunity[224–227]. In this setting, even a single mRNA vaccine dose has shown to trigger significant antibody responses[228], achieving comparable IgG titers to healthy volunteers, thus underscoring the potent recall responses derived from prevailing immune memory.

Our findings on infection-derived immunity might have some implications for the SOT population. First, SARS-CoV-2-specific immune memory derived from previous SARS-CoV-2 infection should be highly considered in future vaccine immunogenicity studies in SOT, given the bias that this might confer. On the other hand, it is still a matter of investigation whether the immune responses elicited by COVID-19 in SOT are protective against re-exposure or disease progression. Notably, in a prospective study encompassing 873 renal transplant patients, while none of the convalescents (N=137) developed symptomatic reinfection during the follow-up, 20 individuals developed

COVID-19 in the un-exposed, vaccinated group (N=736), concluding that infection confers some grade of protection against subsequent COVID-19 in SOT[222].

Due to the increasing number of breakthrough infections combined with varying proportions of three/four booster vaccine doses, SOT patients might exhibit a mixture of infection and vaccine-derived immunity whose impact on protection should be addressed. This knowledge is urgently needed to improve patient-risk stratification, management, and the development of vaccination policies for this more vulnerable at-risk patient population.

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VI. CONCLUSIONS

- Chronic immunosuppression in SOT does not significantly modify the clinical presentation of COVID-19, characterized by systemic inflammation, with fever as the most commonly reported symptom.

- Adult respiratory distress syndrome (ARDS) is the leading cause of mortality in kidney transplant (KT) recipients infected with SARS-CoV-2, which may occur across all age groups, especially among obese and those with underlying pulmonary diseases.

- Hospitalized KT with COVID-19 have a remarkably high mortality rate, whit most deaths converging within the elderly, particularly those over 60 years old.

- SARS-CoV-2 infection elicits robust adaptive immune responses in SOT, comparable to immunocompetent individuals.

- SARS-CoV-2-Specific T-cell responses display a predominant Th1 cytokine pattern, with a clear immunodominance exhibited by the Spike antigen over the other structural viral proteins.

- Compared to immunocompetent patients, SOT show a relative delay in adaptive immune kinetics at the acute infection onset, which could explain the persistence of viral replication in this group of patients.

- A generalized T-cell functional abrogation defines moderate/severe COVID-19 in both immunosuppressed and immunocompetent individuals, and a particularly hampered seroconversion and IL-2-specific T-cell responses may be observed among those SOT with the poorest subsequent clinical outcomes.

- Patients with a solid organ transplant are capable of maintaining long-lasting peripheral immune memory at different adaptive immune compartments after COVID-19 infection, which is directly modulated by the clinical severity degree of COVID-19.

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VII. REFERENCES

1. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol. 2019;17(3):181–92.
2. Tyrrell D.A BML. CULTIVATION OF A NOVEL TYPE OF COMMON-COLD VIRUS IN ORGAN CULTURES. Br Med J. 1965;1(5448):1467–70.
3. https://www.who.int/publications/m/item/summary-of-probable-sars-cases-with-onset-of-illness-from-1-november-2002-to-31-july-2003 .
4. https://apps.who.int/iris/bitstream/handle/10665/361328/WHOEMCSR536E-eng.pdf?sequence=1 .
5. Li, W, Shi, Z, Yu, M, Ren, W, Smith, C, Epstein, J. H et al. Bats are natural reservoirs of SARS-like coronaviruses. Science . 2005;310(5748):676–9.
6. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Feb;382(8):727–33.
7. Wu Z, McGoogan JM. Characteristics of and Important Lessons from the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases from the Chinese Center for Disease Control and Prevention. JAMA - J Am Med Assoc. 2020;323(13):1239–42.
8. Zhou P, Yang X Lou, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature . 2020;579(7798):270–3.
9. Gorbalenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA, et al. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5(4):536–44.
10. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200311-sitrep-51-covid-19.pdf.
11. Helmy YA, Fawzy M, Elaswad A, Sobieh A, Kenney SP, Shehata AA. The COVID-19 pandemic: A comprehensive review of taxonomy, genetics, epidemiology, diagnosis, treatment, and control. J Clin Med. 2020;9(4):1225.
12. Jamison DA, Anand Narayanan S, Trovão NS, Guarnieri JW, Topper MJ, Moraes-Vieira PM, et al. A comprehensive SARS-CoV-2 and COVID-19 review, Part 1: Intracellular overdrive for SARS-CoV-2 infection. Eur J Hum Genet. 2022;30(8):889–98.
13. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell . 2020;181(2):281–92.
14. Lukassen S, Chua RL, Trefzer T et al. SARS-CoV-2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells. EMBO J. 2020;39(10):e105114.

15. Hou YJ, Okuda K, Edwards CE, Martinez DR, Asakura T, Dinnon KH 3rd et al. SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. Cell. 2020;182(2):429–46.

16. He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med. 2020 Apr;26(5):672–5.

17. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet . 2020 Mar;395(10229):1054–62.

18. Lamers MM, Haagmans BL. SARS-CoV-2 pathogenesis. Nat Rev Microbiol. 2022;20(5):270–84.

19. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N Engl J Med. 2020;383(2):120–8.

20. Borczuk AC, Salvatore SP, Seshan S V., Patel SS, Bussel JB, Mostyka M, et al. COVID-19 pulmonary pathology: a multi-institutional autopsy cohort from Italy and New York City. Mod Pathol . 2020;33(11):2156–68.

21. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nat Med . 2020;26(6):842–4.

22. Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med . 2020;26(10):1636–43.

23. Hirsch JS, Ng JH, Ross DW, Sharma P, Shah HH, Barnett RL, et al. Acute kidney injury in patients hospitalized with COVID-19. Kidney Int . 2020 Jul 1;98(1):209–18.

24. Conway EM, Mackman N, Warren RQ, Wolberg AS, Mosnier LO, Campbell RA et al. Understanding COVID-19-associated coagulopathy. Nat Rev Immunol. 2022;22(10):639–49.

25. Barber RM, Sorensen RJD, Pigott DM, Bisignano C, Carter A, Amlag JO, et al. Estimating global, regional, and national daily and cumulative infections with SARS-CoV-2 through Nov 14, 2021: a statistical analysis. Lancet. 2022;399(10344):2351–80.

26. https://covid19.who.int/.

27. COVID-19 Excess Mortality Collaborators. Estimating excess mortality due to the COVID-19 pandemic: a systematic analysis of COVID-19-related mortality, 2020-21. Lancet . 2022;399(10334):1513–36.

28. Ma Q, Liu J, Liu Q, Kang L, Liu R, Jing W, et al. Global Percentage of Asymptomatic SARS-CoV-2 Infections among the Tested Population and Individuals with Confirmed COVID-19 Diagnosis: A Systematic Review and Meta-analysis. JAMA Netw Open. 2021;4(12):1–18.

29. Oran DP, Topol EJ. Prevalence of Asymptomatic SARS-CoV-2 Infection : A Narrative Review. Ann Intern Med. 2020;173(5):362–7.

30. Team C-F. Variation in the COVID-19 infection–fatality ratio by age, time, and geography during the pre-vaccine era: a systematic analysis. Lancet . 2022;399(10334):1469–88.

31. Pastor-Barriuso R, Pérez-Gómez B, Hernán MA, Pérez-Olmeda M, Yotti R, Oteo-Iglesias J, et al. Infection fatality risk for SARS-CoV-2 in community dwelling population of Spain: Nationwide seroepidemiological study. BMJ. 2020;371.

32. Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, et al. OpenSAFELY: factors associated with COVID-19 death in 17 million patients. Nature . 2020 Jul 8;1–11.

33. Kompaniyets L, Pennington AF, Goodman AB et al. Underlying Medical Conditions and Severe Illness Among 540,667 Adults Hospitalized With COVID-19, March 2020-March 2021. Prev Chronic Dis. 2021;18(E66).

34. Chavarot N, Gueguen J, Bonnet G, Jdidou M, Trimaille A, Burger C et al. COVID-19 severity in kidney transplant recipients is similar to nontransplant patients with similar comorbidities. Am J Transpl. 2021;21(3):1285–94.

35. Molnar MZ, Bhalla A, Azhar A, Tsujita M, Talwar M, Balaraman V, et al. Outcomes of critically ill solid organ transplant patients with COVID-19 in the United States. Am J Transplant. 2020;20(11):3061–71.

36. Marx D, Moulin B, Fafi-Kremer S, Benotmane I, Gautier G PP et al. First case of COVID-19 in a kidney transplant recipient treated with belatacept. Am J Transplant. 2020;20(7):1944–6.

37. Willicombe M, Thomas D, McAdoo S. COVID-19 and Calcineurin Inhibitors: Should They Get Left Out in the Storm? J Am Soc Nephrol . 2020 Jun 1;31(6):1145 LP – 1146.

38. Belli LS, Fondevila C, Cortesi PA, Conti S, Karam V, Adam R, et al. Protective Role of Tacrolimus, Deleterious Role of Age and Comorbidities in Liver Transplant Recipients With Covid-19: Results From the ELITA/ELTR Multi-center European Study. Gastroenterology . 2021;160(4):1151-1163.

39. Kemmner S, Guba MO, Schönermarck U, Stangl M, Fischereder M. Cyclosporine as a preferred calcineurin inhibitor in renal allograft recipients with COVID-19 infection. Int J Pharm. 2020;634:2019–20.

40. Fung M, Babik JM. COVID-19 in Immunocompromised Hosts: What We Know so Far. Clin Infect Dis. 2021;72(2):340–50.

41. Kopp E, Medzhitov R. Recognition of microbial infection by Toll-like receptors. Curr Opin Immunol. 2003;15(4):396–401.

42. Collin M, Bigley V. Human dendritic cell subsets: an update. Immunology. 2018;154(1):3–20.

43. García-Sastre A, Biron CA. Type 1 interferons and the virus-host relationship: A lesson in détente. Science . 2006;312(5775):879–82.

44. Lodoen MB, Lanier LL. Natural killer cells as an initial defense against pathogens. Curr Opin Immunol. 2006;18(4):391–8.

45. Andoniou CE, van Dommelen SLH, Voigt V, Andrews DM, Brizard G, Asselin-Paturel C, et al. Interaction between conventional dendritic cells and natural killer cells is integral to the activation of effective antiviral immunity. Nat Immunol. 2005;6(10):1011–9.

46. Seth RB, Sun L, Chen ZJ. Antiviral innate immunity pathways. Cell Res. 2006;16(2):141–7.

47. Worbs T, Hammerschmidt SI, Förster R. Dendritic cell migration in health and disease. Nat Rev Immunol. 2017;17(1):30–48.

48. Parlato S, Santini SM, Lapenta C, Di Pucchio T, Logozzi M, Spada M, et al. Expression of CCR-7, MIP-3β, and Th-1 chemokines in type I IFN-induced monocyte-derived dendritic cells: Importance for the rapid acquisition of potent migratory and functional activities. Blood. 2001;98(10):3022–9.

49. Simmons DP, Wearsch PA, Canaday DH, Meyerson HJ, Liu YC, Wang Y, et al. Type I IFN Drives a Distinctive Dendritic Cell Maturation Phenotype That Allows Continued Class II MHC Synthesis and Antigen Processing. J Immunol. 2012;188(7):3116–26.

50. Kolumam GA, Thomas S, Thompson LJ, Sprent J, Murali-Krishna K. Type I interferons act directly on CD8 T cells to allow clonal expansion and memory formation in response to viral infection. J Exp Med. 2005;202(5):637–50.

51. ZINKERNAGEL, R., DOHERTY P. Immunological surveillance against altered self components by sensitised T lymphocytes in lymphocytes choriomeningitis. Nature. 1974;(251):547–548.

52. Babbitt, B., Allen, P., Matsueda, G., Haber E UE. Binding of immunogenic peptides to Ia histocompatibility molecules. Nature. 1985;(317):359–361.

53. Bretscher P CM. A theory of self-nonself discrimination. Science . 1970;169(3950):1042–9.

54. Linsley PS, Clark EA LJ. T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB-1. Proc Natl Acad Sci U S A. 1990;87(13):5031–5.

55. June CH, Ledbetter JA, Gillespie MM, Lindsten T TC. T-cell proliferation involving the CD28 pathway is associated with cyclosporine-resistant interleukin 2 gene expression. Mol Cell Biol. 1987;7(12):4472–81.

56. Butz EA BM. Massive expansion of antigen-specific CD8+ T cells during an acute virus infection. Immunity. 1998;8(2):167–75.

57. Zotos D, Coquet JM, Zhang Y, Light A, D'Costa K, Kallies A et al. IL-21 regulates

germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. J Exp Med. 2010;207(2):365–78.

58. Imkeller K, Wardemann H. Assessing human B cell repertoire diversity and convergence. Immunol Rev. 2018;284(1):51–66.

59. Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccine Immunol. 2010;17(7):1055–65.

60. Burton DR. Antibodies, viruses and vaccines. Nat Rev Immunol. 2002;2(9):706–13.

61. Pantaleo G, Demarest JF, Vaccarezza M, Graziosi C, Bansal GP, Koenig S, et al. Effect of anti-V3 antibodies on cell-free and cell-to-cell human immunodeficiency virus transmission. Eur J Immunol. 1995;25(1):226–31.

62. Burioni R, Williamson RA, Sanna PP, Bloom FE, Burton DR. Recombinant human Fab to glycoprotein D neutralizes infectivity and prevents cell-to-cell transmission of herpes simplex viruses 1 and 2 in vitro. Proc Natl Acad Sci U S A. 1994;91(1):355–9.

63. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for Preventing Infectious Complications among Hematopoietic Cell Transplantation Recipients: A Global Perspective. Biol Blood Marrow Transplant . 2009;15(10):1143–238.

64. Huber SR, van Beek J, de Jonge J, Luytjes W, van Baarle D. T cell responses to viral infections - opportunities for peptide vaccination. Front Immunol. 2014;5(APR):1–12.

65. Matloubian M, Concepcion RJ, Ahmed R. CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses during chronic viral infection. J Virol. 1994;68(12):8056–63.

66. Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science . 1999;283(5403):857–60.

67. Wilkinson TM, Li CKF, Chui CSC, Huang AKY, Perkins M, Liebner JC, et al. Preexisting influenza-specific CD4 + T cells correlate with disease protection against influenza challenge in humans. Nat Med. 2012;18(2):274–80.

68. Jarque M, Melilli E, Crespo E, Manonelles A, Montero N, Torras J, et al. CMV-specific Cell-mediated Immunity at 3-month Prophylaxis Withdrawal Discriminates D+/R+ Kidney Transplants at Risk of Late-onset CMV Infection Regardless the Type of Induction Therapy. Transplantation. 2018;102(11):e472–80.

69. Jarque M, Crespo E, Melilli E, Gutiérrez A, Moreso F, Guirado L, et al. Cellular Immunity to Predict the Risk of Cytomegalovirus Infection in Kidney Transplantation: A Prospective, Interventional, Multicenter Clinical Trial. Clin Infect Dis. 2020;71(9):2375–85.

70. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science . 2020;369(6504):718–24.

71. Galani IE, Rovina N, Lampropoulou V, Triantafyllia V, Manioudaki M, Pavlos E, et al. Untuned antiviral immunity in COVID-19 revealed by temporal type I/III interferon patterns and flu comparison. Nat Immunol . 2021;22(1):32–40.

72. Kim YM, Shin EC. Type I and III interferon responses in SARS-CoV-2 infection. Exp Mol Med . 2021;53(5):750–60.

73. Channappanavar R, Fehr AR, Vijay R, Mack M, Zhao J, Meyerholz DK, et al. Dysregulated Type I Interferon and Inflammatory Monocyte-Macrophage Responses Cause Lethal Pneumonia in SARS-CoV-Infected Mice. Cell Host Microbe. 2016;19(2):181–93.

74. Kuri-Cervantes L, Pampena MB, Meng W, Rosenfeld AM, Ittner CAG, Weisman AR, et al. Comprehensive mapping of immune perturbations associated with severe COVID-19. Sci Immunol. 2020;5(49).

75. Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. Cell . 2021;184(4):861–80.

76. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Møller R, et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. Cell . 2020;181(5):1036-1045.e9.

77. Miorin L, Kehrer T, Sanchez-Aparicio MT, Zhang K, Cohen P, Patel RS, et al. SARS-CoV-2 Orf6 hijacks Nup98 to block STAT nuclear import and antagonize interferon signaling. Proc Natl Acad Sci U S A. 2020;117(45):28344–54.

78. Kumar A, Ishida R, Strilets T, Cole J, Lopez-Orozco J, Fayad N, et al. SARS-CoV-2 Nonstructural Protein 1 Inhibits the Interferon Response by Causing Depletion of Key Host Signaling Factors. J Virol. 2021;95(13).

79. Zhou R, To KKW, Wong YC, Liu L, Zhou B, Li X, et al. Acute SARS-CoV-2 Infection Impairs Dendritic Cell and T Cell Responses. Immunity . 2020;53(4):864-877.

80. Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann HH, Zhang Y, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science . 2020;370(6515).

81. Zhang Q, Liu Z, Moncada-Velez M, Chen J, Ogishi M, Bigio B, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science . 2020;370(6515).

82. Jing Y, Shaheen E, Drake RR, Chen N, Gravenstein S, Deng Y. Aging is associated with a numerical and functional decline in plasmacytoid dendritic cells , whereas myeloid dendritic cells are relatively unaltered in human peripheral blood. Hum Immunol. . 2009;70(10):777–84.

83. Rosa BA, Ahmed M, Singh DK, Choreño-parra JA, Cole J, Jiménez-álvarez LA, et al. SARS-CoV-2 infection. Commun Biol . 2019;(2021):1–14.

84. Zhao J, Legge K, Perlman S, Zhao J, Zhao J, Legge K, et al. Age-related increases in PGD 2 expression impair respiratory DC migration , resulting in diminished T cell responses upon respiratory virus infection in mice. J Clin Invest. 2011;121(12):4921–30.

85. Salama C, Han J, Yau L, Reiss WG, Kramer B, Neidhart JD, et al. Tocilizumab in Patients Hospitalized with Covid-19 Pneumonia. N Engl J Med. 2021;384(1):20–30.

86. Abani O, Abbas A, Abbas F, Abbas M, Abbasi S, Abbass H, et al. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. Lancet. 2021;397(10285):1637–45.

87. DeDiego ML, Nieto-Torres JL, Regla-Nava JA, Jimenez-Guardeño JM, Fernandez-Delgado R, Fett C, et al. Inhibition of NF-κB-mediated inflammation in severe acute respiratory syndrome coronavirus-infected mice increases survival. J Virol. 2014;88(2):913–24.

88. Wu Y, Ma L, Cai S, Zhuang Z, Zhao Z, Jin S, et al. RNA-induced liquid phase separation of SARS-CoV-2 nucleocapsid protein facilitates NF-κB hyper-activation and inflammation. Sig Transduct Target Ther. 2021;(6):167.

89. Su, CM., Wang, L. & Yoo D. Activation of NF-κB and induction of proinflammatory cytokine expressions mediated by ORF7a protein of SARS-CoV-2. Sci Rep. 2021;(11):13464.

90. Israelow B, Mao T, Klein J, Song E, Menasche B, Omer SB, et al. Adaptive immune determinants of viral clearance and protection in mouse models of SARS-CoV-2. Sci Immunol. 2021;6(64):1–16.

91. Chen J, Lau YF, Lamirande EW, Paddock CD, Bartlett JH, Zaki SR, et al. Cellular Immune Responses to Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) Infection in Senescent BALB/c Mice: CD4 + T Cells Are Important in Control of SARS-CoV Infection . J Virol. 2010;84(3):1289–301.

92. Zhao J, Zhao J, Perlman S. T Cell Responses Are Required for Protection from Clinical Disease and for Virus Clearance in Severe Acute Respiratory Syndrome Coronavirus-Infected Mice. J Virol. 2010;84(18):9318–25.

93. McMahan K, Yu J, Mercado NB, Loos C, Tostanoski LH, Chandrashekar A, et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. Nature . 2021;590(7847):630–4.

94. Grifoni A, Sidney J, Zhang Y, Scheuermann RH, Peters B, Sette A. A Sequence Homology and Bioinformatic Approach Can Predict Candidate Targets for Immune Responses to SARS-CoV-2. Cell Host Microbe . 2020;27(4):671-680.e2.

95. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, 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.

96. Tarke A, Sidney J, Kidd CK, Dan JM, Ramirez SI, Yu ED, et al. Comprehensive

analysis of T cell immunodominance and immunoprevalence of SARS-CoV-2 epitopes in COVID-19 cases. Cell Reports Med . 2021;2(2):100204.

97. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med. 2020;26(6):845–8.

98. Rydyznski Moderbacher C, Ramirez SI, Dan JM, Grifoni A, Hastie KM, Weiskopf D, et al. Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. Cell . 2020;183(4):996-1012.e19.

99. Wajnberg A, Amanat F, Firpo A, Altman DR, Bailey MJ, Mansour M, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science . 2020;370(6521):1227–30.

100. Gudbjartsson DF, Norddahl GL, Melsted P, Gunnarsdottir K, Holm H, Eythorsson E, et al. Humoral Immune Response to SARS-CoV-2 in Iceland. N Engl J Med. 2020;383(18):1724–34.

101. Sokal A, Chappert P, Barba-Spaeth G, Roeser A, Fourati S, Azzaoui I, et al. Maturation and persistence of the anti-SARS-CoV-2 memory B cell response. Cell. 2021;184(5):1201-1213.

102. Piccoli L, Park YJ, Tortorici MA, Czudnochowski N, Walls AC, Beltramello M, et al. Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. Cell . 2020;183(4):1024-1042.e21.

103. Henss L, Scholz T, Von Rhein C, Wieters I, Borgans F, Eberhardt FJ, et al. Analysis of Humoral Immune Responses in Patients with Severe Acute Respiratory Syndrome Coronavirus 2 Infection. J Infect Dis. 2021;223(1):56–61.

104. Robbiani DF, Gaebler C, Muecksch F, Lorenzi JCC, Wang Z, Cho A, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. Nature. 2020;584(7821):437–42.

105. Lucas C, Klein J, Sundaram ME, Liu F, Wong P, Silva J, et al. Delayed production of neutralizing antibodies correlates with fatal COVID-19. Nat Med. 2021;27(7):1178–86.

106. Peng Y, Mentzer AJ, Liu G, Yao X, Yin Z, Dong D, et al. Broad and strong memory CD4+ and CD8+ T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. Nat Immunol. 2020;21(11):1336–45.

107. Zuo J, Dowell AC, Pearce H, Verma K, Long HM, Begum J, et al. Robust SARS-CoV-2-specific T cell immunity is maintained at 6 months following primary infection. Nat Immunol. 2021;22(5):620–6.

108. Le Bert N, Clapham HE, Tan AT, Chia WN, Tham CYL, Lim JM, et al. Highly functional virus-specific cellular immune response in asymptomatic SARS-CoV-2 infection. J Exp Med. 2021;218(5).

109. Sekine T, Perez-Potti A, Rivera-Ballesteros O, Strålin K, Gorin JB, Olsson A, et al.

Robust T Cell Immunity in Convalescent Individuals with Asymptomatic or Mild COVID-19. Cell. 2020;183(1):158-168.e14.

110. Cohen KW, Linderman SL, Moodie Z, Czartoski J, Lai L, Mantus G, et al. Longitudinal analysis shows durable and broad immune memory after SARS-CoV-2 infection with persisting antibody responses and memory B and T cells. Cell Reports Med . 2021;2(7):100354.

111. Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science . 2021;371(6529).

112. Sakharkar M, Rappazzo CG, Wieland-Alter WF, Hsieh CL, Wrapp D, Esterman ES, et al. Prolonged evolution of the human B cell response to SARS-CoV-2 infection. Sci Immunol. 2021;6(56):6916.

113. Thieme CJ, Anft M, Paniskaki K, Blazquez-Navarro A, Doevelaar A, Seibert FS, et al. Robust T Cell Response Toward Spike, Membrane, and Nucleocapsid SARS-CoV-2 Proteins Is Not Associated with Recovery in Critical COVID-19 Patients. Cell Reports Med . 2020;1(6):100092.

114. Zohar T, Loos C, Fischinger S, Atyeo C, Wang C, Slein MD, et al. Compromised Humoral Functional Evolution Tracks with SARS-CoV-2 Mortality. Cell . 2020;183(6):1508-1519.

115. Ren L, Zhang L, Chang D, Wang J, Hu Y, Chen H, et al. The kinetics of humoral response and its relationship with the disease severity in COVID-19. Commun Biol . 2020;3(1):1–7.

116. Tan AT, Linster M, Tan CW, Lye D, Wang L, Bertoletti A, et al. Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. CellReports . 2021;34(6):108728.

117. Chandran A, Rosenheim J, Nageswaran G, Swadling L, Pollara G, Gupta RK, et al. Rapid synchronous type 1 IFN and virus-specific T cell responses characterize first wave non-severe SARS-CoV-2 infections. Cell Reports Med. 2022;3(3).

118. Weiskopf D, Immunol S, Weiskopf D, Schmitz KS, Raadsen MP, Grifoni A, et al. Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. Sci Immunol. 2020;2071:1–15.

119. Vardhana S, Baldo L, Morice WG, Wherry EJ. Understanding T cell responses to COVID-19 is essential for informing public health strategies. Sci Immunol. 2022;7(71):eabo1303.

120. Yao C, Bora SA, Parimon T, Zaman T, Friedman OA, Palatinus JA, et al. Cell-Type-Specific Immune Dysregulation in Severely Ill COVID-19 Patients. Cell Rep. 2021;34(1).

121. Rha MS, Shin EC. Activation or exhaustion of CD8+ T cells in patients with COVID-19. Cell Mol Immunol . 2021;18(10):2325–33.

122. Ng OW, Chia A, Tan AT, Jadi RS, Leong HN, Bertoletti A, et al. Memory T cell

responses targeting the SARS coronavirus persist up to 11 years post-infection. Vaccine . 2016;34(17):2008–14.

123. Cao W-C, Liu W, Zhang P-H, Zhang F, Richardus JH. Disappearance of Antibodies to SARS-Associated Coronavirus after Recovery. N Engl J Med. 2007;357(11):1162–3.

124. Kaech, S., Wherry, E. & Ahmed R. Effector and memory T-cell differentiation: implications for vaccine development. Nat Rev Immunol. 2002;(2):251–262.

125. Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science . 2021;371(6529):1–23.

126. Cohen KW, Linderman SL, Moodie Z, Czartoski J, Lai L, Mantus G, et al. Longitudinal analysis shows durable and broad immune memory after SARS-CoV-2 infection with persisting antibody responses and memory B and T cells. Cell Rep Med. 2021 Jul 20;2(7):100354.

127. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. Cell. 2020 Jun;181(7):1489-1501.

128. Sette A, Crotty S. Pre-existing immunity to SARS-CoV-2: the knowns and unknowns. Nat Rev Immunol . 2020;20(8):457–8.

129. Braun J, Loyal L, Frentsch M, Wendisch D, Georg P, Kurth F, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. Nature. 2020 Nov;587(7833):270-274.

130. Mateus J, Grifoni A, Tarke A, Sidney J, Ramirez SI, Dan JM, et al. Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. Science . 2020;370(6512):89–94.

131. Swadling L, Diniz MO, Schmidt NM, Amin OE, Chandran A, Shaw E et al. Pre-existing polymerase-specific T cells expand in abortive seronegative SARS-CoV-2. Nature. 2022;601(7891):110–7.

132. Bert N Le, Tan AT, Kunasegaran K, Tham CYL, Hafezi M, Chia A, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS , and uninfected controls. Nature. 2020 Aug;584(7821):457-462.

133. Yang Y, Yang M, Peng Y, Liang Y, Wei J, Xing L, et al. Longitudinal analysis of antibody dynamics in COVID-19 convalescents reveals neutralizing responses up to 16 months after infection. Nat Microbiol. 2022;7(3):423–33.

134. Li C, Yu D, Wu X, Liang H, Zhou Z, Xie Y, et al. Twelve-month specific IgG response to SARS-CoV-2 receptor-binding domain among COVID-19 convalescent plasma donors in Wuhan. Nat Commun . 2021;12(1):1–9.

135. Rogers TF, Zhao F, Huang D, Beutler N, Burns A, He WT, et al. Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. Science . 2020;369(6506):956–63.

136. Turner JS, Kim W, Kalaidina E, Goss CW, Rauseo AM, Schmitz AJ, et al. SARS-CoV-2 infection induces long-lived bone marrow plasma cells in humans. Nature. 2021;595(7867):421–5.

137. Guo L, Wang G, Wang Y, Zhang Q, Ren L, Gu X, et al. SARS-CoV-2-specific antibody and T-cell responses 1 year after infection in people recovered from COVID-19: a longitudinal cohort study. The Lancet Microbe . 2022;3(5):e348–56.

138. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, Elliott J, Hofmann C, Hausner MA, et al. Rapid Decay of Anti-SARS-CoV-2 Antibodies in Persons with Mild Covid-19. N Engl J Med. 2020 Sep 10;383(11):1085-1087.

139. Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med. 2020;26(8):1200–4.

140. Zhang J, Lin H, Ye B, Zhao M, Zhan J, Dong S, et al. One-Year Sustained Cellular and Humoral Immunities in Coronavirus Disease 2019 (COVID-19) Convalescents. Clin Infect Dis. 2022 Aug 24;75(1):e1072-e1081.

141. Gaebler C, Wang Z, Lorenzi JCC, Muecksch F, Finkin S, Tokuyama M, et al. Evolution of antibody immunity to SARS-CoV-2. Nature. 2021 Mar;591(7851):639-644.

142. Hodgson SH, Mansatta K, Mallett G, Harris V, Emary KRW, Pollard AJ. What defines an efficacious COVID-19 vaccine? A review of the challenges assessing the clinical efficacy of vaccines against SARS-CoV-2. Lancet Infect Dis . 2021;21(2):e26–35.

143. Leidi A, Koegler F, Dumont R, Dubos R, Zaballa ME, Piumatti G, et al. Risk of Reinfection after Seroconversion to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): A Population-based Propensity-score Matched Cohort Study. Clin Infect Dis. 2022;74(4):622–9.

144. Lumley SF, O'Donnell D, Stoesser NE, Matthews PC, Howarth A, Hatch SB, et al. Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers. N Engl J Med. 2021;384(6):533–40.

145. Hall VJ, Foulkes S, Charlett A, Atti A, Monk EJM, Simmons R, et al. SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicentre, prospective cohort study (SIREN). Lancet. 2021;397(10283):1459–69.

146. Nordström P, Ballin M, Nordström A. Risk of SARS-CoV-2 reinfection and COVID-19 hospitalisation in individuals with natural and hybrid immunity: a retrospective, total population cohort study in Sweden. Lancet Infect Dis. 2022;22(6):781–90.

147. Altarawneh HN, Chemaitelly H, Hasan MR, Ayoub HH, Qassim S, AlMukdad S, et al. Coyle P, Yassine HM, Al-Khatib HA, Benslimane FM, Al-Kanaani Z, Al-Kuwari E, Jeremijenko A, Kaleeckal AH, Latif AN SR. Protection against the Omicron Variant from Previous SARS-CoV-2 Infection. N Engl J Med. 2022;386(13):1288–

90.

148. Madhi SA, Kwatra G, Myers JE, Jassat W, Dhar N, Mukendi CK, et al. Population Immunity and Covid-19 Severity with Omicron Variant in South Africa. N Engl J Med. 2022;386(14):1314–26.

149. Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM, et al. SARS-CoV-2 variants, spike mutations and immune escape. Nat Rev Microbiol . 2021;19(7):409–24.

150. Rössler A, Riepler L, Bante D, von Laer D KJ. SARS-CoV-2 Omicron Variant Neutralization in Serum from Vaccinated and Convalescent Persons. N Engl J Med. 2022;386(7):698–700.

151. Gao Y, Cai C, Grifoni A, Müller TR, Niessl J, Olofsson A et al. Ancestral SARS-CoV-2-specific T cells cross-recognize the Omicron variant. Nat Med. 2022;28(3):472–6.

152. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med . 2021;27(7):1205–11.

153. Kent SJ, Khoury DS, Reynaldi A, Juno JA, Wheatley AK, Stadler E, et al. Disentangling the relative importance of T cell responses in COVID-19: leading actors or supporting cast? Nat Rev Immunol. 2022;22(6):387–97.

154. Barouch DH. Covid-19 Vaccines — Immunity, Variants, Boosters. N Engl J Med. 2022;387(11):1011–20.

155. Britanova O V., Putintseva E V., Shugay M, Merzlyak EM, Turchaninova MA, Staroverov DB, et al. Age-Related Decrease in TCR Repertoire Diversity Measured with Deep and Normalized Sequence Profiling. J Immunol. 2014;192(6):2689–98.

156. Joseph M, Wu Y, Dannebaum R, Rubelt F, Zlatareva I, Lorenc A, et al. Global patterns of antigen receptor repertoire disruption across adaptive immune compartments in COVID-19. Proc Natl Acad Sci U S A. 2022;119(34).

157. Xu K, Chen Y, Yuan J, Yi P, Ding C, Wu W, et al. Factors associated with prolonged viral RNA shedding in patients with coronavirus disease 2019 (COVID-19). Clin Infect Dis. 2020;71(15):799–806.

158. Kim SE, Jeong HS, Yu Y, Shin SU, Kim S, Oh TH, et al. Viral kinetics of SARS-CoV-2 in asymptomatic carriers and presymptomatic patients. Int J Infect Dis . 2020;95:441–3.

159. Wu F, Liu M, Wang A, Lu L, Wang Q, Gu C, et al. Evaluating the Association of Clinical Characteristics with Neutralizing Antibody Levels in Patients Who Have Recovered from Mild COVID-19 in Shanghai, China. JAMA Intern Med. 2020;180(10):1356–62.

160. Yang HS, Costa V, Racine-Brzostek SE, Acker KP, Yee J, Chen Z, et al. Association of Age with SARS-CoV-2 Antibody Response. JAMA Netw Open. 2021;4(3):1–12.

161. Singson JRC, Kirley PD, Pham H, Rothrock G, Armistead I. Factors Associated with Severe Outcomes Among Immunocompromised Adults Hospitalized for COVID-19 — COVID-NET , 10 States , March 2020 – February 2022. 2022;71(27).

162. Maggiore U, Riella L V., Azzi J, Cravedi P. Mortality in solid organ transplant recipients with COVID-19: More than meets the eye. Am J Transplant. 2022;22(5):1496–7.

163. Trapani S, Masiero L, Puoti F, Rota MC, Del Manso M, Lombardini L, et al. Incidence and outcome of SARS-CoV-2 infection on solid organ transplantation recipients: A nationwide population-based study. Am J Transplant. 2021;21(7):2509–21.

164. Ao G, Wang Y, Qi X et al. The association between severe or death COVID-19 and solid organ transplantation: A systematic review and meta-analysis. Transpl Rev. 2021;35(3):100628.

165. Dandachi D, Geiger G, Montgomery MW, Karmen-Tuohy S, Golzy M, Antar AAR, et al. Characteristics, Comorbidities, and Outcomes in a Multicenter Registry of Patients With Human Immunodeficiency Virus and Coronavirus Disease 2019. Clin Infect Dis. 2021;73(7):e1964–72.

166. Nomah DK, Reyes-Urueña J, Díaz Y, Moreno S, Aceiton J, Bruguera A, et al. Sociodemographic, clinical, and immunological factors associated with SARS-CoV-2 diagnosis and severe COVID-19 outcomes in people living with HIV: a retrospective cohort study. Lancet HIV. 2021;8(11):e701–10.

167. Bange EM, Han NA, Wileyto P, Kim JY, Gouma S, Robinson J, et al. CD8+ T cells contribute to survival in patients with COVID-19 and hematologic cancer. Nat Med . 2021;27(7):1280–9.

168. Scherer EM, Babiker A, Adelman MW, Allman B, Key A, Kleinhenz JM, et al. SARS-CoV-2 Evolution and Immune Escape in Immunocompromised Patients. N Engl J Med. 2022;386(25):2436–8.

169. Caillard S, Benotmane I, Gautier Vargas G, Perrin P, Fafi-Kremer S. SARS-CoV-2 viral dynamics in immunocompromised patients. Am J Transplant. 2021;21(4):1667–9.

170. Benotmane I, Gautier-Vargas G, Wendling MJ, Perrin P, Velay A, Bassand X, et al. In-depth virological assessment of kidney transplant recipients with COVID-19. Am J Transplant. 2020;20(11):3162–72.

171. Benotmane I, Risch S, Doderer-Lang C, Caillard S, Fafi-Kremer S. Long-term shedding of viable SARS-CoV-2 in kidney transplant recipients with COVID-19. Am J Transplant. 2021;21(8):2871–5.

172. Cristelli MP, Viana LA, Dantas MTC, Martins SBS, Fernandes R, Nakamura MR, et al. The Full Spectrum of COVID-19 Development and Recovery Among Kidney Transplant Recipients. Transplantation. 2021;105(7):1433–44.

173. Babel N, Anft M, Blazquez-Navarro A, Doevelaar AAN, Seibert FS, Bauer F, et al.

Immune monitoring facilitates the clinical decision in multifocal COVID-19 of a pancreas-kidney transplant patient. Am J Transplant. 2020;20(11):3210–5.

174. Candon S, Guerrot D, Drouot L, Lemoine M, Lebourg L, Hanoy M, et al. T cell and antibody responses to SARS-CoV-2: Experience from a French transplantation and hemodialysis center during the COVID-19 pandemic. Am J Transplant. 2021;21(2):854–63.

175. Benotmane I, Wendling GGM, Perrin P, Velay A, Bassand X, Bedo D, et al. In-depth virological assessment of kidney transplant recipients with COVID-19. Am J Transplant. 2020;(20):3162–72.

176. Burack D, Pereira MR, Tsapepas DS, Harren P, Farr MA, Arcasoy S, et al. Prevalence and predictors of SARS-CoV-2 antibodies among solid organ transplant recipients with confirmed infection. Am J Transplant. 2021;21(6):2254–61.

177. Boyarsky BJ, Ou MT, Werbel WA, Avery RK, Clarke WA, Tobian AAR, et al. Early Development and Durability of SARS-CoV-2 Antibodies among Solid Organ Transplant Recipients: A Pilot Study. Transplantation. 2021;105(5):E52–3.

178. Caballero-Marcos A, Salcedo M, Alonso-Fernández R, Rodríguez-Perálvarez M, Olmedo M, Graus Morales J, et al. Changes in humoral immune response after SARS-CoV-2 infection in liver transplant recipients compared to immunocompetent patients. Am J Transplant. 2021;21(8):2876–84.

179. Akalin E, Azzi Y, Bartash R, Seethamraju H, Parides M, Hemmige V, et al. Covid-19 and Kidney Transplantation. N Engl J Med. 2020 Jun 18;382(25):2475-2477.

180. Columbia University Kidney Transplant Program. Early Description of Coronavirus 2019 Disease in Kidney Transplant Recipients in New York. J Am Soc Nephrol. 2020 Jun;31(6):1150-1156.

181. Heldman MR, Kates OS, Safa K, Kotton CN, Multani A, Georgia SJ, Steinbrink JM, et al. Delayed mortality among solid organ transplant recipients hospitalized for COVID-19. Clin Infect Dis. 2022 Feb 25:ciac159.

182. Ranzani OT, Bastos LSL, Gelli JGM, Marchesi JF, Baião F, Hamacher S, et al. Characterisation of the first 250 000 hospital admissions for COVID-19 in Brazil: a retrospective analysis of nationwide data. Lancet Respir Med. 2021;9(4):407–18.

183. Favà A, Cucchiari D, Montero N, Toapanta N, Centellas FJ, Vila-Santandreu A, et al. Clinical characteristics and risk factors for severe COVID-19 in hospitalized kidney transplant recipients: A multicentric cohort study. Am J Transplant. 2020 Nov;20(11):3030-3041.

184. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, 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 Jul 1;180(7):934-943.

185. https://www.idsociety.org/practice-guideline/covid-19-guideline-treatment-

and-management/

186. Cravedi P, Suraj SM, Azzi Y, Haverly M, Farouk S, Pérez-Sáez MJ, et al. COVID-19 and Kidney Transplantation: Results from the TANGO International Transplant Consortium. Am J Transplant. 2020 Nov;20(11):3140-3148.

187. Kates OS, Haydel BM, Florman SS, Rana MM, Chaudhry ZS, Ramesh MS, et al. Coronavirus Disease 2019 in Solid Organ Transplant: A Multicenter Cohort Study. Clin Infect Dis. 2021;73(11):E4090–9.

188. Masset C, Gautier-vargas G, Cantarovich D, Ville S, Dantal J, Delbos F, et al. Occurrence of De novo Donor-Specific Antibodies After COVID-19 in Kidney Transplant Recipients Is Low Despite Immunosuppression Modulation. Kidney Int Rep. 2022 May;7(5):983-992.

189. Villanego F, Mazuecos A, Pérez-Flores IM, Moreso F, Andrés A, Jiménez-Martín C, et al. Predictors of severe COVID-19 in kidney transplant recipients in the different epidemic waves: Analysis of the Spanish Registry. Am J Transplant. 2021;21(7):2573–82.

190. Requião-moura LR, Gustavo L, Andrade M De, Sandes-freitas TV De, Cristelli MP, Viana LA, et al. The Mycophenolate-based Immunosuppressive Regimen Is Associated With Increased Mortality in Kidney Transplant Patients With COVID-19. Transplantation. 2022 Oct 1;106(10):e441-e451.

191. Prendecki M, Clarke C, Gleeson S, Greathead L, Santos E, McLean A, et al. Detection of SARS-CoV-2 antibodies in kidney transplant recipients. J Am Soc Nephrol. 2020;31(12):2753–6.

192. Favà A, Donadeu L, Sabé N, Pernin V, González-Costello J, Lladó L, 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. 2021 Aug;21(8):2749-2761.

193. Weiskopf D, Schmitz KS, Raadsen MP, Grifoni A, Okba NMA, Endeman H, et al. Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. Sci Immunol. 2020;5(48):1–11.

194. Cravedi P, Ahearn P, Wang L, Yalamarti T, Hartzell S, Azzi Y, et al. Delayed kinetics of IgG, but Not IgA, antispike antibodies in transplant recipients following SARS-CoV-2 Infection. J Am Soc Nephrol. 2021 Oct 1;32(12):3221–30.

195. van Kampen JJA, van de Vijver DAMC, Fraaij PLA, Haagmans BL, Lamers MM, Okba N, et al. Duration and key determinants of infectious virus shedding in hospitalized patients with coronavirus disease-2019 (COVID-19). Nat Commun. 2021;12(1):8–13.

196. Libster R, Pérez Marc G, Wappner D, Coviello S, Bianchi A, Braem V, et al. Early High-Titer Plasma Therapy to Prevent Severe Covid-19 in Older Adults. N Engl J Med. 2021;384(7):610–8.

197. Gupta A, Gonzalez-Rojas Y, Juarez E, Crespo Casal M, Moya J, et al. Early

Treatment for Covid-19 with SARS-CoV-2 Neutralizing Antibody Sotrovimab. N Engl J Med. 2021;385(21):1941–50.

198. ACTIV-3/Therapeutics for Inpatients with COVID-19 (TICO) Study Group. Efficacy and safety of two neutralising monoclonal antibody therapies, sotrovimab and BRII-196 plus BRII-198, for adults hospitalised with COVID-19 (TICO): a randomised controlled trial. Lancet Infect Dis. 2022;22(5):622–35.

199. Thieme CJ, Anft M, Paniskaki K, Blazquez-Navarro A, Doevelaar A, Seibert FS, et al. The Magnitude and Functionality of SARS-CoV-2 Reactive Cellular and Humoral Immunity in Transplant Population Is Similar to the General Population Despite Immunosuppression. Transplantation. 2021 Oct 1;105(10):2156-2164.

200. Le Bert N, Tan AT, Kunasegaran K, Tham CYL, Hafezi M, Chia A , et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. Nature. 2020;584(7821):457–62.

201. Remy KE, Moldwawer LL, Hotchkiss RS, Remy KE, Mazer M, Striker DA, et al. Severe immunosuppression and not a cytokine storm characterizes COVID-19 infections. JCI Insight. 2020 Sep 3;5(17):e140329.

202. Zheng M, Gao Y, Wang G, Song G, Liu S, Sun D, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. Cell Mol Immunol. 2020 May;17(5):533-535.

203. Mazzoni A, Annunziato F, Cosmi L, Mazzoni A, Salvati L, Maggi L, et al. Impaired immune cell cytotoxicity in severe COVID-19 is IL-6 dependent. J Clin Invest. 2020 Sep 1;130(9):4694-4703.

204. Gatto I, Biagioni E, Coloretti I, Farinelli C, Avoni C, Caciagli V, et al. Cytomegalovirus blood reactivation in COVID-19 critically ill patients: risk factors and impact on mortality. Intensive Care Med. 2022 Jun;48(6):706-713.

205. Le P, Pinceaux K, Pronier C, Seguin P, Tadié J, Reizine F. Herpes simplex virus and cytomegalovirus reactivations among severe COVID-19 patients. Crit Care. 2020 Aug 28;24(1):530.

206. De Biasi S, Meschiari M, Gibellini L et al. Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. Nat Commun. 2020;11(1):3434.

207. Rha M, Jeong HW, Ko J, Choi SJ, Seo I, Lee JS, et al. Report PD-1-Expressing SARS-CoV-2-Specific CD8 + T Cells Are Not Exhausted , but Functional in Patients with PD-1-Expressing SARS-CoV-2-Specific CD8 + T Cells Are Not Exhausted , but Functional in Patients with COVID-19. Immunity . 2021;54(1):44-52.

208. Søfteland JM, Gisslén M, Liljeqvist JÅ, Friman V, de Coursey E, Karason K, et al. Longevity of anti-spike and anti-nucleocapsid antibodies after COVID-19 in solid organ transplant recipients compared to immunocompetent controls. Am J Transplant. 2022;22(4):1245–52.

209. Fernández-Ruiz M, Olea B, Almendro-Vázquez P, Giménez E, Marcacuzco A, San

Juan R, et al. T cell–mediated response to SARS-CoV-2 in liver transplant recipients with prior COVID-19. Am J Transplant. 2021;21(8):2785–94.

210. Chang CC, Vlad G, Vasilescu ER, Husain SA, Liu YN, Sun WZ, et al. Disparity between levels of anti-RBD IgG and anti-nucleocapsid protein IgG antibodies in COVID-19–recovered patients who received a kidney transplant. Kidney Int [Internet]. 2021;100(1):240–1.

211. Chavarot N, Leruez-Ville M, Scemla A, Burger C, Amrouche L, Rouzaud C, et al. Decline and loss of anti–SARS-CoV-2 antibodies in kidney transplant recipients in the 6 months following SARS-CoV-2 infection. Kidney Int. 2021;99(2):486–8.

212. Boyarsky BJ, Werbel WA, Avery RK, Tobian AAR, Massie AB, Segev DL, et al. Antibody response to 2-dose sars-cov-2 mrna vaccine series in solid organ transplant recipients. JAMA - J Am Med Assoc. 2021;325(21):2204–6.

213. Stumpf J, Siepmann T, Lindner T, Karger C, Schwöbel J, Anders L, et al. Humoral and cellular immunity to SARS-CoV-2 vaccination in renal transplant versus dialysis patients: A prospective, multicenter observational study using mRNA-1273 or BNT162b2 mRNA vaccine. Lancet Reg Heal - Eur [Internet]. 2021;9:100178.

214. Kamar N, Abravanel F, Marion O, Esposito L, Hebral AL, Médrano C, et al. Anti-SARS-CoV-2 spike protein and neutralizing antibodies at 1 and 3 months after three doses of SARS-CoV-2 vaccine in a large cohort of solid organ transplant patients. Am J Transplant. 2022;22(5):1467–74.

215. Fajnzylber J, Regan J, Coxen K, Corry H, Wong C, Rosenthal A, et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. Nat Commun. 2020;11(1):1–9.

216. Lyudovyk O, Kim JY, Qualls D, Hwee MA, Lin Y-H, Boutemine SR, et al. Impaired humoral immunity is associated with prolonged COVID-19 despite robust CD8 T cell responses. Cancer Cell. 2022;1–16.

217. Benotmane I, Gautier Vargas G, Velay A, Wendling MJ, Perrin P, Fafi-Kremer S, et al. Persistence of SARS-CoV-2 antibodies in kidney transplant recipients. Am J Transplant. 2021;21(6):2307–10.

218. Benning L, Morath C, Bartenschlager M, Reineke M, Töllner M, Nusshag C, et al. Natural SARS-CoV-2 infection results in higher neutralization response against variants of concern compared with 2-dose BNT162b2 vaccination in kidney transplant recipients. Kidney Int. 2022;101(3):639–42.

219. Yang J, James E, Gates TJ, DeLong JH, LaFond RE, Malhotra U, et al. CD4+ T cells recognize unique and conserved 2009 H1N1 influenza hemagglutinin epitopes after natural infection and vaccination. Int Immunol. 2013;25(8):447–57.

220. Sridhar S, Begom S, Hoschler K, Bermingham A, Adamson W, Carman W, et al. Longevity and determinants of protective humoral immunity after pandemic influenza infection. Am J Respir Crit Care Med. 2015;191(3):325–32.

221. L'Huillier AG, Ferreira VH, Hirzel C, Nellimarla S, Ku T, Natori Y, et al. T-cell responses following Natural Influenza Infection or Vaccination in Solid Organ Transplant Recipients. Sci Rep. 2020;10(1):1–9.

222. Charmetant X, Espi M, Benotmane I, Barateau V, Heibel F, Buron F, et al. Infection or a third dose of mRNA vaccine elicits neutralizing antibody responses against SARS-CoV-2 in kidney transplant recipients. Sci Transl Med. 2022;14(636):1–15.

223. Reischig T, Kacer M, Vlas T et al. Insufficient response to mRNA SARS-CoV-2 vaccine and high incidence of severe COVID-19 in kidney transplant recipients during pandemic. Am J Transplant. 2022;22(3):801–12.

224. Cucchiari D, Egri N, Bodro M, Herrera S, Del Risco-Zevallos J, Casals-Urquiza J, et al. Cellular and humoral response after MRNA-1273 SARS-CoV-2 vaccine in kidney transplant recipients. Am J Transplant. 2021;21(8):2727–39.

225. Charmetant X, Espi M, Barba T, Ovize A, Morelon E, Mathieu C, et al. Predictive factors of a viral neutralizing humoral response after a third dose of COVID-19 mRNA vaccine. Am J Transplant. 2022;22(5):1442–50.

226. Kemlin D, Lemy A, Pannus P, Desombere I, Gemander N, Goossens ME, et al. Hybrid immunity to SARS-CoV-2 in kidney transplant recipients and hemodialysis patients. Am J Transplant. 2022;22(3):994–5.

227. Magicova M, Zahradka I, Fialova M, Neskudla T, Gurka J, Modos I, et al. Determinants of Immune Response to Anti-SARS-CoV-2 mRNA Vaccines in Kidney Transplant Recipients: A Prospective Cohort Study. Transplantation. 2022;106(4):842–52.

228. Benotmane I, Gautier-Vargas G, Gallais F, Gantner P, Cognard N, Olagne J, et al. Strong antibody response after a first dose of a SARS-CoV-2 mRNA-based vaccine in kidney transplant recipients with a previous history of COVID-19. Am J Transplant. 2021;21(11):3808–10.