ISSN 2220-3230 (online)

World Journal of *Transplantation*

World J Transplant 2022 October 18; 12(10): 313-330

Published by Baishideng Publishing Group Inc

World Journal of Transplantation

Contents

Monthly Volume 12 Number 10 October 18, 2022

World Journal of Transplantation

Monthly Volume 12 Number 10 October 18, 2022

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INDEXING/ABSTRACTING

The *WJT* is now abstracted and indexed in PubMed, PubMed Central, Scopus, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Transplantation

ISSN
ISSN 2220-3230 (online)

LAUNCH DATE
December 24, 2011

FREQUENCY
Monthly

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World J Transplant 2022 October 18; 12(10): 313-324

DOI: 10.5500/wjt.v12.i10.313

ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Prospective Study

Effect of panel reactive antibodies on T cell immunity reinstatement following renal transplantation

Lampros Vagiotas, Maria Stangou, Efstratios Kasimatis, Aliki Xochelli, Grigorios Myserlis, Georgios Lioulios, Vasiliki Nikolaidou, Manolis Panteli, Konstantinos Ouranos, Nikolaos Antoniadis, Daoudaki Maria, Aikaterini Papagianni, Georgios Tsoulfas, Asimina Fylaktou

Specialty type: Transplantation

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Chan WYK, China; Ghazanfar A, United Kingdom; Sahin TT, Turkey

Received: July 3, 2022
Peer-review started: July 3, 2022
First decision: August 4, 2022
Revised: August 5, 2022
Accepted: September 9, 2022
Article in press: September 9, 2022
Published online: October 18, 2022

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Abstract

BACKGROUND

Chronic kidney disease is associated with immunological disorders, presented as phenotypic alterations of T lymphocytes. These changes are expected to be restored after a successful renal transplantation; however, additional parameters may contribute to this process.

AIM

To evaluate the impact of positive panel reactive antibodies (PRAs) on the restoration of T cell phenotype, after renal transplantation.

METHODS

CD4CD28null, CD8CD28null, natural killer cells (NKs), and regulatory T cells (Tregs) were estimated by flow cytometry at T0, T3, and T6 which were the time of transplantation, and 3- and 6-mo follow-up, respectively. Changes were estimated regarding the presence or absence of PRAs.

RESULTS
Patients were classified in two groups: PRA(-) (n = 43) and PRA(+) (n = 28) groups. Lymphocyte and their subtypes were similar between the two groups at T0, whereas their percentage was increased at T3 in PRA(-) compared to PRA(+) [23 (10.9-47.9) *vs* 16.4 (7.5-36.8 μ/L, respectively; P = 0.03]. Lymphocyte changes in PRA(-) patients included a significant increase in CD4 cells (P < 0.0001), CD8 cells (P < 0.0001), and Tregs (P < 0.0001), and a reduction of NKs (P < 0.0001). PRA(+) patients showed an increase in CD4 (P = 0.008) and CD8 (P = 0.0001), and a reduction in NKs (P = 0.07). CD4CD28null and CD8CD28null cells, although initially reduced in both groups, were stabilized thereafter.

CONCLUSION
Our study described important differences in the immune response between PRA(+) and PRA(-) patients with changes in lymphocytes and lymphocyte subpopulations. PRA(+) patients seemed to have a worse immune profile after 6 mo follow-up, regardless of renal function.

Key Words: Chronic kidney disease; Panel reactive antibodies; Lymphocyte subpopulation; CD4CD28null cells; CD8CD28null cells

Core Tip: Chronic kidney disease is associated with phenotypic and functional changes in the immune system. This study evaluated the impact of positive panel reactive antibodies (PRAs) on restoration of the T cell phenotype after renal transplantation. Our study described important differences in the immune response between PRA(+) and PRA(-) patients with changes in lymphocytes and lymphocyte subpopulations. PRA(+) patients seemed to have a worse immune profile after 6 mo follow-up, regardless of renal function.

Citation: Vagiotas L, Stangou M, Kasimatis E, Xochelli A, Myserlis G, Lioulios G, Nikolaidou V, Panteli M, Ouranos K, Antoniadis N, Maria D, Papagianni A, Tsoulfas G, Fylaktou A. Effect of panel reactive antibodies on T cell immunity reinstatement following renal transplantation. *World J Transplant* 2022; 12(10): 313-324
URL: https://www.wjgnet.com/2220-3230/full/v12/i10/313.htm
DOI: https://dx.doi.org/10.5500/wjt.v12.i10.313

INTRODUCTION

Chronic kidney disease (CKD) is associated with phenotypic and functional changes in the immune system, including both innate and adaptive immunity, causing detrimental clinical consequences. Total lymphopenia is one of the major concerns in CKD, whereas changes in T lymphocytes include both elimination of their population and alterations of their subtypes. Some of these phenotypic and functional changes have been described by investigators[1,2]. We previously showed that CKD, even at the pre-dialysis stage, results in reduced levels of CD4, CD8, and regulatory T cells (Tregs). Furthermore, it affects the expression of CD28 molecule on T lymphocytes, leading to an increased proportion of CD4CD28null and CD8CD28null cells[1,2].

The CD28 molecule constitutes a primary co-stimulatory receptor, which is essential for successful T cell activation, proliferation, and survival. It is mainly expressed on naive T cells in humans, but its expression on memory T cells depends on their differentiation status. Expansion of circulating T lymphocytes lacking the CD28 molecule represents an adaptive mechanism following repeated antigenic stimulation, and has been considered an age-associated immunological alteration[3-7].

Initiation of hemodialysis (HD) cannot restore these structural changes of lymphocytes. Even more, the HD itself, as an extracorporeal circulation, use of dialyzers, may have an additive deleterious effect [1]. Conversely, successful renal transplantation allows patients to stop dialysis and reinstates kidney function. Accordingly, as part of returning to normality, it is also expected to restore patients' immune profile[8,9].

However, despite the indisputable beneficial effect of renal transplantation on immune status, there may be parameters that affect the outcome of graft function and potentially influence the reestablishment of immunological disorders. Most of these parameters are closely associated with the patient's immune status at the time of transplantation. Immune status of the CKD patient is determined by phenotypic and functional alterations of lymphocytes due to CKD, and even more interesting for those patients undergoing renal transplantation, by the presence of human leukocyte antigen (HLA) sensit-

ization. HLA sensitization refers to the presence of antibodies in the potential recipient against HLA molecules of the selected donor. While on the waiting list, CKD patients may develop antibodies against HLA antigens as a result of blood transfusions, previous transplantations, or pregnancies[10,11], generally described as panel reactive antibodies (PRAs)[12]. The risk of sensitization increases as there is exposure to more than one sensitizing factor[9,13]. PRA screening is routinely performed in CKD patients before renal transplantation to assess recipients' exposure and sensitization. PRA titers before kidney transplantation may be used to predict acute rejection and guide the immunosuppressive treatment, including induction treatment. The presence of PRAs is not uncommon, as patients have to wait long for a kidney transplant, and meanwhile, are exposed to blood transfusions or get pregnant [12]. The purpose of this study was to evaluate the effect of positive PRA on restoration of the immunological T cell phenotype following successful renal transplantation.

MATERIALS AND METHODS

Study population

The study was conducted between January 2020 and October 2021 at the Department of Renal Transplantation, Hippokration General Hospital (Thessaloniki, Greece). Seventy-eight kidney transplantations were performed, from which seventy-one fulfilled the criteria and were included in the study. Three of the recipients were adolescents, aged 13, 16, and 17 years; the rest were adults. All participants provided informed consent before their enrollment in the study. The trial was approved by the local ethics committee and followed the general principles of the Declaration of Helsinki (2008 Amendment).

Inclusion criteria: Patients eligible for the study were 13-70-years-old, and had undergone a living or deceased donor kidney transplantation. Regarding the deceased donors, we included only Donation after Brain Death and not Donation after Cardiac Death transplants. All transplantations were ABO-compatible with a negative complement-dependent crossmatch. The patients were followed for 6 mo in the outpatient clinic, and all were treated with the same treatment protocol.

Exclusion criteria: Patients were excluded from the study in case of recent (less than 3 mo) cytomegalovirus (CMV) or bacterial infection; recent malignancy (less than 5 years); or active autoimmune, inflammatory disease, or hematological disorder. Also, patients who had been on immunosuppressive treatment during the last 12 mo prior to kidney transplantation were excluded, as were patients not compliant with the treatment instructions.

Schedule of the study

Each patient receiving a kidney transplantation was assessed for eligibility to be included in the study. For patients who fulfilled the inclusion criteria, as described above, the day of enrollment in the study was the day of transplantation. Blood samples were taken in the morning, before the administration of any immunosuppressive treatment, and used for laboratory and immunological assessments. During the posttransplant period, renal function, medication, and possible side effects were recorded. Following discharge from the hospital, after renal transplantation, all patients were regularly followed up at the outpatient clinic on a monthly basis. Their immune profile was recorded on the day of transplantation (T0), and at the 3- and 6-mo follow-up (T3 and T6, respectively). At the same time intervals, the function of the renal graft was evaluated and the results were correlated with the immunophenotype.

Demographic, clinical data from donors and recipients, HLA mismatches, and cold ischemia time were recorded at T0, and delayed graft function (DGF), acute rejection episodes, infections, and hospitalization time were recorded and analyzed at T3 and T6, 3 and 6 mo after transplantation. All patients received the same immunosuppressive regimen, according to the Immunosuppressive Protocol, including basiliximab or antithymocyte globulin (ATG), steroids, tacrolimus, and multimode fiber. Eleven patients (15.5%) received ATG, reasons to receive ATG were as follows: 4/11 because of retransplant and 7/11 because of the presence of PRA(+). Seven patients had DGF during the first 7 d following transplantation. Basiliximab was used as induction immunosuppression in 84.5% of the patients.

Laboratory measurements

Flow cytometry: T cell subsets were identified using multicolor flow cytometry with standard techniques on the Navios EX flow cytometer (Beckman Coulter, Sykesville, MD, United States). Whole blood samples were drawn from patients at the scheduled time points (T0, T3, and T6), collected in EDTA tubes, and processed for the evaluation of lymphocyte count and their subpopulations. T lymphocyte subsets determined were CD3+CD4+, CD3+CD8+, CD3-CD16+CD56+, CD3+CD4+CD28-, and CD3+CD8+CD28-, using the following monoclonal antibodies: CD3-FITC (clone: UCHT1; Beckman Coulter), CD16 (clone: 3G8; Beckman Coulter), CD56 clone: N901(NKH-1)-PE; Beckman Coulter), CD4-APC (clone: 13B8.2; Beckman Coulter), CD8 PC5.5 (clone: B9.11l Beckman Coulter), CD28-ECD (clone:

CD28.2; Beckman Coulter), and CD45-PC7 (clone: J33; Beckman Coulter). Peripheral blood mononuclear cells were obtained by Ficoll density gradient centrifugation. Immunophenotyping of Tregs was performed with the combination of the following monoclonal antibodies: CD45-PC7 (clone: J33; Beckman Coulter), CD4-FITC (clone: 13B8.2; Beckman Coulter), CD25-PC5 (clone: B1.49.9; Beckman Coulter), and FOXP3-PE (clone: 259D; Beckman Coulter).

Statistics

Statistical analyses were performed using the Statistical Package for Social Sciences for Windows, version 27.0 (SPSS Inc., Chicago, IL, United States). The Shapiro-Wilk or Kolmogorov-Smirnov test was applied to examine the normality of distribution for continuous variables. For all comparisons, $P < 0.05$ was considered statistically significant. Mean ± SD and medians and interquartile range were used to describe data from normally distributed and non-parametric variables, respectively. Similarly, the student's *t*-test for non-paired and paired variables, and Mann-Whitney *U* test and Wilcoxon signed-rank test were respectively performed to compare differences between groups. To investigate the change in subpopulations among T0, T3, and T6, repeated measures analysis of variance (ANOVA) for parametric variables or Friedman's ANOVA for non-parametric variables was used.

RESULTS

Seventy-one recipients of a kidney transplant were included in the study. Characteristics of patients are depicted in Table 1.

Differences between PRA(-) and PRA(+) patients

Differences in clinical and laboratory findings: Of the study population, 43 patients had negative PRA, and were classified as PRA(-), whereas 28 had positive PRA, and were classified as PRA(+). There were no differences between the two groups in terms of age, sex, and time on HD, [defined as HD vintage (HDV)]. Also, no differences were found between the two groups in the proportion of patients who underwent preemptive transplantation, had an episode of acute rejection or were administered ATG, as well as in those who had DGF (Table 2).

No significant differences in lymphocyte numbers and T lymphocyte subpopulations were noticed between PRA(-) and PRA(+) patients at the time of transplantation. An increase in percentage of CD4CD28null and CD8CD28null cell within PRA(+) patients did not reach statistical difference (Table 3).

Correlations of immunological parameters at time point T0

In the whole cohort of patients, age was significantly correlated with the percentage of CD4CD28null ($r = 0.3$, $P = 0.03$), percentage and number of CD8CD28null (r = 0.4, $P < 0.001$ and $r = 0.3$, $P = 0.03$, respectively) and percentage of NK cells ($r = 0.3$, $P = 0.02$). HDV had a negative correlation with total lymphocyte number ($r = -0.3$, $P = 0.04$), CD4+ lymphocytes ($r = -0.3$, $P = 0.01$), and Tregs ($r = -0.4$, $P = 0.006$). Patients who underwent preemptive kidney transplantation had a better immune profile than patients already enrolled in HD or continuous ambulatory peritoneal dialysis. In these patients, a significantly increased percentage and number of lymphocytes was observed, 27.9 (14%-37.7%) *vs* 18 (6.4%-40%) $P = 0.03$, and 1705 (100-2800) *vs* 1200 (700-2700) cells/μL, $P = 0.03$, respectively. Reduction in the percentage of CD4CD28null, 1.7 (0.4%-2.9%) *vs* 6.7 (0%-33.7%), $P = 0.04$ and CD8CD28null, [14.9 (6.1%-22.1%) *vs* 39.7 (114%-91%), $P = 0.002$, 207 (85-266) *vs* 477 (105-1131), $P = 0.002$] were also noticed as well as a significant increase in Tregs, affecting both percentage, 5.6 (1.7%-8.3%) *vs* 3.9 (0.1%-11.5%) $P = 0.05$, and total number of Tregs, 32.1 (24-47) cells/μL *vs* 18.9 (0.5-74) cells/μL, $P = 0.006$.

Differences in the outcome of subpopulations depending on the existence of PRA

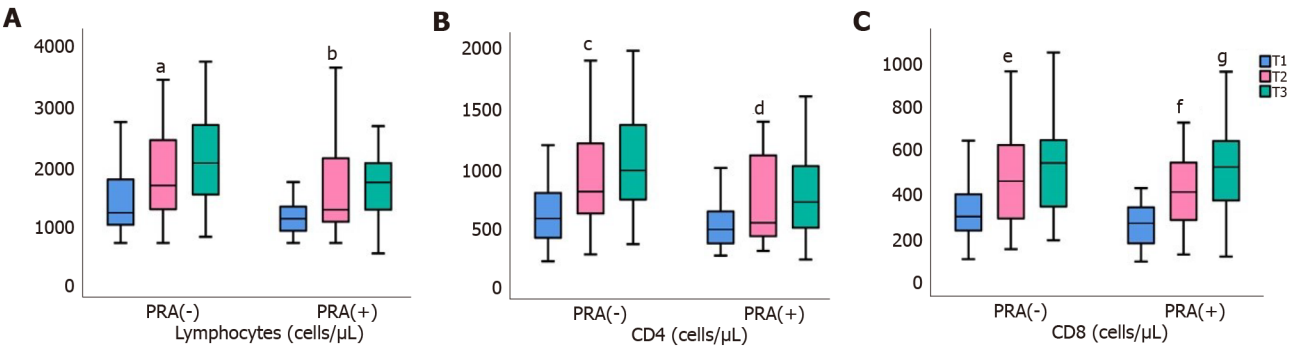
Changes in lymphocytes and their subpopulations following renal transplantation are depicted in Tables 4 and 5, for PRA(-) and PRA(+) patients, respectively. In both groups, PRA(-) and PRA(+), the percentage and total number of lymphocytes were increased. However, the response of lymphocyte changes was earlier and stronger in PRA(-) patients, as their percentage raised from T0 to T3, mean rank 15.35 to 20.98, $P = 0.002$, compared to 10.2 and 13.9, P = NS in PRA(+). This prompt response resulted in a significant increase in the number of total lymphocytes, in PRA(-), during the period T0 to T3, mean rank 10.57 to 20.41, $P < 0.0001$.

Although at time point T0, there was no significant difference in the percentage or total number of lymphocytes between the two groups of patients, at T3, PRA(-) had significantly increased percentage of lymphocytes, compared to PRA(+), 23 (10.9-47.9) *vs* 16.4 (7.5-36.8) μ/L, respectively, $P = 0.03$. At time T6, although there was still a superiority in PRA(-) patients the difference did not reach statistical significance, $P = 0.06$.

Table 1 Clinical and demographic characteristics of kidney transplant recipients

Characteristics	
Age, yr, median (range)	46 (13-70)
Male/female	49/22
Living kidney donor	22.5%
Deceased kidney donor	77.5%
Previous kidney transplant	7.0%
Preemptive transplantation	4.2%
PRA(-)	60.5%
Early rejection, within first 6 mo after KT	4.2%
Induction therapy	
Basiliximab	84.5%
ATG	15.5%
Maintenance immune suppression	
Tacrolimus/mycophenolate/prednisone	100.0%
Other	0.0%
Distribution of underlying kidney disease	
Polycystic kidney disease	22.5%
Primary glomerulopathies	21.1%
Reflux nephropathy	12.6%
Diabetes mellitus	4.2%
Nephrosclerosis/hypertension	4.2%
Urinary tract infections/ stones	3.7%
Other	16.2%
Unknown	15.5%

ATG: Antithymocyte globulin; KT: Kidney transplant; PRA: Panel reactive antibody.



DOI: 10.5500/wjt.v12.i10.313

Figure 1 Sequential changes. A: Total lymphocyte populations; B: CD4 cells; C: CD8 cells. Differences between panel reactive antibody (PRA)(-) and PRA(+) patients. [a]*P* < 0.001 *vs* T0; [b]*P* = 0.003 *vs* T0; [c]*P* < 0.001 *vs* T0; [d]*P* = 0.006 *vs* T0; [e]*P* < 0.001 *vs* T0; [f]*P* = 0.003 *vs* T0; [g]*P* = 0.03 *vs* T0.

Changes in CD4(+) and CD8(+) cells and CD4CD28null and CD8CD28null subtypes

Both CD4 and CD8 cells were significantly increased in the two groups of patients, from T0 to T3. Figure 1 depicts changes of total lymphocytes, and also, in CD4 and CD8 cells after transplantation in PRA(-) and PRA(+) patients. There was a definite increase and gradual increase of total lymphocytes, together with CD4 and CD8 cells, from T0 towards T6 in both groups of patients, with changes in all

Table 2 Differences between panel reactive antibodies (-) and (+) patients

Parameter	T0 (at renal transplantation)		
	PRA(-)	PRA(+)	*P* value
Age, yr	45 (13-65)	47 (14-70)	NS
HDV, mo	82.5 (0-251)	112 (0-165)	NS
Time of cold ischemia, h	18 (0-30)	16.5 (0-30.5)	NS
Pre-emptive RT, %	6 (13.6)	1 (3.6)	NS
Acute rejection episode, %	2 (4.5)	1 (3.6)	NS
ATG administration, %	5 (11.4)	7 (25)	NS
DGF, %	7 (15.9)	4 (14.3)	NS

ATG: Antithymocyte globulin; DGF: Delayed graft function; HDV: Hemodialysis vintage; PRA: Panel reactive antibody; RT: Renal transplantation.

Table 3 Lymphocyte numbers and subpopulations in panel reactive antibodies (-) and (+) patients at time of transplantation (T0)

Parameter	T0, at renal transplantation		
	All patients	PRA(-)	PRA(+)
n	71	43	28
Lymphocyte, %	18.1 (6.4-40)	18.8 (6.4-38.4)	17.8 (11.2-40)
Lymphocyte, cells/μL	1200 (700-2800)	1200 (700-2800)	1100 (700-2600)
CD4+, %	42.0 (20.6-68.6)	44.4 (20.6-68.6)	41.5 (25.3-59.5)
CD4+, cells/μL	515 (206-1453.2)	557 (206-1453.2)	435 (253-1362.4)
CD8+, %	24.55 (10.5-53.1)	25.1 (12,2-37.7)	23.4 (10.5-53.1)
CD8+, cells/μL	301.5 (91.7-665.6)	301.5 (102.9-641.7)	294.9 (91.7-665.6)
CD4+/CD8+	1.7 (0.6-5.6)	1.5 (0.9-5.6)	2 (0.6-5)
CD4+CD28-, %	5.4 (0.0-33.7)	4.8 (0.2-33.7)	7.2 (0-32.1)
CD4+CD28-, cells/μL	26.9 (0.0-206)	26.7 (0-160)	27.3 (0-206)
CD8+CD28-, %	38.6 (6.1-91.5)	38.3 (6.1-68.2)	48.4 (15.1-91.5)
CD8+CD28-, cells/μL	121.5 (13-583)	113.6 (17-315)	122 (13-583)
CD16/56, %	18 (3.6-50.6)	17.7 (3.6-50.6)	18.4 (4.4-34.2)
CD16/56, cells/μL	198.1 (50.4-750.5)	210 (50.4-750.5)	190.4 (94.8-393.6)
Tregs, %, on CD4	4 (0.1-11.5)	3.9 (0.1-11.5)	4.2 (1.5-7.3)
Tregs, cells/μL	20 (0.52-74.38)	20.2 (0.5-74.3)	18.9 (5.8-73.5)

PRA: Panel reactive antibody; Tregs: Regulatory T cells.

three cell types being statistically significant even during the first 3 mo following transplantation.

Regarding CD4CD28null cells, although there was a significant reduction in the percentage of CD4CD28null subtypes from T0 to T3, in both PRA(-) and PRA(+) patients, $P = 0.04$ and 0.01, respectively, population of cells and their percentage were stabilized thereafter, until T6, leading to no significant changes in these cell types during follow up, regardless of the presence of PRA. The results are descried in Tables 3 and 4 and depicted at Figure 2. On the other hand, there was a marked reduction in CD8CD28null cells, both percentage and numbers only in PRA(-) patients, from T0 to T3, $P = 0.03$, and from T3 to T6, $P = 0.02$. Such changes were not evident in PRA(+) patients, in contrast there was a significant increase in these cells during the first 3 mo (from T0 to T3).

Changes in NK cells and Tregs

In PRA(-) there was a significant reduction in the percentage of NKs after renal transplantation, from T0 to T3 and from T3 to T6, $P < 0.0001$ and $P = 0.006$, respectively, and this was accompanied by significant

Table 4 Changes in T lymphocyte subpopulations at T0, T3, and T6 time points in patients with panel reactive antibodies (-)

Parameter	T0	T3	T6	*P* value
Lymphocyte, %	18.8 (6.4-38.4)	23 (10.9-47.9)	25.4 (8.4-52)	0.001
Lymphocyte, cells/μL	1200 (700-2800)	1650 (700-4100)	1900 (800-3700)	< 0.0001
CD4+, %	44.4 (20.6-68.6)	49.8 (22.7-77.1)	49.1 (16.2-71.4)	0.004
CD4+, cells/μL	557 (206-1453.2)	782 (261.8-1951.6)	872 (330-2001.6)	< 0.0001
CD8+, %	25.1 (12.2-37.7)	26.9 (12.4-50.1)	27.4 (13.3-49)	NS
CD8+, cells/μL	301.5 (102.9-641.7)	456.3 (148.6-1402.8)	514.5 (189.2-1397.8)	< 0.0001
CD4CD28null, %	4.8 (0.2-33.7)	2.8 (0-21.1)	2.7 (0.1-36.4)	NS
CD4CD28null, cells/μL	26.7 (0.9-149)	27.5 (0-160)	26.5 (09-241)	NS
CD8CD28null, %	38.3 (6.1-68.2)	28.4 (8.3-80.5)	32.8 (6.7-90.7)	NS
CD8CD28null, cells/μL	113.6 (17-315)	112.6 (28-1129)	158 (18-1267)	NS
CD16/56, %	17.7 (3.6-50.6)	6.6 (1.9-24.2)	9.3 (2.9-28.6)	< 0.0001
CD16/56, cells/μL	210 (50.4-750.5)	121.6 (33-622.2)	151.2 (44-774.4)	< 0.0001
Tregs, %, on CD4	3.9 (0.1-11.5)	3.3 (0.9-6.8)	4.1 (1.4-8.8)	NS
Tregs, cells/μL	20.2 (0.5-74.3)	29.4 (7.5-122.9)	38.4 (8-104)	< 0.0001

Tregs: Regulatory T cells.

Table 5 Changes in T lymphocyte subpopulations at T0, T3, and T6 time points in patients with panel reactive antibodies (+)

Parameter	T0	T3	T6	*P* value
Lymphocyte, %	17.8 (11.2-40)	16.4 (7.5-36.8)	20.9 (12.2-36.4)	0.07
Lymphocyte, cells/μL	1100 (700-2600)	1300 (700-3600)	1700 (525-3200)	0.009
CD4+, %	41.5 (25.3-59.5)	42.3 (29.2-65.3)	46.5 (27.4-62)	NS
CD4+, cells/μL	435 (253-1362.4)	548.9 (292-1371.3)	744 (220-1888)	0.008
CD8+, %	23.4 (10.5-53.1)	27.4 (10.3-53.6)	29.9 (11.6-56.2)	0.005
CD8+, cells/μL	294.9 (91.7-665.6)	408 (123.6-1234.8)	504.9 (114.4-955.4)	< 0.0001
CD4CD28null, %	7.2 (0-32.1)	5.3 (0.2-24.8)	4 (0.1-28.6)	NS
CD4CD28null, cells/μL	27.3 (0-206)	22.8 (1.5-234)	24.2 (1.3-244)	NS
CD8CD28null, %	48.4 (15.1-91.5)	47.1 (10.7-82.1)	36.5 (7.7-82)	NS
CD8CD28null, cells/μL	122.2 (13-583)	200 (19-547)	160 (22-726)	NS
CD16/56, %	18.4 (4.4-34.2)	11.4 (2.9-26)	7.9 (3-24.6)	< 0.0001
CD16/56, cells/μL	190.4 (94.8-393.6)	157.5 (34.8-450)	135.7 (23.76-385.7)	0.07
Tregs, %, on CD4	4.2 (1.5-7.3)	3.3 (1.2-6.8)	4.4 (1.4-8.6)	NS
Tregs, cells/μL	18.9 (5.8-73.5)	20.9 (7.4-65.8)	26.7 (8.5-103.8)	NS

Tregs: Regulatory T cells.

elimination in the number of NK cells, (P = 0.002 and P = 0.005, respectively) in Figure 2. In contrast, within PRA(+) patients, the only significant changes were reported in the percentage of NK cells, during the time period, from T0 to T3, P = 0.001.

Similar differences were noticed between the two groups of patients regarding Tregs. The percentage of Tregs was increased only in PRA(-) patients, and this alteration was restricted only in the time period 3 to 6 mo, from T3 to T6, P = 0.02. Regulatory T cell population, however, was increased significantly in the same group, from T0 to T3, P = 0.01 and from T3 to T6, P = 0.003, while these cells showed no difference in PRA(+) patients from T0 to T3, and only mild restoration fromT3 to T6 (Figure 3).

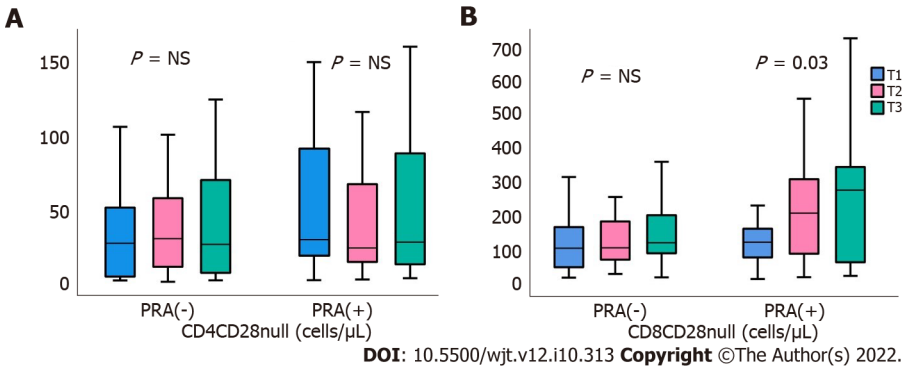


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Figure 2 Changes in the number of CD4CD28null and CD8CD28null cells during follow up in panel reactive antibody patients. A: CD4CD28null cells; B: CD8CD28null cells.

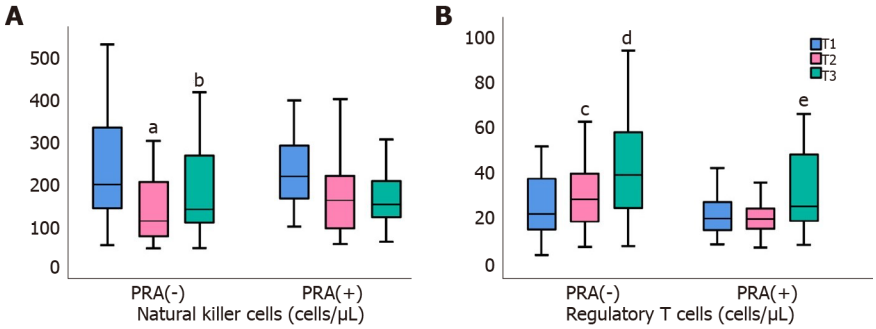

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Figure 3 Changes in natural killer cells and regulatory T cells during follow-up in panel reactive antibody (-) and panel reactive antibody (+) patients. A: Natural killer cells; B: Regulatory T cells. [a]*P* = 0.002 *vs* T0; [b]*P* = 0.005 *vs* T3; [c]*P* = 0.01 *vs* T0; [d]*P* = 0.003 *vs* T3; [e]*P* = 0.04 *vs* T3. PRA: Panel reactive antibody.

DISCUSSION

The presence of high PRA levels, as a consequence of previous exposure to foreign HLAs[13], represents an increased possibility of preformed DSA occurrence, which is associated with the highest likelihood of graft loss[9,14]. Sensitization leads to the production of antibodies against HLA class I and HLA class II antigens, and activates different cell subpopulations, inducing immune response and possible rejection. The presence of HLA antibodies in the early term of transplantation may be more harmful to allografts, as they are associated with a higher incidence of acute rejection compared to patients who may develop antibodies later[12].

In this study, we evaluated the effect of PRA on the alterations of total lymphocytes and their subpopulations, following successful renal transplantation. For this reason, patients undergoing renal transplantation were divided in two groups, PRA(+) and PRA(-), according to the presence or absence of PRA at time of transplantation. All patients were followed prospectively for 6 mo at the Renal Transplant Outpatient clinic, and their renal function, medication, and clinical and laboratory parameters were assessed every month. Likewise, total lymphocytes, CD4, CD8, their subsets, CD4CD28null and CD 8CD28null, natural killer (NK) cells and Tregs were estimated by flow cytometry at the time of transplantation, and the 3- and 6-mo follow-up.

Although lymphocyte number was significantly and rapidly increased very early during follow-up, there were important differences in the immune response between PRA(-) and PRA(+) patients. The percentage and total number of lymphocytes were significantly improved during the first 3 mo in PRA(-) patients after transplantation. By contrast, the former showed a delayed and weak response in PRA(+) patients. Also, changes in lymphocyte subpopulations showed differences between the two groups. PRA(+) patients were characterized by a shift towards the CD8+ cell population, while in PRA(-) patients, CD4+ cells predominated during follow-up. As the presence of PRA was not associated with sex, age, time on HD, or impaired renal function, we anticipated that differences in T lymphocytes between PRA(-) and PRA(+) patients could not be attributed to other parameters such as HDV or renal function impairment, but rather were directly connected to the effect of PRA.

Interestingly, the expression of CD28 antigen on both CD4 and CD8 cells was not substantially affected by transplantation. CD28 loss is related to normal aging, but is also a consequence of chronic

autoimmune and inflammatory diseases[15-19], while recently, CD28 elimination has been described in patients with CKD. The reduction of this receptor in CKD patients has been attributed to uremia, chronic inflammation, oxidative stress, CMV infection, and chronic dialysis[1,17-20].

We found that the percentage of CD4CD28null cells showed a reduction in both groups during the first 3 mo, yet they were subsequently stabilized until the end of follow-up. Regarding CD8CD28null cells, the beneficial effect was proven only in PRA(-) and not in PRA(+) patients, in whom there was a significant increase after the 3rd mo posttransplantation. This is in accordance with previous studies, which showed that CD28 antigen was significantly eliminated in both CD4 and CD8 cells after renal transplantation[21]. In a recent study, lymphocytes from renal transplant patients, who were followed for up to 5 years posttransplant, showed a tendency towards senescent phenotype, including a gradual increase in CD4CD28null and CD8CD28null cells. These findings indicate that despite restoring renal function with a successful renal transplantation, immune phenotype cannot be completely retained. Apparently, immunosuppression and steroid administration have a crucial role in this phenomenon, and this has been proved by the alterations in T cell phenotypes, after the withdrawal of steroids[22].

CD4+CD28null T cells are differentiated from classic T helper cells and share many features of cytotoxic CD8+ T cells and NK cells. They express a cytotoxic profile by producing proinflammatory cytokines, such as interferon gamma (IFN-γ), tumor necrosis factor alpha, and cytotoxic molecules[18,23,24]. CD28null T cells are considered terminally differentiated senescent cells, with shortened telomeres and great ability of cytotoxicity[19]. Thus, any alloreactivity of these cells may be detrimental for the transplant[20]. The gradual disappearance of CD28 following transplantation is controversial, with some investigators showing that loss of CD28 on CD4 T cells promotes immunosuppression resistance and allograft rejection[25,26], while others showing that loss of CD28 on T cells is related to immunosuppressive activity[17], leading to allograft tolerance and stabilization and is also associated with a lower frequency of late rejection and graft loss[27-29]. The role of PRA in CD28 expression seems crucial; however, there is a shortage of related information in the literature. The presence of anti-HLA antibodies may simply reflect the activation of adaptive immunity; however, they can induce endothelial damage, leading to *de novo* expression of endothelial neoantigens and vascular remodeling, as well as immune activation and chronic inflammation[30]. Therefore, the indirect effects of PRAs on the persistence of lymphocytes with cytotoxic activity may explain the increased levels of CD28null cells, but also their correlation with NK cells and regulatory T cells.

Changes in NK cells after transplant were more prominent. In both groups of patients, the percentage of NK cells was rapidly reduced during the first 3 mo, but only in PRA(-) patients was a reduction in the percentage of cells followed by the elimination of NK cell absolute numbers. NK cells play a crucial role in antibody-mediated rejection as occurs by the presence of HLA-DSAs[31-33]. NK cells are a source of IFN-γ production and they stimulate the T helper type 1 immune response. A direct interaction of NK cells with CD4+ T lymphocytes[34] increases their reactivity, which may motivate the mechanisms of acute rejection[33].

Most investigators support a mutual antagonism between NK and Treg cells[35]. Tregs seem to play major role in the long-term outcome of renal transplantation, as their population in the 6th and 12th mo posttransplantation was found to maintain immune tolerance in transplantation and is associated with better long-term graft survival[28,36-38], and some investigators have proven a time-dependent reduction of Tregs after kidney transplantation as a result of immunosuppressive treatment[28]. In our study, Tregs were almost spontaneously increased in PRA(-) patients during the first 3 mo of follow up, and continued to improve thereafter until the end of follow-up; by contrast, they showed only a delayed increase in PRA(+) patients.

CONCLUSION

In conclusion, this study demonstrated that T cell reinstatement following renal transplantation was closely affected by the presence of PRAs. Although lymphocyte population increased early after transplant, this beneficial effect did not involve all subpopulations. NK cells were reduced in both groups, Tregs were increased, but only in PRA(-) patients, whereas CD28null cells were not significantly restored regardless of the presence of PRAs.

ARTICLE HIGHLIGHTS

Research background

It is essential to try to both understand and evaluate the effect of panel reactive antibodies (PRAs) on T cell immunity reinstatement, which follows renal transplantation. The potential association between subset changes and posttransplant graft function should be studied further.

Research motivation

This study demonstrated that T cell reinstatement following renal transplantation was closely affected by the presence of PRAs. Although the lymphocyte population increased early after kidney transplantation, this beneficial effect did not involve all subpopulations. Natural killer (NK) cells are reduced in both groups, regulatory T cells (Tregs) were increased, but only in PRA(-) patients, whereas CD28null cells were not significantly restored regardless of the presence of PRAs.

Research objectives

Patients were classified into two groups: PRA(-) (n = 43) and PRA(+) (n = 28). Patients who underwent preemptive kidney transplantation had a better immune profile than those already enrolled in hemodialysis or continuous ambulatory peritoneal dialysis.

Research methods

Flow cytometry analysis was performed in 71 recipients of kidney transplantation at the time of transplantation, and at 3 and 6 mo after transplantation to estimate CD4CD28null, CD8CD28null, NK, and Treg cells.

Research results

The impact of positive PRA on the restoration of T cell phenotype after renal transplantation was evaluated.

Research conclusions

Given the fact that PRA screening is a widely used test performed routinely in patients with chronic kidney disease (CKD) before renal transplantation to assess recipients' exposure and sensitization, we believe it is essential to try to both understand and carefully evaluate the effect of PRA on T cell immunity reinstatement, which follows renal transplantation.

Research perspectives

CKD is associated with phenotypic and functional changes in the immune system, including both innate and adaptive immunity, with detrimental clinical consequences. A successful renal transplantation will allow patients to stop dialysis and reinstates kidney function. Accordingly, as part of returning to normality, it is also expected to restore patients' immune profile.

FOOTNOTES

Author contributions: Fylaktou A, Papagianni A, and Tsoulfas G designed the research study; Xochelli A, Nikolaidou V, Panteli M, and Ouranos K contributed new reagents and analytic tools; Vagiotas L, Kasimatis E, and Lioulios G performed the study; Antoniadis N, Miserlis G, and Vagiotas L provided the patients; Vagiotas L, Stangou M, and Daoudaki M analyzed the data and wrote the manuscript; and all authors have read and approved the final manuscript.

Institutional review board statement: The study was reviewed and approved by the Aristotle University of Thessaloniki. This registration identification number is 611/30-09-2020.

Clinical trial registration statement: This study is registered at the Aristotle University of Thessaloniki. This registration identification number is 611/30-09-2020.

Informed consent statement: All participants provided informed consent before their enrollment to the study.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: Participants gave informed consent for data sharing. The relevant document was given.

CONSORT 2010 statement: The authors have read the CONSORT 2010 Statement, and the manuscript was prepared and revised according to the CONSORT 2010 Statement.

Country/Territory of origin: Greece

ORCID number: Lampros Vagiotas 0000-0002-5176-6781; Maria Stangou 0000-0003-2496-9863; Grigorios Myserlis 0000-0003-1985-9336; Konstantinos Ouranos 0000-0001-9034-5026; Nikolaos Antoniadis 0000-0001-6939-3373; Aikaterini Papagianni 0000-0003-0437-5208; Georgios Tsoulfas 0000-0001-5043-7962.

S-Editor: Wang JJ
L-Editor: Filipodia
P-Editor: Wang JJ

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World Journal of Transplantation

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World J Transplant 2022 October 18; 12(10): 325-330

DOI: 10.5500/wjt.v12.i10.325

ISSN 2220-3230 (online)

CASE REPORT

COVID-19 in a pregnant kidney transplant recipient - what we need to know: A case report

Roberta Angelico, Maria Luisa Framarino-dei-Malatesta, Giuseppe Iaria

Specialty type: Transplantation

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Gonzalez FM, Chile; Tarris G, France

Received: April 5, 2022
Peer-review started: April 5, 2022
First decision: May 31, 2022
Revised: June 7, 2022
Accepted: September 7, 2022
Article in press: September 7, 2022
Published online: October 18, 2022

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Abstract

BACKGROUND

In the era of the coronavirus disease 2019 (COVID-19) pandemic, kidney transplant recipients are more susceptible to severe acute respiratory syndrome coronavirus (SARS-CoV-2) infection, developing severe morbidity and graft impairment. Pregnant women are also more likely to develop severe COVID-19 disease, causing pregnancy complications such as preterm births and acute kidney injury.

CASE SUMMARY

Herein, we report the case of a pregnant woman with a third kidney transplantation who developed COVID-19 disease. The reduction of immunosuppressive drugs and strict monitoring of trough blood levels were needed to avoid severe SARS-CoV-2-related complications, and permitted to continue a healthy pregnancy and maintain good graft function. In such a complex scenario, the concomitance of COVID-19-related morbidity, the risk of acute rejection in the hyperimmune recipient, graft dysfunction and pregnancy complications make the management of immunosuppression a very difficult task and clinicians must be aware.

CONCLUSION

Tailoring the immunosuppressive regimen is a key factor affecting both the graft outcome and pregnancy safety.

Key Words: Kidney transplantation; Pregnancy; SARS-CoV-2 infection; COVID-19 disease; Immunosuppression; Complications; Case report

Core Tip: Kidney transplant (KT) recipients are susceptible to coronavirus disease 2019 (COVID-19). Pregnant women are more likely to develop severe COVID-19, causing pregnancy complications such as preterm births and acute kidney injury. The management of immunosuppression in pregnant KT recipients with severe acute respiratory syndrome coronavirus infection is crucial for the avoidance of severe morbidity to the patient and the fetus, and to escape renal graft dysfunction.

Citation: Angelico R, Framarino-dei-Malatesta ML, Iaria G. COVID-19 in a pregnant kidney transplant recipient - what we need to know: A case report. *World J Transplant* 2022; 12(10): 325-330
URL: https://www.wjgnet.com/2220-3230/full/v12/i10/325.htm
DOI: https://dx.doi.org/10.5500/wjt.v12.i10.325

INTRODUCTION

Kidney transplant (KT) recipients are susceptible to coronavirus disease 2019 (COVID-19), with an associated 18%-39% intensive care admission rate and 13%-39% mortality[1]. Pregnant women are more likely to develop severe COVID-19, causing pregnancy complications such as preterm births and acute kidney injury[2,3].

CASE PRESENTATION

Chief complaints

In October 2020, a 37-year-old woman at 20 wk of gestation, who had received a third KT 2 years ago, presented with fever, cough, and anosmia.

History of present illness

The patient presented with fever, cough, and anosmia.

History of past illness

Her past medical history consisted of end-stage chronic kidney disease due to focal and segmental glomerulosclerosis, requiring three sequential KTs due to chronic rejections with a panel reactive antibody titer of 100%.

Personal and family history

The patient's personal and family histories were unremarkable.

Physical examination

At presentation, the severe acute respiratory syndrome coronavirus (SARS-CoV-2) polymerase chain reaction (PCR) test was positive.

Laboratory examinations

Biochemical tests showed 7.640/μL white blood cells, C-reactive protein of 10.1 mg/L and creatinine of 1.18 mg/dL (baseline at pregnancy: 1.1 mg/dL). The immunosuppression (IS) regimen consisted of steroids (5 mg/d), once-daily tacrolimus (extended-released Envarsus, target level: 7-8 μmol/L) and azathioprine (1 mg/kg/d), the latter started 1 year previously, replacing mycophenolate acid as she declared the intent to become pregnant.

Imaging examinations

Chest X-ray was negative for pneumonia.

FINAL DIAGNOSIS

SARS-CoV-2 infection in a KT pregnant lady.

TREATMENT

At diagnosis of SARS-CoV-2 infection, azathioprine was suspended, while steroids and tacrolimus were maintained at unchanged doses. During the infection, the patient developed moderate respiratory symptoms and close clinical monitoring was performed, showing persistent stable graft function, steady tacrolimus blood levels and regular fetal growth. One month later, the patient achieved a complete clinical recovery. The SARS-CoV-2 swab became negative after 40 d. At 39 wk of gestation, she had an uneventful delivery of a healthy male infant (weight: 3.2 kg; Apgar score: 9/10) by caesarean section.

OUTCOME AND FOLLOW-UP

At the time of delivery, the placenta and the newborn were not tested for SARS-CoV-2. The patient's renal graft function remained stable throughout the post-delivery period, and after 17 mo of follow-up the creatinine was 1.09 mg/dL (Table 1). During pregnancy, anti-human leukocyte antigen donor-specific antibody (DSA) screening was performed and these antibodies were not detected. In particular, no evidence of post-COVID-19 DSA was identified. Graft biopsy was not done. At the last follow-up, both the mother and the child were in good clinical condition.

DISCUSSION

The reduction of the immune response due to both IS drugs and pregnant status render pregnant KT recipients vulnerable to viral infections such as SARS-CoV-2[1,2]. In our case, this was further enhanced by her non-vaccinated status, since at that time the vaccine for SARS-CoV-2 was not available yet. Therefore, the concomitance of COVID-19-related morbidity, the risk of acute rejection in hyperimmune re-KT, graft dysfunction and pregnancy complications make the management of IS a very difficult task.

In KT recipients, recommendations suggest the modification of IS drugs according to the severity of COVID-19, ranging from no modification in asymptomatic patients, antimetabolite withdrawal in mild/moderate symptomatic disease, to complete drug discontinuation in severely ill patients requiring mechanical respiratory support[4,5]. In this case, we decided to withdraw azathioprine, which inhibits purine synthesis, aiming to avoid the depletion of T- and B-cells during the SARS-CoV-2 infection. Tacrolimus and steroids at low-doses remained the only IS drugs, without increasing their blood target-levels. The extended-released formula of tacrolimus Envarsus, which provides effective and stable blood concentration with less toxic levels compared to other Tacrolimus formulae[6], permitted the safe control of rejection risk and the avoidance of severe COVID-19. Thus, a recent report suggested that a mammalian target of rapamycin inhibitor may have potential antiviral benefits in SARS-CoV-2 infection [7].

In this case, strict monitoring of DSA was performed before and after COVID-19, since the IS regimen had been reduced. Despite the significant decrease of the IS and the high risk of rejection due to the hyperimmune status of third-KT recipients, our patient did not develop new DSA or rejection episodes. These data confirm a recent report investigating the alloreactive immune response during and after SARS-CoV-2 infection in KT recipients, which showed that the incidence of acute rejection is about 1.3% (all in hospitalized patients) and the occurrence of post-COVID-19 DSA is 4% overall, ranging from 0% to 8% in non-hospitalized and hospitalized patients, respectively[8]. Despite the immunosuppressed status of a third KT pregnant lady, our patient was very lucky because she was in this group of patients who do not develop severe COVID-19 disease. Since the stable kidney function and the pregnant status, we did not perform a graft biopsy in order to avoid possible biopsy-related complications. Additionally, venous thromboembolism prophylaxis was not administrated as no evidence was present, but its utility should be explored in pregnant COVID-19 KT recipients.

Pregnancy in KT recipients may be associated with a high-risk of maternal complications and decreased graft function, which could further deteriorate in the presence of COVID-19[9]. In fact, the occurrence of acute kidney injury in infected pregnant KT recipients could be due to the SARS-CoV-2 infection or to other pregnancy-related causes, which need to be differentiated[10]. In immunosuppressed transplant recipients as well as pregnant women, SARS-CoV-2 showed the potently to replicate into the kidney causing renal disfunction[11,12]. Lastly, despite the fact that the risk of acquiring SARS-CoV-2 infection during pregnancy seems to be similar to that of non-pregnant patients, severe maternal COVID-19 is associated with acute kidney injury and preterm birth.

The risk of congenital infection with SARS-CoV-2 to the newborn is still unknown[2,13]. In our case, the placenta and the baby were not tested for SARS-CoV-2 PCR, therefore unfortunately we do not have these interesting data. Moreover, despite KT pregnant recipients are more susceptible to chronic infection such as cytomegalovirus (CMV) infection, we didn't detect any CMV infection during pregnancy. This is the first report focusing on IS management in SARS-CoV-2-positive pregnant KT recipients.

Table 1 Patients' characteristics

Variables at presentation	Values
Demographics	
Age, yr	37
Sex	Female
Race	White
Number of KT	3
Primary nephropathy	Focal and segmental glomerulosclerosis
Causes of previous KT losses	Chronic rejection
Time from last KT	24 mo
Comorbidities	Arterial hypertension
Pregnancy	
Gestation age, wk	20
Fetal grow	Regular
Symptoms/signs	
Fever, T > 37.5 °C	Yes
Dyspnea	Yes
Anosmia	Yes
Myalgias	Yes
SARS-CoV-2 status	
SARS-CoV-2 swab test positive	Yes (positivity for 40 d)
SARS-CoV-2 vaccination	No
Biochemical tests	
At infection diagnosis	
Creatinine, mg/dL	1.18
WBC as × 10^3/mmc	7.640
Lymphocytes, cells/mmc	1.590
PTL as × 10^3/mmc	202
C-reactive protein, mg/L	10.1
Procalcitonin, ng/mL	0.52
Peak during infection	
Creatinine, mg/dL	1.3
WBC as × 10^3/mmc	12.700
Lymphocytes, cells/mmc	3.400
PTL as × 10^3/mmc	250
C-reactive protein, mg/L	20.2
Procalcitonin, ng/mL	2.01
Immunosuppression regimen	
Tacrolimus	Continued at unchanged doses (target levels: 7-8 μmol/L)
Azathioprine	Withdrawal
Steroids	Continued at unchanged doses (5 mg/d)
Outcomes	
Recovery from COVID-19 disease, mo	1

De novo DSA after SARS-CoV-2 infection	No
Rejection episode	No
Delivery	
Time of delivery, wk	39
Newborn status	Healthy, no complication
Time of follow-up after infection, mo	17
Renal function at last follow-up	
Creatinine, mg/dL	1.09

COVID-19: Coronavirus disease 2019; DSA: Donor-specific antibody; KT: Kidney transplant; PTL: Primary testicular lymphoma; SARS-CoV-2: Severe acute respiratory syndrome coronavirus; WBC: White blood cell.

CONCLUSION

We suggest that all efforts should be made to avoid severe maternal COVID-19 disease through tailored adjustment of the IS regimen and close monitoring of calcineurin inhibitor trough-blood levels, graft function and fetal parameters. Currently, mRNA vaccines against SARS-CoV-2 are recommended both in KT recipients and pregnant women, and may help in preventing severe COVID-19 disease[14,15]. However, KT patients have been shown to frequently be poor responders to the vaccines, thus remaining at high risk of developing severe COVID-19[16], especially in pregnancy. In fact, recent data suggest that only selected KT recipients seem to respond to the third booster dose of SARS-CoV-2 vaccine (assessed by anti-receptor binding domain immunoglobulin G titers and/or positive interferon-gamma-releasing assay)[17]. Moreover, in pregnancy, the boosting effect of a third vaccine dose is suggested to have a potential benefit only in those who completed the two-dose vaccine series in early pregnancy or prior to conception[16]. We feel that, although no data are yet available on the efficacy of the vaccine in preventing COVID-19 disease in pregnant KT recipients, a complete vaccine cycle against SARS-CoV-2 with three doses should preferably be performed before pregnancy. In addition, clinicians should be ready to tailor IS drugs when a member of this rare population is infected by SARS-CoV-2.

FOOTNOTES

Author contributions: Angelico R contributed to the study conception and design, writing; Framarino-dei-Malatesta ML was involved in the acquisition of clinical data, analysis, and interpretation; Iaria G contributed to the study conception; and all authors were involved in critical revision.

Informed consent statement: Informed consent was obtained from the patient for publication of their information.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

Country/Territory of origin: Italy

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S-Editor: Wang JJ
L-Editor: Filipodia
P-Editor: Wang JJ

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