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Evaluation of Quantiferon®-Monitor as a biomarker of immunosuppression and predictor of infection in lung transplant recipients

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Abbreviations: CLAD, chronic lung allograft dysfunction. QFM, Quantiferon®-Monitor. LTR, lung transplant recipients. BAL, bronchoalveolar lavage. CMV, cytomegalovirus. D, donor. R, recipient. OI,

opportunistic infection. IQR, interquartile range. ROC, receiver-operating characteristic. HR, hazard ratio.

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Running head: Quantiferon®-Monitor in lung transplantation

Authorship statement: BG, AP & GW participated in all aspects of this project including study design, data collection, analysis, interpretation and manuscript preparation. YC & BL coordinated patient recruitment and data collection, SL with analysis. GS and LS were involved in project development, interpretation and manuscript editing. All authors have reviewed the manuscript and are in agreement with its content.

Abstract

Background: Optimizing immunosuppression in lung transplant recipients (LTR) is crucially important in minimizing the risk of infection and rejection. Quantiferon®-Monitor (QFM) is a candidate immune function biomarker which has not yet been rigorously evaluated in the lung transplant setting. The aim of this prospective cohort study was to explore relationships between QFM results, immunosuppression and infection/rejection in LTR.

Methods: QFM, which measures interferon-γ after stimulation with innate and adaptive immune antigens, was tested before and at 2, 6, 12, 24- and 52-weeks post-transplant. Immunosuppression relationships were assessed with linear mixed effects models. Clinical outcomes were analyzed based on the preceding QFM result.

Results: Eighty LTR were included. Median pre-transplant QFM levels were 171 IU/mL (IQR 45-461), decreasing to 3 IU/mL (IQR 1-8) at 2 weeks post-transplant then progressively recovering towards baseline with time from transplant. Prednisolone was strongly inversely associated with QFM level (0.1mg/kg dose increase correlating with 88 IU/mL QFM decrease, 95% CI 61-114, p<0.001). Patients with QFM values <10 and <60 IU/mL were more likely to develop a serious opportunistic infection between 3-6 months (HR 6.38, 95% CI 1.37-29.66, p=0.02) and 6-12 months (HR 3.25, 95% CI 1.11-9.49, p=0.03) post-transplant respectively.

Conclusions: QFM values declined significantly post-transplant, with patients recovering at different rates. Prednisolone dose significantly impacted QFM results. Low levels were associated with infection beyond 3 months post-transplant, suggesting that QFM may be able to identify overly

immunosuppressed patients who could be targeted for dose reduction. Larger prospective studies are needed to further evaluate this promising assay.

Background

Lung transplantation is a life-saving procedure for patients with end-stage lung disease, however survival remains limited by infection and rejection, both of which contribute to chronic lung allograft dysfunction (CLAD) and subsequent graft failure (1-3). Optimizing the level of immunosuppression to minimize the risk of these outcomes is of key importance. While a number of potential biomarkers have been explored, the current standard of care is mostly limited to monitoring of calcineurin inhibitor levels, with precise targets that achieve appropriate immunosuppression for individual patients difficult to define (4-7).

The Quantiferon®-Monitor (QFM, Qiagen, Germantown, MD, USA) is a novel whole blood immune function assay that measures plasma interferon- γ levels following stimulation with innate and adaptive immune antigens. Clinical studies of this promising biomarker are emerging. Low QFM levels have been described in a small study of cirrhotic patients and liver transplant recipients, with patients testing <214 IU/mL more likely to develop pre-transplant infection (HR 4.1, p=0.01) and those testing <30 IU/mL experiencing higher pre-transplant mortality (HR 56.6, p=0.003) (8, 9). In another cohort of liver transplant recipients, low QFM values early post-transplant were associated with infection, and high values with rejection, however clinical cutoffs could not be easily identified because of high numbers of false positives and false negatives (10). A larger prospective observational study of 137 transplant recipients (50 lung, 44 kidney, 42 liver, 1 small bowel) who underwent testing at 1, 3- and 6-months post-transplant identified an association between low QFM results and higher prednisolone doses and thymoglobulin use. Patients who developed infections had lower QFM values prior to their infection, but there was no association with rejection (11). More recently, QFM has been evaluated in bone marrow transplant recipients and low levels were associated with infections during the pre-engraftment period (12).

An improved ability to measure net state of immunosuppression would allow for individualization of immunosuppressive therapy before clinical events occur, potentially minimizing the risk of both under-immunosuppression leading to rejection and over-immunosuppression resulting in infection and drug toxicity. The aims of this study were to evaluate QFM both as a biomarker of

immunosuppression and as a tool for the prediction of infection and rejection in a prospective observational cohort of lung transplant recipients (LTR).

Methods

Study design & population

Patients on the lung transplant waiting list were recruited from August 2015 to July 2017. The Alfred Health Ethics Committee approved the study (no. 276/15) and written informed consent was obtained from all participants. Patients were excluded if they were unable to consent, <18 years old, previously received a transplant, or if long-term follow-up was planned elsewhere. Blood was collected before and at 2, 6, 12, 24 and 52 weeks following transplant. Clinical data were obtained from hospital medical records. A panel of lung transplant/infectious diseases physicians reviewed all cases to determine whether they experienced infection, blinded to QFM results. All patients underwent surveillance bronchoscopy with bronchoalveolar lavage (BAL) at the same timepoints as blood draws and when clinically indicated. BAL fluid was sent for bacterial/mycobacterial/fungal cultures, cytomegalovirus (CMV) viral load testing, cytology and other diagnostics as indicated. Transbronchial lung biopsy was performed at all bronchoscopies except 9 months and sent for histopathologic evaluation. Serial lung function testing was performed at least monthly coinciding with outpatient clinic attendance.

Laboratory methods

The QFM assay was performed according to the manufacturer's instructions without protocol variations. In brief, 1mL of blood was collected in a dedicated tube. A lysosphere containing anti-CD3 (T-cell stimulant) and R848 (a Toll-like receptor 7/8 ligand) was added within 8 hours and the sample incubated for 16-24 hours at 37°C, then centrifuged to obtain plasma. An enzyme-linked immunosorbent assay for interferon-γ was then performed. The package insert recommends two cutoffs of <15 and >1000 IU/mL, dividing results into low, moderate and high categories. Testing was batched, performed weekly, and not available to treating clinicians in real-time.

Immunosuppression & rejection

Patients received maintenance immunosuppression with a calcineurin inhibitor (typically tacrolimus), an antiproliferative drug (typically azathioprine) and prednisolone. Basiliximab induction was used in patients at high risk of renal dysfunction. Acute cellular rejection was diagnosed on transbronchial biopsy according to the International Society for Heart and Lung Transplantation (ISHLT) scoring system (13). Patients with rejection ≥ grade A2 were treated with intravenous methylprednisolone 15mg/kg daily for 3 days, as were patients with clinically suspected acute rejection in the absence of a confirmatory biopsy.

Antimicrobial prophylaxis & infection

All patients received perioperative antibacterial prophylaxis for 10-14 days, typically an antipseudomonal beta-lactam (eg. piperacillin-tazobactam), tailored to donor/recipient microbiology. *Pneumocystis* prophylaxis with trimethoprim-sulfamethoxazole was commenced 2 weeks post-transplant and continued indefinitely. Routine antifungal prophylaxis was not used, instead patients with a mold identified on pre- or post-transplant microbiologic testing received a mold-active azole for 3 months. Otherwise, routine oral nystatin was used to prevent oral candidiasis. All CMV seropositive recipients (R+) and those receiving an organ from a CMV seropositive donor (D+/R-) received primary prophylaxis for 5 or 11 months respectively with IV ganciclovir then oral valganciclovir 450mg twice daily (renally dose-adjusted), generally discontinued a month before a planned bronchoscopy. D+/R- patients also received 7 doses of CMV immune globulin. D-/R- patients received valaciclovir for 3 months.

Three different infection endpoints were used: (1) any clinically significant infection, (2) serious infection requiring hospitalization and (3) opportunistic infection (OI). The OI definition was designed to identify infections related to immunosuppression that don't usually affect healthy individuals (see *Supplementary Methods, Table S1*). Some infections were classified as both serious and opportunistic. These definitions were adapted from previous studies and modified to suit our patient population and local diagnostic testing (14-18).

Statistical analysis

Descriptive statistics were calculated, with categorical data reported as counts and percentages, continuous data as means ± standard deviations if normally distributed and medians with interquartile ranges (IQR) if non-normal. Groups were compared using the Chi-squared/Fishers' exact test for

categorical variables, Student's T-test for normally distributed continuous variables, and Wilcoxon rank-sum test for non-normal continuous variables. Relationships between QFM results and immunosuppression were assessed using univariate and multivariate linear mixed effects models, with random effects fitted for patient.

To analyze infection and rejection outcomes, the follow-up year was split into four time periods (2-6 weeks, 6 weeks-3 months, 3-6 months and 6-12 months), with the events occurring within each period analyzed based on QFM results from the beginning of that period. Receiver-operating characteristic (ROC) curves were constructed to determine optimal cutoffs for each time period and outcome. Kaplan-Meier survival curves representing time to infection/rejection were compared using the log-rank test. Cox proportional hazard models were used to calculate hazard ratios (HR) for each time period and outcome, with patients divided into two groups based on calculated cutoffs. For each analysis the reference group was those testing greater than or equal to the cutoff value. Censoring occurred at death, loss to follow-up, re-transplantation, the end of the time period or one-year post-transplant. We performed several sensitivity analyses to confirm our primary findings (*Supplementary Methods)*. P-values of <0.05 were considered statistically significant. Analyses were performed using both the R statistical software platform version 3.5.3 (RStudio version 1.2.1335) and Stata version 15.1 (StataCorp, TX, USA).

Results

Cohort description

Eighty LTR were included (*Figure S1*). Median age was 61 years, 48 (60%) were male, 69 (86%) underwent bilateral lung transplantation and 40 (50%) received basiliximab induction (*Table 1*). The most common indications for transplant were chronic obstructive pulmonary disease (42%) and pulmonary fibrosis (30%). Thirty-two patients (40%) were receiving prednisolone immediately prior to their transplant, for a range of indications mostly related to their underlying pulmonary disease. There was no loss to follow-up although a few patients did not have QFM testing at all timepoints (*Table S2*). One patient who died 12 days post-transplant from a cardiac event was included in the pre-transplant analyses only, leaving 79 patients with post-transplant data available. There were 4 additional deaths and one patient who required re-transplantation during the 1-year follow-up period. Valganciclovir prophylaxis was continued for a median of 10.7 months (IQR 6.7-11.9) in the 20 CMV D+/R- patients and 5.5 months (IQR 5.1-6.6) in the 51 R+ patients.

Pre-transplant QFM results

QFM was performed at a median of 69 days (IQR 21-141) prior to transplantation in 79/80 patients. There was significant variability between patients, with a median value of 171 IU/mL (IQR 45-461). Twenty-two patients (28%) had a result <50 IU/mL. The 32 patients receiving prednisolone (median dose 10mg/day, IQR 5-15) had much lower results than the 47 who were not (median 62 IU/mL, IQR 9-213 versus 299 IU/mL, IQR 67-503, p=0.01, *Figure S2*). The 29 patients who tested CMV seronegative also had lower QFM values than the 50 seropositive patients (71 IU/mL, IQR 5-262 versus 231 IU/mL, IQR 58-499, p=0.01,). There were no significant differences by age, sex, underlying respiratory or cardiac disease, hypertension, diabetes, other immunosuppression or use of intravenous immunoglobulin.

Post-transplant QFM results & relationships to immunosuppression

QFM values declined precipitously following transplantation in almost all patients to a median of 3 IU/mL (IQR 1-8), which was significantly lower than pre-transplant (p<0.00001). Levels then progressively increased over the subsequent year back towards baseline (*Figure 1, Table S2)*. There were 17 patients who had a QFM value of 0 at 2 weeks post-transplant, and over the following year, this subgroup had persistently lower values compared to the others (p<0.001). Patients without prior immunity to CMV (n=29) also consistently had lower post-transplant results than R+ patients (p<0.01).

Tacrolimus levels and prednisolone doses were both inversely associated with QFM levels (*Table 2*). After adjusting for other immunosuppressive medications, prednisolone dose was strongly independently associated with QFM results, with every 0.1mg/kg increase in prednisolone dose correlating with an 88 IU/mL decrease in the QFM level (95% CI 61-114, p<0.001). For every 1µg/L decrease in tacrolimus level, there was a 6 IU/mL increase in QFM (95% CI 0.5-11.5, p=0.03). This means that for example, in a 70kg patient, an increase in prednisolone from 10mg to 20mg would on average decrease the QFM level by 125 IU/mL. However even doubling the tacrolimus trough level from 5 to 10 µg/L would result in a reduction in QFM of only 30 IU/mL. There was no significant effect of other immunosuppressive agents.

Infection

Infections were extremely common, with 77 patients experiencing 313 infections in the first post-transplant year, including 153 (49%) serious infections in 62 patients and 80 (26%) opportunistic infections in 54 patients (*Table S3*). Forty-one episodes of infection in 34 patients were classified as both serious and opportunistic (*Table S4*). The median number of infections per patient was 4 (IQR 2-5), and median time from transplant to first infection was 35 days (IQR 14-80). Respiratory tract infections were the most common (221/313, 71%). An infecting organism was identified in 222/313 cases (71%), more than one in 29 (*Table S5)*. The most common OI was CMV, with 39 episodes in 30 patients. There were 43 fungal infections and an additional 19 episodes of mold colonization. Because of the high number of infection episodes, we restricted our primary analyses to serious and opportunistic infections only.

Early post-transplant, QFM was unable to reliably distinguish between patients with/without infections due to low values with substantial overlap (*Figures S3-S7, Table S6*). However beyond 3 months, patients with lower QFM values were more likely to develop serious and opportunistic infections, although these findings did not all reach statistical significance (*Figures 2-4, Table 3*). Importantly, QFM was able to identify a key subgroup of patients at high risk of late serious OI (*Figure 4*). Patients with QFM values <10 IU/mL at 3 months were much more likely to develop serious OI (HR 6.38, 95% CI 1.37-29.66, p=0.02), as were those testing <60 IU/mL at 6 months (HR 3.25, 1.11-9.49, p=0.03). Sensitivity, specificity, positive and negative predictive values also improved in later periods (*Table S7)*. Our calculated cutoffs changed over time and performed much better than those recommended by the package insert (*Figure S4, Tables S7-S8*). For example, using a higher cutoff of 60 IU/mL at 6 months rather than the package insert cutoff of 15 IU/mL resulted in an improvement in sensitivity from 29% to 65%.

Rejection

Twenty-one patients (27%) were treated with high-dose steroids for a total of 30 episodes of suspected/proven rejection (19 one episode, 11 two or more). Two patients also received an antilymphocyte agent. Biopsies were performed in 15 cases, 4 showed grade 2 rejection, 1 grade 1 and 10 grade 0. Median time from transplant to first rejection was 126 days (IQR 51-222). There were an additional 6 episodes of low-grade rejection that were managed with oral immunosuppression alone. Overall there was a low number of rejection events within each time period and no clear relationship was identified between these and QFM values (*Table S6-S9, Figure S8*). Altering our

definition between any suspected/confirmed episode of rejection and just those treated with pulse methylprednisolone did not significantly change our findings.

Discussion

Optimizing the level of immunosuppression in LTR is challenging but crucially important in minimizing infection and rejection, events which not only cause direct morbidity and mortality but also contribute to the development of CLAD. Current methods for titrating immunosuppression are crude and empiric, based mainly around therapeutic drug monitoring for calcineurin inhibitors. An accurate, reliable, affordable, simple biomarker that is able to measure the overall level of immunosuppression would represent a major step forward in transplant medicine and could inform immunosuppression dose titration, risk profiling, and clinical decision-making around monitoring for infections and antimicrobial prophylaxis. In this study, we have described the trajectory of QFM levels following lung transplantation. QFM results were much more strongly corelated with prednisolone dose than tacrolimus levels or other immunosuppressive agents, suggesting that small increases in prednisolone dose may result in higher levels of immunosuppression that generally thought. This was supported by the finding that patients receiving prednisolone prior to transplant also had lower QFM levels.

The relationship between QFM and clinical outcomes is more complex. QFM values were all extremely low early post-transplant, a period of intense immunosuppression. During this time, there was significant overlap between patients who did and did not develop infections, many of which were not necessarily directly related to immunosuppression. Beyond 3-6 months post-transplant, a period of immune recovery, there was more variability in QFM results. Here we identified a much stronger association between lower QFM levels and infectious complications, particularly for serious opportunistic infections, which were less common earlier post-transplant. These findings suggest that QFM may be able to identify a subgroup of patients who continue to be overly immunosuppressed and could be targeted for dose reduction. Due to the low number of patients within each time period experiencing an episode of rejection, interpreting the relationship between QFM values and rejection was difficult. Prior infection with CMV appears to influence QFM results, with higher levels seen both before and after transplant. This may be related to a higher proportion of terminally differentiated NK and T-cells secreting more interferon-γ in response to mitogen stimulation in CMV seropositive patients (19, 20).

While our findings are overall similar to previous studies, there are some important differences (11). The QFM values in our patients were lower and infections of all types were more common. We suspect that a key reason for this is that ours was a homogenous cohort of LTR, who are more immunosuppressed than other solid organ transplant recipients and at higher risk of infection. In fact the QFM levels seen in our cohort were more similar to bone marrow transplant recipients (12). The ImmuKnow® assay (Cylex/Viracor-Eurofins, USA) is a widely studied immune biomarker that measures adenosine triphosphate (ATP) release from CD4+ T-lymphocytes after phytohemagglutinin-L (PHA) stimulation, which is thought to represent T-cell function (4, 21, 22). Despite being FDA approved in 2002, there has not been widespread clinical uptake in this test mainly due to difficulties in defining cutoffs and interpreting results at a single timepoint in an individual patient (23). There are a range of published studies exploring this test, some of which have shown associations between low levels and infection (24-26). However, many of these are limited by retrospective data collection, small sample size, limited follow-up, low number of outcomes, and lack of longitudinal measurements. Data in lung transplant recipients are limited (27-30). In a randomized clinical trial of ImmuKnow®-guided immunosuppression management versus standard of care, liver transplant recipients who had their immunosuppression regimens modified in response to assay results had a reduced incidence of infection and improved 1-year survival (31). While QFM has not been compared directly to the ImmuKnow® or other immune function tests, it aims to improve on earlier assays by targeting different cell types that capture both innate and adaptive immune responses.

The QFM package insert recommends two cutoff points (15 and 1000 IU/mL), which divides patients into low, moderate and high categories. This is based on limited clinical data. Our findings suggest that like the ImmuKnow® assay, designating simple cutoffs would be overly simplistic and of limited value given the significant changes that occur over time and between different organ types. There was substantial overlap in values between patients who do and do not develop the various outcomes of interest, resulting in many false positives/negatives. One potential alternative strategy may be to develop organ-specific reference curves that plot the predicted post-transplant QFM trajectories with an expected range against which individual patients could be compared and outliers could be identified. Patients could then have their immunosuppression titrated aiming to normalize return them to a 'normal' middle zone. We are currently designing a larger prospective study to further evaluate this approach.

This is the largest study to evaluate QFM in LTR, a complex highly immunosuppressed group of patients. The cohort was characterized in detail, with multiple QFM tests performed over the first

post-transplant year and detailed infection/rejection data collected and analyzed using robust statistical methods. We carefully accounted for the increase in QFM occurring over time when comparing values in patients with/without infection. However, there are some limitations that should be considered when interpreting our results. It was a single-center study with statistical power limited by the sample size and number of outcomes, particularly rejection and serious opportunistic infections, which were relatively rare early post-transplant. While we measured QFM at 6 intervals over the first post-transplant year, monitoring more frequently or at different times may provide additional diagnostic information. Although we identified a strong correlation between prednisolone dose and QFM values, dose reduction occurred at a similar rate after transplant so we could not completely exclude confounding by time or another factor. Additionally, we could not account for dosing alterations between study visits. Events in earlier time periods could have influenced those occurring later, which was not fully accounted for in our analyses. CMV D+/R- patients remained on antiviral prophylaxis for the majority of the follow-up period so CMV events were rare in this high-risk subgroup. There was significant variation in time between the first test and transplant, which occurred for logistic reasons but limited our ability to analyze this as a predictor of early outcomes. Thymoglobulin use in our patients was rare which may have influenced results. Finally, our follow-up was limited to one year and with our findings suggesting a stronger relationship between QFM results and infections occurring beyond the first 6 months, exploring this over a longer time period may be warranted.

In conclusion, QFM may represent an important step towards the goal of individualizing immunosuppression dosing in LTR. It has the potential to monitor global levels of immunosuppression beyond medication doses or serum levels and may be able to identify overly immunosuppressed patients at high risk for infection. However, before this test can be implemented into routine clinical care, interventional studies are required to evaluate it in more detail.

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Potential conflicts of interest: Qiagen provided the QFM assays free-of-charge, supported the study with a research grant and provided GW with financial and travel support to attend Qiagen Advisory Board meetings, but did not have any input into the study design, analysis or manuscript preparation. BL is a Lungitude Foundation board member. All other authors report no conflicts of interest.

Figure legends

Figure 1: Quantiferon®-Monitor results before and after lung transplantation (n=80).

Figure 2: Unadjusted Kaplan-Meier estimates of freedom from serious infection (A) 3 to 6 months and (B) 6 to 12 months following transplantation, stratified by Quantiferon®-Monitor (QFM) results from the beginning of the period. P-values refer to log-rank test results.

Figure 3: Unadjusted Kaplan-Meier estimates of freedom from opportunistic infection (A) 3 to 6 months and (B) 6 to 12 months following transplantation, stratified by Quantiferon®-Monitor (QFM) results from the beginning of the period. P-values refer to log-rank test results.

Figure 4: Unadjusted Kaplan-Meier estimates of freedom serious opportunistic infection (A) 3 to 6 months and (B) 6 to 12 months following transplantation, stratified by Quantiferon®-Monitor (QFM) results from the beginning of the period. P-values refer to log-rank test results.

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Table 1: Characteristics of the 80 lung transplant recipients in our cohort.

Characteristic	Details (n=80)
Age at transplant (years), median, IQR	61, 57-65
Male sex, no. (%)	48 (60%)
Underlying disease, no. (%)	
COPD	34 (42%)
Pulmonary fibrosis	24 (30%)
Cystic fibrosis	5 (6%)
Pulmonary hypertension	3 (4%)
α_1-antitrypsin deficiency	4 (5%)
Other	10 (12%)
Pre-transplant comorbidities, no. (%)	
Hypertension	21 (26%)
Diabetes	8 (10%)
Underlying cardiac disease	21 (26%)
Previous thoracic surgery	8 (10%)
Prednisolone use	32 (40%)
Other immunosuppression	3 (4%)
Intravenous immune globulin use	7 (9%)
Transplant details	
Bilateral (vs. single) lung transplant, no. (%)	69 (86%)
Average ischemic time (mins), mean ± SD	284 ± 105
Surgery duration (mins), median, IQR	236, 103-656
Cardiopulmonary bypass, no. (%)	10 (12%)
ECMO, no. (%)	7 (9%)
Time intubated (hours), median, IQR	22, 15-48
Reintubated, no. (%)	10 (12%)
Initial ICU LOS (days), median, IQR	4, 3-8
ICU readmission, no. (%)	10 (12%)
Hospital LOS (days), median, IQR	19, 16-28
CMV serogroup, no. (%)	
D+/R-	20 (25%)
D+/R+	32 (40%)
D-/R+	19 (24%)
D-/R-	9 (11%)
EBV serogroup, no. (%)	

D+/R-	3 (4%)
D+/R+	74 (93%)
D-/R+	3 (4%)
Immunosuppression, no. (%)	
Basiliximab induction	40 (50%)
Azathioprine (vs mycophenolate)	63 (79%)

IQR, interquartile range. COPD, chronic obstructive pulmonary disease. SD, standard deviation. ECMO, extracorporeal membrane oxygenation. ICU, intensive care unit. LOS, length of stay. CMV, cytomegalovirus. D, donor. R, recipient. EBV, Epstein-Barr virus.

Table 2: Univariable and multivariable linear mixed effects models exploring relationships between immunosuppression and Quantiferon®-Monitor results.

Immunosuppressive medication	Unadjusted		Adjusted*	
	Beta (95% CI)	p-value	Beta (95% CI)	p-value
Tacrolimus level (µg/L)	-10.2 (-15.8, -4.6)	<0.001	-5.9 (-11.3, -0.5)	0.03
Prednisolone dose (mg/kg)	-970 (-1226, -710)	<0.0001	-875 (-1138, -607)	<0.0001
Azathioprine dose (mg/kg)	-20.7 (-62.3, 19.9)	0.27	-28.7 (-79.4, 24.8)	0.26
Mycophenolate mofetil dose (mg/kg)	-1.38 (-3.38, 0.64)	0.18	-1.91 (-4.69, 0.93)	0.18
Basiliximab induction	1.33 (-47.9, 50.5)	0.96	0.5 (-52.1, 53.1)	0.98

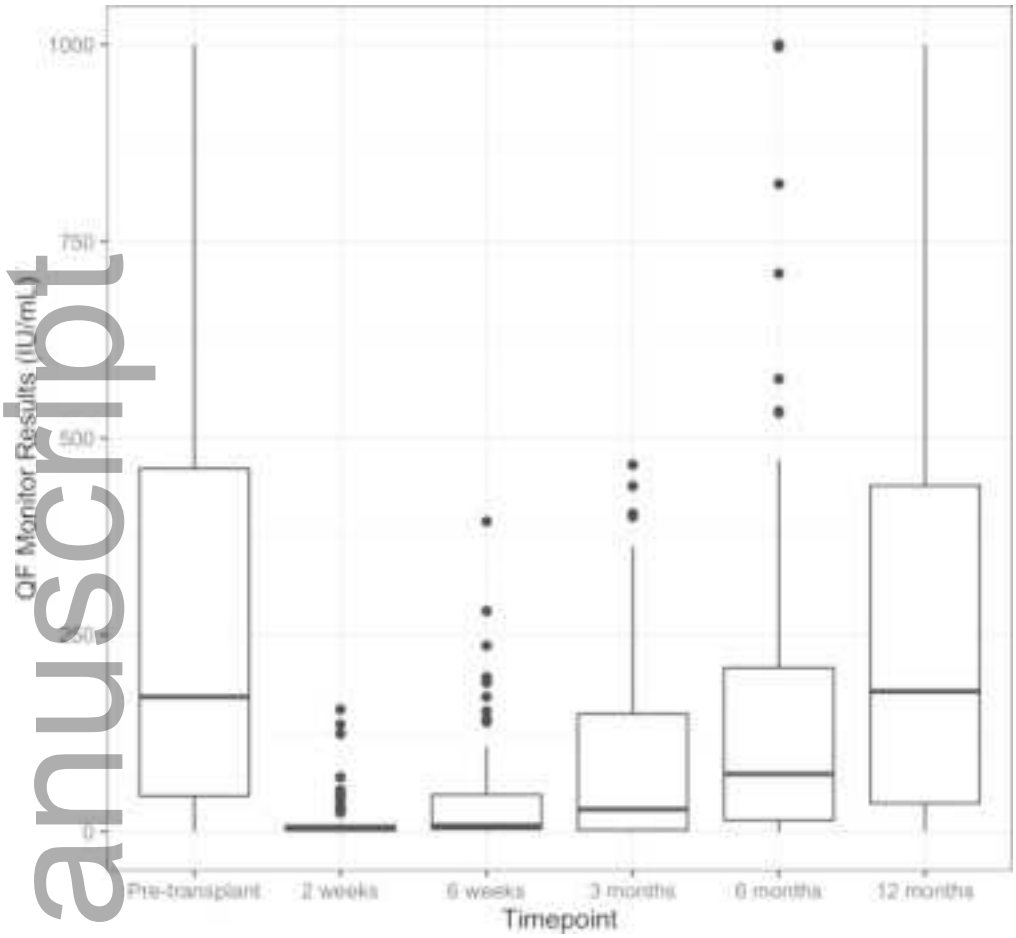
*Adjusted for other immunosuppressive medications.

CI, confidence interval.

Table 3: Hazard ratios for each time period and infection outcome for patients testing below the stated cutoff relative to those testing above.

Period	Outcome	No. patients (%) with outcome	Cutoff (IU/mL)	HR (95% CI)	P-value
2-6 weeks (n=79)	*Serious infection*	16 (20%)	2	1.41 (0.52-3.78)	0.50
	OI	9 (11%)	4	2.28 (0.56-9.33)	0.25
	Serious OI	5 (6%)	2	2.77 (0.46-16.59)	0.26
6 weeks – 3 months (n=78)	*Serious infection*	17 (22%)	6	1.03 (0.39-2.71)	0.96
	OI	4 (5%)	6	0.49 (0.05-4.67)	0.53
	Serious OI	2 (3%)	194	0.06 (0-0.92)	0.04
3-6 months (n=79)	*Serious infection*	24 (30%)	16	2.22 (0.97-5.09)	0.06
	OI	16 (20%)	48	2.53 (0.80-7.95)	0.11
	Serious OI	11 (14%)	10	6.38 (1.37-29.66)	0.02
6-12 months (n=77)	*Serious infection*	30 (39%)	60	2.90 (1.34-6.25)	0.01
	OI	36 (47%)	92	1.68 (0.84-3.37)	0.14
	Serious OI	17 (22%)	60	3.25 (1.11-9.49)	0.03

HR, hazard ratio. OI, opportunistic infection.



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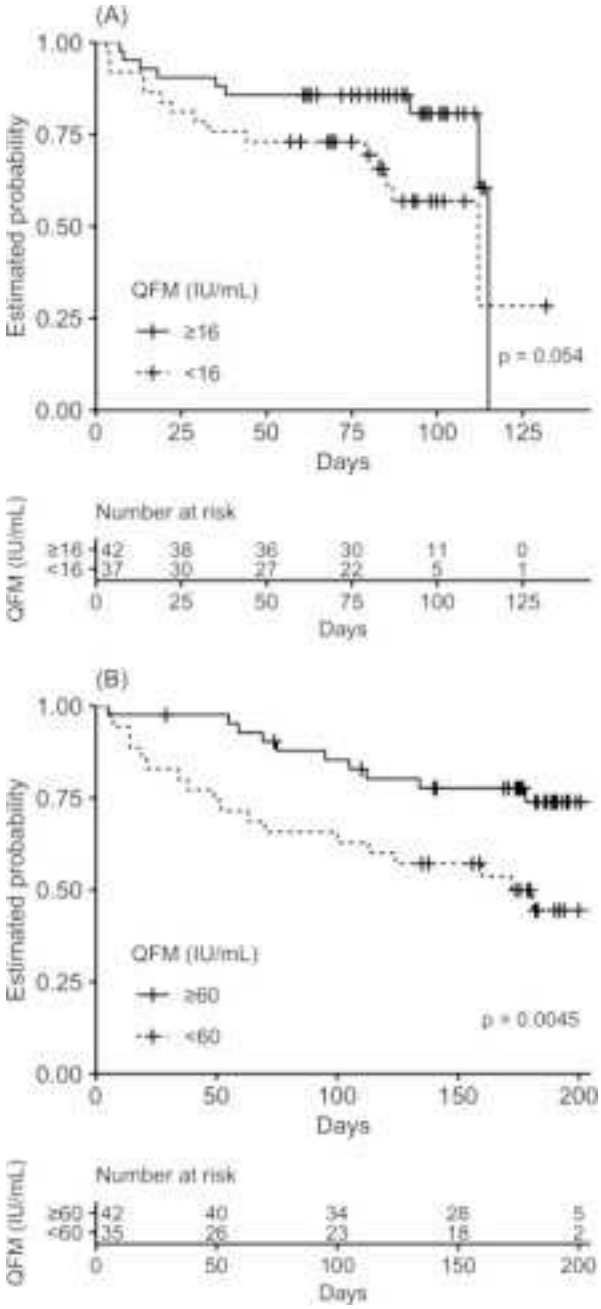

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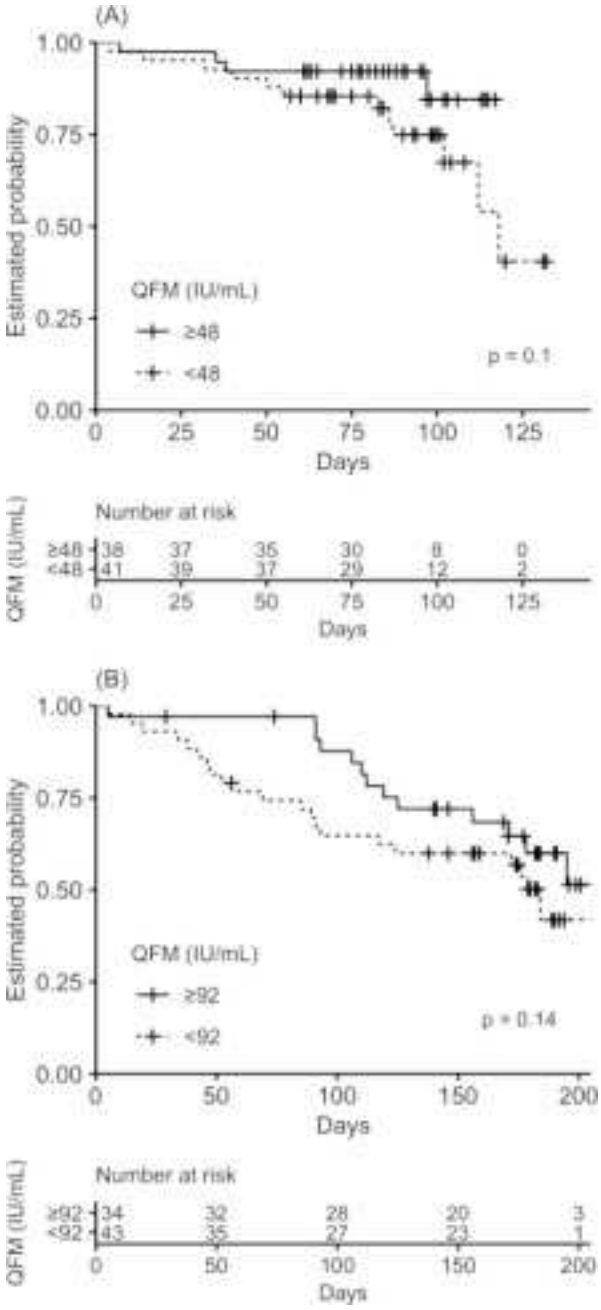

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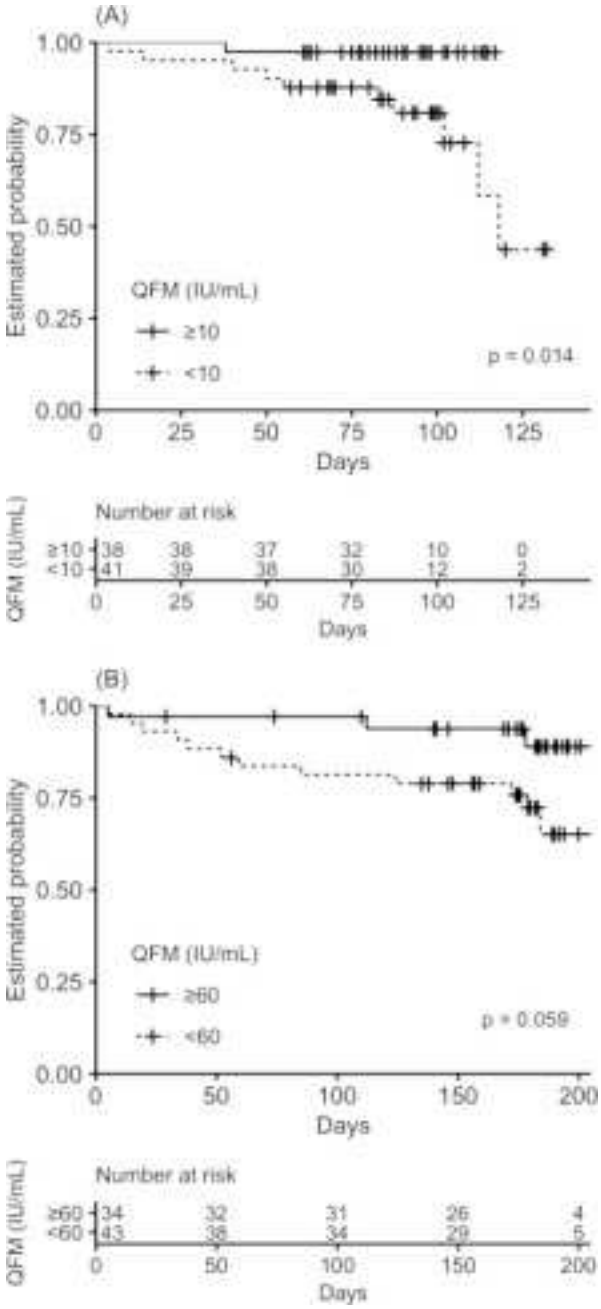

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