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Evaluation of cell-mediated immune response by QuantiFERON Monitor Assay in kidney transplant recipients presenting with infective complications

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Abstract

The net level of immunosuppression in kidney transplant recipients is difficult to assess. QuantiFERON Monitor (QFM) is an in vitro diagnostic test that detects interferon-γ (IFN-γ) release in peripheral blood. The aim of our study was to compare QFM testing results in stable kidney transplant recipients and kidney transplant recipients with infection, in a single-centre cohort.

We enrolled 71 kidney transplant recipients from our transplantation centre. They were divided into 2 groups according to clinical presentation (Stable kidney transplant recipients or Infection).

There were no significant differences in interferon-γ release between the 2 groups (Stable kidney transplant recipients 140.59 ± 215.28 IU/ml, Infection group 78.37 ± 197.03 IU/ml, $P = .24$). A further analysis revealed that kidney transplant recipients presenting with bacterial infection had significantly lower IFN-γ release when compared to stable kidney transplant recipients (26.52 ± 42.46 IU/ml vs 140.59 ± 215.28 IU/ml, $P = .04$).

Kidney transplant recipients presenting with bacterial infection had lower IFN-γ release when compared to stable kidney transplant recipients. The QFM test may be useful as a tool to help guide immunosuppression dosing in kidney transplant recipients, but further studies are required to confirm its diagnostic value.

Abbreviations: CICFA = Cylex ImmuKnow cell function assay, CNI = Calcineurin-inhibitor, ds = Donor-specific, eGFR = Estimated glomerular filtration rate, ELISPOT = T-cell Enzyme-linked Immunosorbent Spot assay, IFN-γ = Interferon-gamma, MPA = Mycophenolic acid, PRT = Panel of reactive T cells, QFM = QuantiFERON Monitor.

Keywords: immunosuppression, infection, kidney transplantation, QuantiFERON Monitor

1. Introduction

All kidney transplant recipients require immunosuppression to ensure graft survival. An individual kidney transplant recipients net immunosuppression can be difficult to assess, which in turn can be problematic because under-immunosuppression is associated with increased risk of kidney rejection and over-immunosuppression can lead to infectious or malignant complications.[1,2] The current practice in assessing NI is by monitoring concentrations of calcineurin-inhibitors (CNIs) or mTOR inhibitors and doses of mycophenolic acid (MPA).[3] Despite years of experience with this approach, patients are often over- or under -immunosuppressed, because there is imprecise relationship between a CNI, or mTOR inhibitor exposure and rejection.[4] In addition, by monitoring just one drug, it is impossible to assess net state of immunosuppression, which hinders individualization of immunosuppression in organ transplant recipients.[5] There are currently no available tools for measuring net immunosuppression, which would allow more precise individual tailoring of immunosuppression.

One of the studied potential tools for measuring net immunosuppression is the alloreactive T-cell Enzyme-linked Immunosorbent Spot assay (ELISPOT). It is a diagnostic test which measures the frequency of allo-specific cytokine secreting cells.[6,7] Interferon-gamma (IFN-γ) ELISPOT was found to be able to identify patients who are at risk for early acute rejection.[8] Also, it was shown that IFN-γ ELISPOT had a significant inverse correlation with allograft function at 6 and 12 months post-transplantation.[9] However, a recent observational multi-centric study did not show correlation of IFN-γ ELISPOT results with either acute rejection or kidney function at 6 and 12 months post-transplantation, although it did find that there was a correlation of lower kidney function in patients who had positive IFN-γ ELISPOT and did not receive ATG induction therapy.[10]

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The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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Pre- and post-transplant viral specific ELISPOT (CMV, BK) assays can be effective in determining risk of developing viral infections post-transplant as well as help to individualize immunosuppressive treatments by identifying patients with viral-specific T-cell recovery. These assays also may highlight patients who either need to have immunosuppression doses lowered or are at risk of acquiring long-term viral infections.[11]

Another potential for monitoring net immunosuppression in transplant recipients is the Cylex ImmuKnow cell function assay (CICFA). It is a diagnostic tool which monitors CD4+ T cell function by measuring the intracellular concentration of adenosine triphosphate.[12] A meta-analysis suggested that CICFA was not able to identify transplant recipients at risk for rejection or infection.[13] CICFA mostly measures the adaptive immune response. Hence, a test which measures both the adaptive and innate immune response could provide a better approximation of net immunosuppression.

In this study, we used QuantiFERON Monitor assay (QFM, Qiagen, Hilden, Germany). QFM is an in-vitro diagnostic test that measures IFN-γ release in response to stimulation of patients whole blood with both adaptive and innate stimulants of the cellular immune response system.[14] A high IFN-γ release is associated with a stronger immune response, while a low IFN-γ release is suggestive of a weaker immune response or higher net immunosuppression state. Therefore, QFM may be useful to measure a kidney transplant recipients overall degree of immunosuppression. Unfortunately, prospective trials with QFM in kidney transplant recipients are lacking. Therefore, we performed a cross-sectional study in order to determine the discriminating value of QFM assay results for infection in a single-center cohort of kidney transplant recipients.

2. Materials and methods

The cross-sectional study was approved by the University Hospital "Merkur" Ethics Committee and patients provided informed consent. Study was conducted in accordance with the Helsinki declaration. Adult kidney transplant recipients from our center were recruited between April 2017 and February 2019.

Blood samples were collected during regular outpatient visits and at hospital admission for infection. The samples were collected independent of time after transplantation, meaning both patients in early and late-post transplant period were included. For patients with repeated testing, only the first QFM assay result for each patient was included in statistical analysis.

The QFM assay was performed according to the manufacturers instructions at the Department of Clinical and Molecular Microbiology, University Hospital Center Zagreb, Croatia and validated by clinical microbiologist that was not aware of patient clinical condition. Briefly, 1 ml of blood was collected in the provided test tube. The blood was then incubated with cellular immune response stimulants, anti-CD3 and R848.[14] After incubation an enzyme-linked immunosorbent assay (ELISA) was performed to quantify IFN-γ release. Level of IFN-γ was expressed in IU/ml. QFM assay results were not revealed to treating transplant physicians and did not influence further patient treatment.

Kidney transplant recipients were divided into 2 groups: stable kidney transplant recipients and kidney transplant recipients with infection. The stable kidney transplant recipient group was defined by absence of clinical, laboratory, or biopsy findings associated with infection or rejection. Kidney transplant recipients with rejection, or previous episodes of rejection were excluded from the study. Minimal time after transplantation for inclusion was 60 days. Infection inclusion criteria for CMV infection were defined by the American Society of Transplantation recommendations.[15] Both CMV active disease and CMV infection, as defined by the AST criteria, were included in the infection group. For BKV we used both the presumptive and definitive BKV nephropathy diagnosis as inclusion criteria into our infection group.[16] Other infections were defined as an elevated CRP (>20 mg/dl), clinical and laboratory signs and symptoms of infections (e.g., positive urine culture, positive blood culture, fever, chest x-ray suggestive of infection), and prescription of antimicrobial drugs by the transplant physician.

Induction immunosuppression consisted of basiliximab, CNI, MPA, and steroids. Patients with panel reactive antibodies >20% were given a single course of rituximab (375 mg/m^2) pre-transplant. Immunosuppression consisted mostly of a CNI (tacrolimus or cyclosporine) and MPA, with or without steroids. A few patients had an mTOR inhibitor instead of CNI in their immunosuppression regimen.

2.1. Statistics

Numerical values are shown as means with standard deviation. T- test were used to detect differences between the 2 indication groups. Descriptive statistics were used for demographics. Correlation matrices were used to detect association of continuous variables with IFN-γ release. Multivariate analysis was performed using logistic regression. Variables were included in a multivariate analysis, if being associated in a univariate analysis with the exposure variable with p value of ≤ 0.1 Data were analyzed using Statistica version 13 (TIBCO software Inc., Tulsa, OK 74104, USA). A *P* value of <.05 was considered significant.

3. Results

We collected samples from 71 kidney transplant recipients from April 2017 to February 2019. The majority of our patients were male. There were 47 patients in the stable kidney transplant recipients group and 24 patients in the infection group. Our cohort included both patients in early and late post-transplant period. Main immunosuppression regimen consisted of tacrolimus and MPA ± steroids. Baseline patient characteristics and differences between the 2 groups are shown in Table 1.

Most common bacterial infections were UTIs and pneumonia, there were single cases of Bartonella henselae infection and Clostridium difficile colitis. Patients with viral infection had either BKV or CMV infection. There were no other opportunistic infections diagnosed in our cohort.

Patients in the stable kidney transplant recipient group had a numerically higher IFN-γ release than patients in the infection group, but it was not statistically significant (140.59 ± 215.28 vs 78.37 ± 197.03 IU/ml, *P* = .24). An analysis comparing kidney transplant recipients presenting with bacterial infections and stable kidney transplant recipients was also conducted. We found a significant difference between these 2 groups of patients (Fig. 1). There was no significant difference between patients with viral infection and stable kidney transplant recipients (182.06 ± 324.43 vs 140.59 ± 215.28 IU/ml, *P* = .65). There was borderline significant difference when comparing patients with bacterial and patients with viral infection (26.52 ± 42.46 vs 182.06 ± 324.43, *P* = .07). These results are shown in Table 2.

Table 1

Baseline characteristics.

	Stable KTRs (N=47)	Infection group (N=24)	*P* value
Age, years	52.6±13.2	55.3±13.4	.42
Male, n (%)	30 (63.8)	15 (62.5)	.91
Time after tx, years	2.7±3.1	2.6±2.8	.93
eGFR, ml/min/1.73 m^2	58.8±23.0	51.3±26.4	.22
Tac concentration, μg/L	6.0±1.7	6.4±3.9	.53
MPA dose, mg	1834±641	1507±691	.05
Steroid dose, mg	3.2±3.0	5.2±2.2	.01

Data is presented as number (percentage), mean ± SD.
eGFR = estimated glomerular filtration rate, MPA = mycophenolic acid, Tac = tacrolimus, Tx = transplantation.

Tacrolimus concentration had a borderline significant negative correlation with IFN-γ release (Fig. 2). Patient age, time after transplantation, estimated glomerular filtration rate (eGFR), MPA dose, and steroid dose had no significant correlation with IFN-γ release (Table 2). Patients who received rituximab in induction therapy had no differences in IFN-γ release.

Multivariate analysis was performed with infection as the dependent variable. We included variables which were statistically significant or borderline significant –MMF dose and steroid dose. We also used forced entry for IFN-γ release. Only higher steroid dose remained significantly associated with infection. Afterwards we performed multivariate analysis excluding viral infections using the IFN-γ release, steroid dose, and Tacrolimus concentration as variables. All of the selected variables had significant or borderline difference when excluding patients with viral infections. Using this analysis steroid dose, tacrolimus concentration and IFN-γ release did not reach statistically significant association with bacterial infection (Table 3).

4. Discussion

We conducted this cross-sectional study to determine if the QFM assay could be used to help identify overimmunosuppressed kidney transplant recipients at risk for infection. When comparing stable kidney transplant recipients and patients with infection there was no statistical difference between the 2 groups.

Table 2

IFNγ release according to indication groups and correlation of baseline characteristics and IS concentrations/doses with IFNγ release.

	IFNγ release (IU/ml), or correlation (r) with IFNγ release	*P* value
Age	−0.03	.80
Female vs Male gender	55.93±106.48 vs 156.32±244.79	.05
Time after tx	0.11	.37
eGFR	−0.03	.81
Tac concentration	−0.21	.08
MPA dose	−0.08	.48
Steroid dose	0.04	.70
Stable KTRs vs Infection	140.59±215.28 vs 78.37±197.03	.24
Stable KTRs vs Bacterial infection	140.59±215.28 vs 26.52±42.46	.04
Stable KTRs vs Viral infection	140.59±215.28 vs 182.06±324.43	.65
Bacterial vs Viral infection	26.52±42.46 vs 182.06±324.43	.07
Stable KTRs vs Infection	140.59±215.28 vs 78.37±197.03	.24

eGFR = estimated glomerular filtration rate, IFN-γ = interferon gamma, KTRs = kidney transplant recipients, MPA = Mycophenolic acid, Tac = Tacrolimus, Tx = transplantation.

However, our results showed that kidney transplant recipients presenting with bacterial infections had lower IFN-γ release when compared to stable kidney transplant recipients. IFN-γ release was also numerically lower in patients with bacterial infections, as compared to the patients with viral infections, but this was only borderline significant. However, our multivariate analysis showed that only higher steroid dose was associated with infection risk. Even when excluding viral infections IFN-γ release did not reach statistically significant association with bacterial infections.

Some recent studies have shown that QFM can detect solid organ transplant recipients at higher risk of infection, although a multivariate analysis was not preformed.[17] Lower IFN-γ release was strongly associated with infections in liver transplant recipients, which also remained statistically significant in multivariate analysis.[18] The reason for this discrepancy between our results and previously published ones are unclear, but our study included only kidney transplant recipients, whereas others compared all solid organ transplant recipients or only liver transplant recipients.

What drives magnitude of IFN-γ release measured by QFM has been incompletely evaluated. When analyzing IS drug concen-

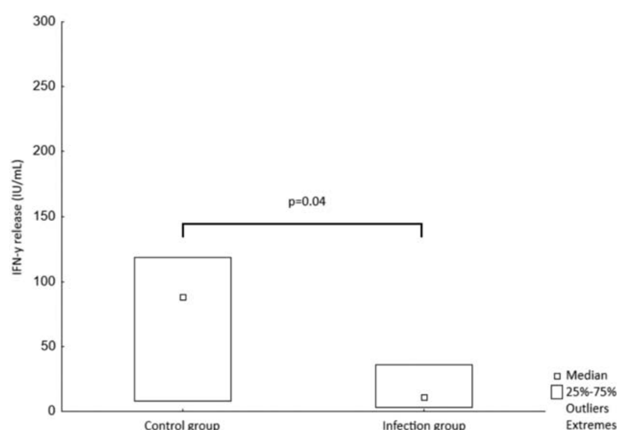

Figure 1. IFN-γ release difference between control and infection group exluding viral infections.

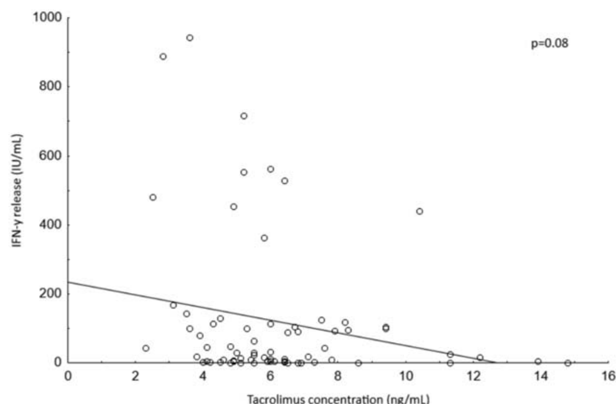

Figure 2. Correlation of Tacrolimus concentration and IFN-γ release.

Table 3

Multivariate logistic regression analysis with bacterial infection as a dependent variable.

	Odds ratio	−95% CI	+95% CI	*P* value
IFN-γ release	0.98	0.97	1.00	.07
Tac concentration	1.11	0.85	1.44	.44
Steroid dose	1.24	0.98	1.56	.07

IFN-γ = interferon gamma, MPA = mycophenolic acid, Tac = tacrolimus.

trations and doses we found a borderline significant negative correlation between IFN-γ release and tacrolimus concentration. In contrast, there was no correlation between IFN-γ release and MPA or steroid dose. These results are opposite to other similar studies which found significant correlation between IFN-γ release and MPA and steroid doses and no correlation of IFN-γ release with tacrolimus concentration.[17,18] Another interesting fact was that there was no significant correlation of time after transplantation and IFN-γ release which was found in some other studies.[13,17,18] This may be due to our transplant center immunosuppression regimen which uses higher long-term MPA doses.

Bacterial infection-induced immunosuppression is another factor which may lower IFN-γ release. It is mostly associated with severe sepsis.[19] However, as our cohort had a very low number of sepsis patients, we feel that bacterial infection-induced immunosuppression might not have been dominant case of our IFN-γ release results. The opposite explanation may be that excessive immune suppression, evidenced as a low IFN-γ release in some patients, might have increased their risk for bacterial infections. However, a prospective study would be required to assess causal relationship between bacterial infections and IFN-γ release.

QFM adds to some other modalities of cell-mediated immune function assessment, such as IFN-γ release determined by ELISPOT, and the Cylex ImmuKnow cell function assay (CICFA). There is large body of evidence that IFN-γ release in response to allospecific stimulation, measured by ELISPOT, may predict kidney graft rejection[8,20] and worse graft function.[21] We have not found a study which used ELISPOT for detecting kidney transplant recipients at higher risk of bacterial infection.

CICFA was developed as a diagnostic tool which monitors CD4+ T cell function by measuring the intracellular concentration of adenosine triphosphate.[12] Reports about CICFA are conflicting. Smaller studies have found that lower CICFA values are associated with an increased incidence of infections,[22,23] while higher values had predictive value for acute rejection.[24] However, a larger retrospective study which analyzed 1330 CICFA values failed to show association between CICFA values and development of infection or rejection.[25] This was confirmed by 2 meta-analyses which also found no association between CICFA values and adverse effects.[12,26]

There were certain limitations to our study. Our patient recruitment was done at the discretion of the treating transplant physician. Due to relatively lower number of patients with infection, our stable kidney transplant group was overrepresented, which diminished the power of our study to detect small differences in IFN-γ release between the groups.

In conclusion, kidney transplant recipients presenting with bacterial infection had lower IFN-γ release when compared to stable kidney transplant recipients. The QFM test may be useful as a tool to help guide immunosuppression dosing in kidney transplant recipients, esp. to avoid over-immunosuppression, but further randomized multicenter prospective studies are required to confirm its diagnostic value in such patients.

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Partial results of the study were presented as a mini-oral presentation at TTS Madrid 2018. Abstract with partial results was published in Transplantation (July 2018 - Volume 102 - Issue - p S130, doi: 10.1097/01.tp.0000542746.59298.61).

Author contributions

IM and MK designed the study, analyzed the data and wrote the paper; I. Mareković and AP performed the laboratory testing; IM, MZ, MD and I. Mrnjec collected the data.

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