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Cytomegalovirus Immunity Assays Predict Viremia but not Replication Within the Lung Allograft

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Background: Cytomegalovirus (CMV) infection causes significant morbidity and mortality in lung transplant recipients. Current guidelines use pretransplant donor and recipient CMV serostatus to predict the risk of subsequent CMV replication and length of antiviral prophylaxis. Immunological monitoring may better inform the risk of CMV infection in patients, thereby allowing for improved tailoring of antiviral prophylaxis. In this study, we compared 2 commercially available assays, the QuantiFERON-CMV (QFN-CMV) and T-Track-CMV (enzyme-linked immunosorbent spot assay), to predict the risk of CMV disease in lung transplant recipients. **Methods:** We performed CMV immunity assays on 32 lung transplant recipients at risk of CMV disease as defined by serostatus (CMV-seropositive recipients, n=26; or CMV-seronegative lung transplant recipient receiving a CMV-seropositive donor organ, n=6). QFN-CMV and T-Track were performed on peripheral blood mononuclear cells, and episodes of CMV replication in both serum and bronchoalveolar lavage were found to be correlated to the CMV immune assays. The predictive ability of the assays was determined using Kaplan–Meier curves. **Results:** There was a degree of concordance between tests, with 44% of recipients positive for both tests and 28% negative for both tests; however, test results were discordant in 28% of cases. A negative result in either the QFN-CMV ($P < 0.01$) or T-Track ($P < 0.05$) assays was obtained in a significantly higher number of recipients who experienced CMV replication in the blood. Using these assays together gave higher predictability of CMV replication, with only 1 recipient experiencing CMV replication in the blood who obtained a positive test result for both assays. Neither assay was able to predict recipients who experienced CMV replication in the lung allograft. **Conclusions:** Our study demonstrates that CMV immunity assays can predict viremia; however, the lack of association with allograft infection suggests that CMV-specific T-cell immunity in the circulation is not associated with the control of CMV replication within the transplanted lung allograft.

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Cytomegalovirus (CMV) is a double-stranded DNA virus of the beta subfamily of herpesviruses. It has a high seroprevalence in the human population with 50% to 90% of people seroconverting by adulthood.[1] During primary CMV infection, a strong adaptive immune response is stimulated, including a well-characterized CD8 T-cell response toward CMV epitopes derived from pp65 and IE-1.[2] CMV-specific memory T cells often constitute >10% of both CD8 and CD4 memory compartments in the peripheral blood of CMV-seropositive individuals.[2]

In transplant recipients, immunosuppressive therapy diminishes the cellular immune response, often resulting in active CMV replication, which can result in invasive diseases such as pneumonitis or colitis.[3] CMV replication after transplantation has been associated with the development of chronic rejection, including chronic lung allograft dysfunction after lung transplantation (LTx).[3,4] Notably, the detection of actively replicating CMV is associated with a reduced frequency of CMV-specific $CD8^+$ or $CD4^+$ T cells.[5] The consensus guidelines for CMV management in LTx recipients includes antiviral prophylaxis for "at-risk" patients (donor or recipient CMV seropositive), most commonly with (val)ganciclovir, although the duration varies among centers from 6 to 12 mo.[6] The detection of active CMV replication is monitored at routine surveillance by quantitative polymerase chain reaction (qPCR), normally in both the blood and bronchoalveolar

lavage (BAL).[7] Although these strategies have reduced the incidence of CMV infection post–LTx, widespread use of antivirals is associated with side effects including neutropenia, bone marrow toxicity, antiviral resistance, and late-onset CMV disease.[8-10] Therefore, there is an increasing interest to use CMV immune monitoring tests to guide and provide an individualized approach to antiviral prophylaxis.

The QuantiFERON-CMV assay (QFN-CMV) measures the cell-mediated immune response to CMV, specifically interferon-gamma (IFN-γ) release from CMV-specific $CD8^+$ T cells after ex vivo stimulation with a panel of T-cell CMV epitopes.[11] A study of solid organ transplant recipients showed that the QFN-CMV assay is a useful predictor of the development of late-onset CMV disease.[12] Moreover, studies from our own group have shown the utility of QFN-CMV assay for predicting CMV replication in the blood after LTx.[13] T-Track-CMV (Lophius Biosciences GmbH, Regensburg, Germany) also measures IFN-γ release; however, it uses an enzyme-linked immunosorbent spot (ELISpot) platform using CMV antigens IE-1 and pp65.[3] A single-center study of LTx recipients has shown that ELISpot nonresponders have earlier onset and longer duration of CMV infection,[14] and in a cohort study of kidney transplant patients, ELISpot has been shown to be better than the QFN-CMV at stratifying the risk of CMV disease.[15] This may be in part because of the ability of ELISpot to monitor both CD4 and CD8 responses to CMV.

This study aims to compare the efficacy of QFN-CMV and T-Track-CMV in predicting CMV infection in the blood and allograft in the same cohort of LTx recipients. Moreover, we aimed to assess whether using both tests in combination demonstrated enhanced power to predict CMV replication over using 1 test alone.

MATERIALS AND METHODS

Ethics

All patients gave written informed consent. The study was approved by The University of Melbourne Human Research Ethics Committee (Project 1953932) and The Alfred Hospital Ethics Committee (Project 401/13).

Study Population

The study population included 32 adult patients who received a bilateral LTx at The Alfred Hospital from 2014–2015. The participants also took part in a randomized study comparing standard 5-mo antiviral prophylaxis with QFN-CMV–directed antiviral prophylaxis and consented to samples being used for additional research.[13] Immunosuppression consisted of tacrolimus, azathioprine, and prednisolone. All patients received at least 7 d of intravenous ganciclovir (5 mg/kg BID) and then oral valganciclovir (450 mg BID, adjusted according to renal function) up to 11 mo posttransplant. Patients at high risk of CMV disease (donor CMV seropositive and recipient CMV seronegative [D^+/R^-]) also received CMV hyperimmune gammaglobulin (1.5 million units) on days 1, 2, 3, 7, 14, 21, and 28. The final selection of the cohort of patients for this study is shown in Figure 1.

Timing of Clinical Sample Collection Used in the Assays

QFN-CMV Analysis was performed on samples collected between 126 and 196 d post-LTx (mean 157; Table 1). The collection of the samples used in the T-Track-CMV (ELISpot) assay was performed between 91 and 245 d post-LTx (with a mean at 184; Table 1).

Detection of CMV Infection

Before LTx, CMV serology was determined for each donor (D) and recipient (R) by CMV IgG ELISA. Recipients were grouped into moderate risk (recipient CMV seropositive; R^+) or high risk (D^+/R^-) of CMV disease. Serum and BAL samples were tested for actively replicating virus by qPCR using the Cobas Ampliprep/Cobas Taqman assay (Roche), which has a lower limit of detection of 137 international units (IU)/mL. After cessation of antiviral prophylaxis, patient serum samples were tested monthly for 6 mo, then additionally at 15 and 18 mo posttransplant. BAL samples were analyzed for CMV replication at 6, 9, 12, and 18 posttransplant. Additional blood and BAL samples were collected at any other time points for CMV qPCR if the patient developed clinical features of CMV infection.

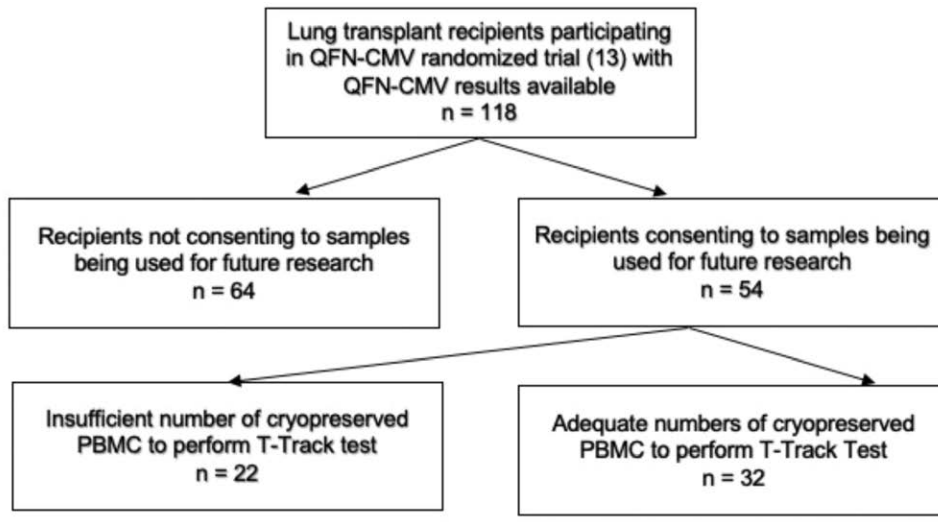

FIGURE 1. Selection of patient cohort. PBMC, peripheral blood mononuclear cell; QFN-CMV, QuantiFERON-cytomegalovirus.

TABLE 1.
Study cohort demographics

Characteristic	All patients (N = 32)
Age, y, mean (range)	57.4 (23–70)
Sex, n/n, male/female	18/14
Primary diagnosis	
Chronic obstructive pulmonary disease	14
Idiopathic pulmonary fibrosis	4
Interstitial lung disease	2
Usual interstitial pneumonia	4
Cystic fibrosis	2
Bronchiectasis	3
Other	3
CMV donor (D)/recipient (R) serostatus (%)	
R^+	26 (81)
D^+/R^-	6 (19)
Antiviral therapy arm	
Standard care	14 (44)
QFN-CMV–directed care	18 (56)
QFN-CMV assay, days posttransplant, mean (range)	157 (126–196)
Antivirals ceased, days posttransplant, median (range)	168 (147–343)
Antivirals ceased at 5 mo posttransplant	22 (69)
T-Track-CMV (ELISpot), days posttransplant, mean (range)	184 (91–245)
CMV infection outcomes	
Any CMV in blood	22 (69)
CMV blood >600 IU/mL	8 (25)
Any CMV in BAL	20 (63)
CMV in BAL >600 IU/mL	14 (44)

BAL, bronchoalveolar lavage; CMV, cytomegalovirus; QFN-CMV, QuantiFERON-CMV.

QFN-CMV Assay

The QFN-CMV assay was performed as recommended by the manufacturer (QIAGEN, Hilden, Germany). The results of the QFN-CMV assay were performed as published in our previous study.[13] Briefly, whole blood from LTx recipients was collected in 3 separate tubes: a CMV-antigen tube, a mitogen control tube, and a negative control tube. The CMV-antigen tube contains a pool of peptides that will elicit an IFN-γ response from CMV-specific CD8 T cells if they are present and are functional. IFN-γ production is measured by ELISA, and the test is considered positive if the IFN-γ response to the CMV antigens is >0.2 IU/mL above the negative control. Results are considered indeterminant when the IFN-γ response to the mitogen control is <0.5 IU/mL. In this study, indeterminant results were grouped with negative assay results because a lack of response to the mitogen control was demonstrated to occur early in the posttransplant period due to strong immunosuppression.[16]

T-Track-CMV (ELISpot) Assay

The T-Track-CMV ELISpot assay (Lophius Biosciences GmbH, Regensburg, Germany) uses whole IE-1 and pp65 antigens to deduct antigen-specific CD4 and CD8 T-cell responses. Important for this study, the T-Track-CMV assay had previously been determined as having identical results when using fresh and frozen peripheral blood mononuclear cells (PBMCs; Lophius Biosciences, personal communication, 21 August 2018). Cryopreserved PBMCs were thawed and cultured overnight at 37 °C/5% CO_2 in media containing Roswell Park Memorial Institute (RPMI) media supplemented with 10% human AB serum and 50 IU/mL recombinant human interelukin-2. PBMCs were added to the 96-well ELISpot plate provided in the T-Track-CMV kit at a dilution of 200 000 cells/100 µL in duplicates. Media-only control, a phytohemagglutinin A–positive control, and an operator control containing IFN-γ bound to capture antibodies were used as technical controls. The plate was incubated for 17 to 21 h at 37 °C/5% CO_2, washed, and incubated with alkaline phosphatase monoclonal antibody followed by staining substrate for 6 to 7 min. The spots were counted using the AID ELISpot reader (Autoimmun Diagnostika GmbH, Strassberg, Germany), and the results were interpreted by the provided T-Track-CMV calculator. The T-Track was considered positive if the pp65 and/or the IE-1 results were interpreted by the software as yielding a positive result.

Statistical Analysis

Descriptive statistics were used such as median, mean, and SD depending on the data distribution. Proportions or counts were used to describe categorical variables. The Student *t* test was used for group comparisons. Kaplan–Meier survival curve analysis was used to show CMV infection incidence during the follow-up period, and the log-rank test was used to determine whether the difference between curves was significant. Statistical analysis was performed using GraphPad Prism version 8 software.

RESULTS

Patient Characteristics

Thirty-two adult LTx recipients transplanted between 2014 and 2015 at The Alfred Hospital, Melbourne, were included in the final cohort for this study. The demographics of the cohort are shown in **Table 1**. The final cohort where both QFN-CMV and ELISpot results were available included 6 CMV D^+/R^- recipients (19%) and 26 CMV R^+ recipients (81%). Of the 32 patients, 22 (69%) received standard duration prophylaxis (5 mo) and 10 (31%) received extended duration prophylaxis (11 mo; **Table 1**). Of the 10 recipients on extended prophylaxis, 2 were CMV D^+/R^- (33% of D^+/R^-) and 8 R^+ (31% of R^+).

Cytomegalovirus Outcomes Posttransplant

In keeping with our previous study, we defined higher CMV replication as >600 IU/mL.[13] This cutoff is based on our clinical protocol in which CMV <600 IU/mL is not treated and patients with CMV loads of >600 IU/mL are given oral valganciclovir or intravenous ganciclovir (if associated clinical symptoms) to control replication. No patients developed any detectable CMV replication in the serum or BAL while on antiviral prophylaxis. In the 18-mo follow-up, 22 of 32 patients (69%) developed detectable CMV replication in the blood, and 8 of 32 patients (25%) developed CMV loads of >600 IU/mL (Table 1). Twenty of 32 patients (63%) developed CMV replication in the lung allograft with 14 of 32 patients (44%) developing higher levels of CMV replication in the BAL (>600 copies/mL; Table 1).

Concordance of Test Results Between QFN-CMV and T-Track Assays

Chi-square tests were performed to evaluate whether there was an association between test results. There was a significant association between QFN-CMV and T-Track when

considering T-Track results overall rather than pp65/IE-1 results separately ($\chi^2 = 5.8$, $P = 0.02$; Table 2). Surprisingly, there was no association between the pp65 and IE-1 T-Track results (Table 3) nor between the pp65 and IE-1 QFN-CMV results when considering pp65 (Table 4) or IE-1 (Table 5) independently.

QFN-CMV Analysis

For comparison purposes, we used a single QFN-CMV assay result for each recipient, which was performed at 5 mo posttransplant,[13] obtained at a mean of 157 d (Table 1). At this time point, all of the D^+/R^- recipients in this study were QFN-CMV negative. Overall, the value of the QFN-CMV level was not significantly different between D^+/R^- and R^+ patients (Figure 2A). When considering only recipients who had a shorter period of antiviral prophylaxis (5 mo), there was no difference between the incidence of any level of CMV replication (>0 IU/mL) in the blood between QFN-CMV–negative or –positive individuals (Figure 2B). However, in the same group of recipients on shorter prophylaxis, there were a significantly higher number of QFN-CMV–negative individuals experiencing >600 IU/mL replication in the blood (Figure 2C). However, this effect was no longer significant when including all recipients (ie, those on both 5 and 11 mo of prophylaxis) either with any level of CMV replication (Figure 2D) or >600 IU/mL (Figure 2E). Strikingly, the QFN-CMV test did not adequately stratify recipients who will experience CMV replication in the lung allograft, either on shorter periods of prophylaxis (Figure 2F and G) or in the cohort as a whole (Figure 2H and I). In summary, QFN-CMV test results were only able to predict high-level CMV replication in the blood of recipients on a shorter period of antiviral prophylaxis but not in the lung allograft.

TABLE 2.

Correlation between QFN-CMV and T-Track

	QFN-CMV positive, n (%)	QFN-CMV negative, n (%)
T-Track positive, n (%)	14 (44)	5 (16)
T-Track negative, n (%)	4 (12)	9 (28)

$\chi^2 = 5.8$, $P = 0.02$.
QFN-CMV, QuantiFERON-cytomegalovirus.

TABLE 3.

Correlation between T-Track pp65 and IE-1

	pp65 positive, n (%)	pp65 negative, n (%)
IE-1 positive, n (%)	6 (19)	3 (9)
IE-1 negative, n (%)	10 (31)	13 (41)

$\chi^2 = 1.4$, $P = 0.24$.
QFN-CMV, QuantiFERON-cytomegalovirus.

TABLE 4.

Correlation between pp65 T-Track and QFN-CMV

	QFN-CMV positive, n (%)	QFN-CMV negative, n (%)
pp65 positive, n (%)	11 (34)	5 (16)
pp65 negative, n (%)	7 (22)	9 (28)

$\chi^2 = 2.0$, $P = 0.15$.
QFN-CMV, QuantiFERON-cytomegalovirus.

TABLE 5.

Correlation between IE-1 T-Track and QFN-CMV

	QFN-CMV positive, n (%)	QFN-CMV negative, n (%)
IE-1 positive, n (%)	7 (22)	2 (6)
IE-1 negative, n (%)	11 (34)	12 (38)

$\chi^2 = 2.4$; $P = 0.12$.
QFN-CMV, QuantiFERON-cytomegalovirus.

T-Track-CMV (ELISpot) Analysis

The ELISpot assay was performed on thawed, cryopreserved PBMCs collected at a time point as close to the QFN-CMV as possible, with a mean of 184 d posttransplant (Table 1). All samples that were analyzed for T-Track were taken before any episode of CMV replication. Similar to QFN-CMV test, the mean number of spot-forming units was not statistically different between D^+/R^- patients and R+ patients (Figure 3A). Although the ELISpot assay failed to predict patients on shorter prophylaxis who experienced >0 IU/mL CMV replication in the blood (Figure 3B), similar to the QFN-CMV assay, the T-Track assay predicted higher-level (>600 IU/mL) CMV replication in the blood of the same cohort such that there were significantly fewer recipients developing higher levels of replication in T-Track–positive patients (Figure 3C). Moreover, the same effect was seen in the whole patient cohort without stratifying by shorter prophylaxis (Figure 3D and E). However, similar to the QFN-CMV test, the ELISpot assay was also unable to predict CMV replication in the lung allograft. There was no significant difference in the T-Track assay results between patients with CMV replication in the allograft when stratifying by any detectable replication (Figure 3F and H) or >600 IU/mL (Figure 3G and I), even when stratifying by shorter periods of prophylaxis (Figure 3F and G). In summary, the T-Track ELISpot test was able to predict high-level CMV replication in the blood but had no ability to predict CMV replication in the lung.

Combining QFN-CMV and ELISpot Tests to Predict CMV Replication

Next, we assessed whether combined results from both assays were superior at predicting freedom from CMV replication. Overall, 9 of 32 recipients tested negative for both QFN-CMV and ELISpot tests and 14 of 32 tested positive for both tests (Table 2). In contrast, 9 of 32 recipients tested positive for either QFN-CMV or ELISpot test (Table 2). When considering CMV replication of >0 IU/mL in the blood, there was a relatively even distribution in the number of recipients with zero, 1, or 2 positive tests (Figure 4A). Moreover, many recipients without higher-level (>600 IU/mL) CMV replication were both QFN-CMV and ELISpot positive (13/14 recipients), whereas only 1 recipient with 2 positive tests had high-level CMV replication in the blood (Figure 4B). Conversely, 11 of 14 recipients who were both QFN-CMV and ELISpot positive had CMV replication of >0 IU/mL (Figure 4A), and additionally, the majority of these (8/14 recipients) had higher-level CMV replication in the BAL (Figure 4B). Therefore, combining QFN-CMV with ELISpot appears to have a superior ability to predict high-level CMV replication in the blood but still has limited clinical utility for predicting CMV replication in the allograft.

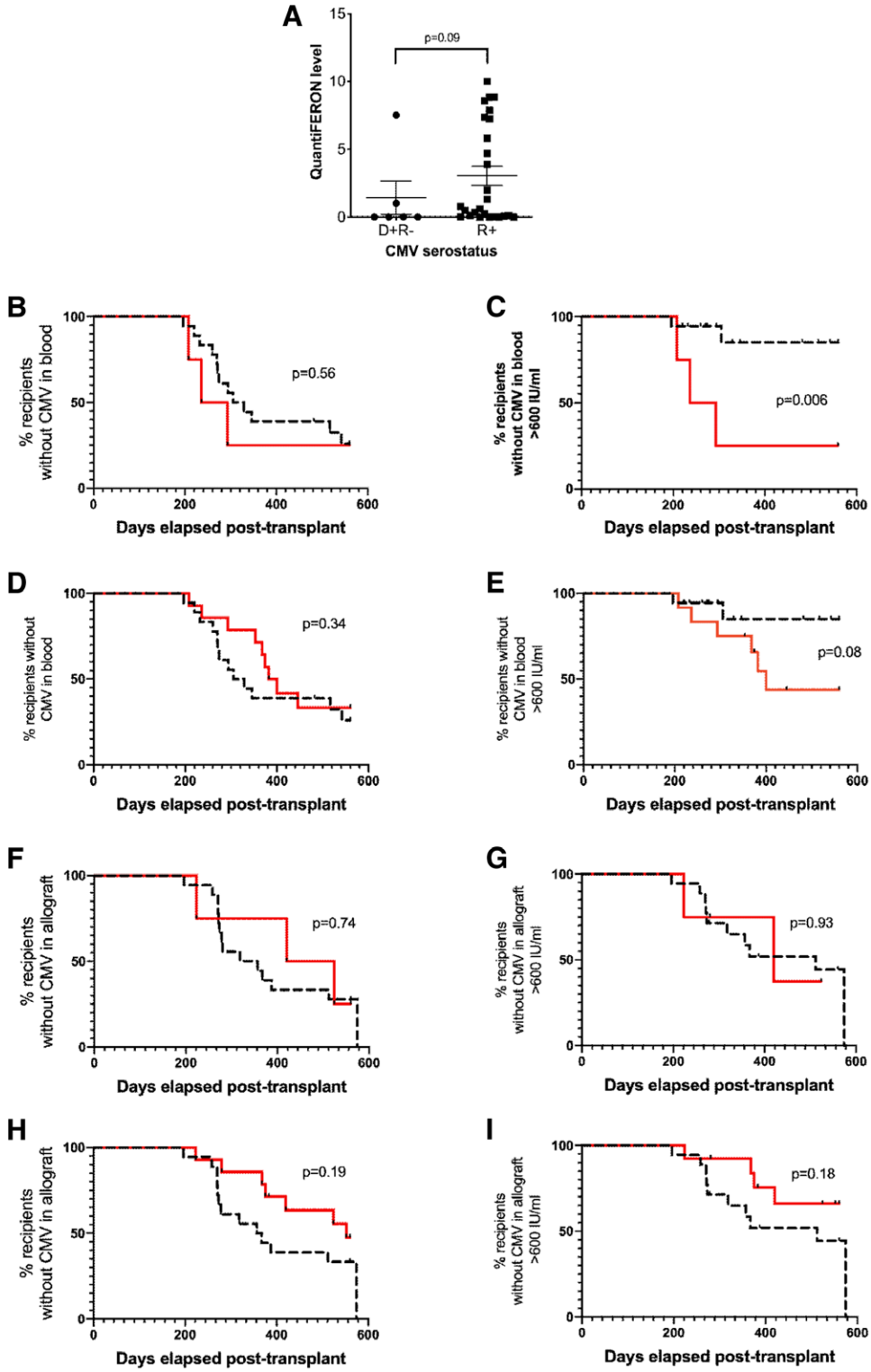

FIGURE 2. QFN-CMV predicts protective immunity against high-level CMV infection in the blood but not in the lung allograft. QFN-CMV results were obtained at 5 mo posttransplant, and recipients with an indeterminate result were considered QFN-CMV negative. Recipients were followed up for 18 mo posttransplant. A, Measured QFN-CMV levels separated by D^+/R^- or R^+ serostatus. Kaplan–Meier survival curves of CMV replication with (B) threshold of >0 copies/mL in the blood of recipients who ceased prophylaxis at 5 mo post-LTx, (C) threshold of >600 IU/mL in the blood of recipients who ceased prophylaxis at 5 mo post-LTx, (D) threshold of >0 copies/mL in the allograft of recipients who ceased prophylaxis at 5 mo post-LTx, (E) threshold of >600 IU/mL in the allograft of recipients who ceased prophylaxis at 5 mo post-LTx, (F) threshold of >0 copies/mL in the blood of all recipients post-LTx, (G) threshold of >600 IU/mL in the blood of all recipients post-LTx, (H) threshold of >0 copies/mL in the allograft of all recipients post-LTx, and (I) threshold of >600 IU/mL in the allograft of all recipients post-LTx. Recipients who were QFN-negative are shown in red lines, and QFN-CMV–positive recipients are shown in black lines. To calculate the significance of different QFN-CMV levels between R^+ and D^+/R^- individuals, the unpaired *t* test was used. For Kaplan–Meier survival curves, the log-rank test was used to assess the difference between QFN-CMV–negative and –positive recipients. Data for this figure has been acquired from a previously published study.[13] LTx, lung transplantation; QFN-CMV, QuantiFERON-cytomegalovirus.

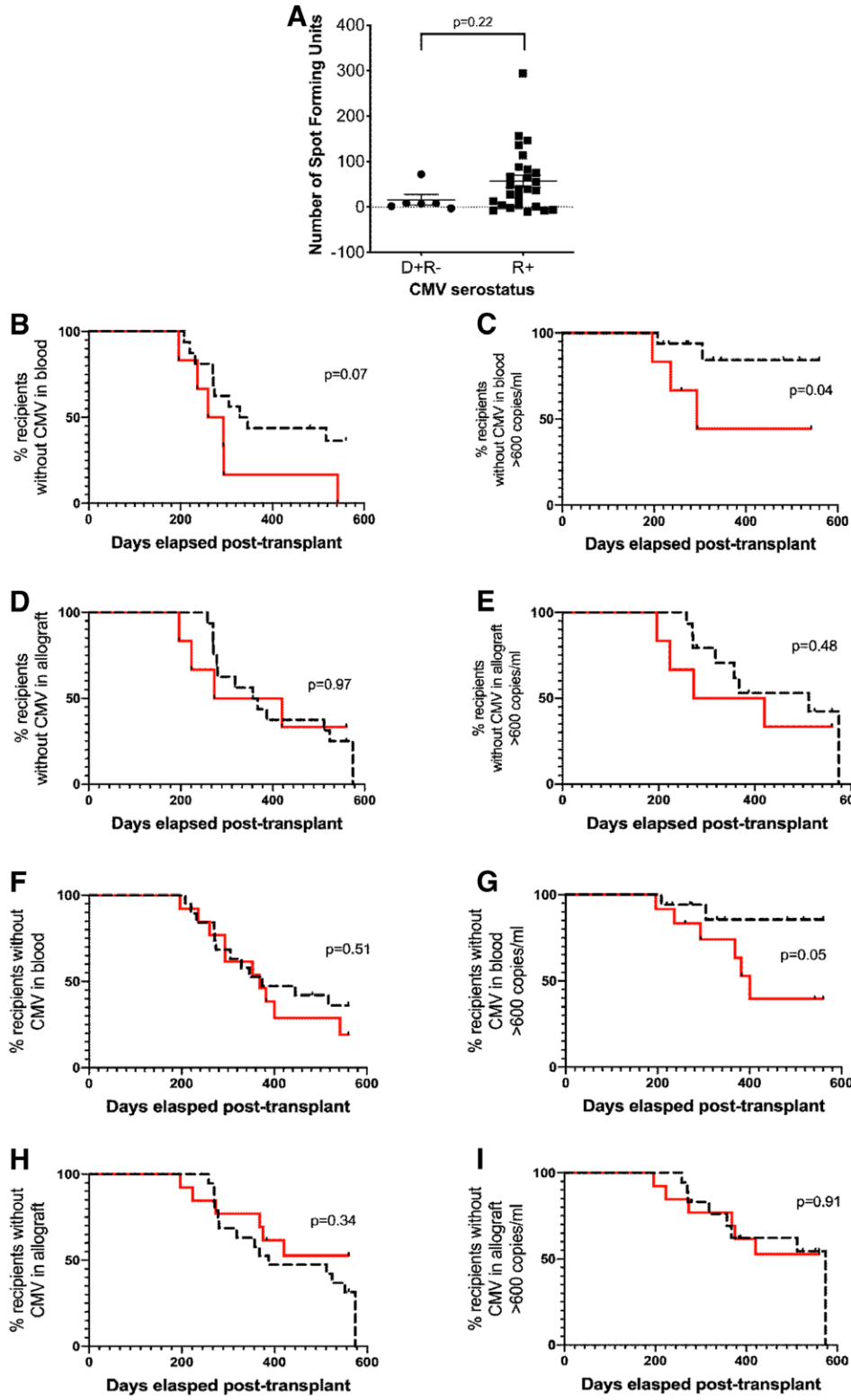

FIGURE 3. T-Track-CMV (ELISpot) predicts immunity against high-level CMV in the blood. Frozen PBMCs collected posttransplant were thawed and cultured and then tested using the ELISpot assay provided in the T-Track-CMV kit. A, SFUs per 200 000 cells stratified by serostatus. Kaplan–Meier survival curves of incidence of CMV infection with (B) threshold of >0 copies/mL in the blood of recipients who ceased prophylaxis at 5 mo post-LTx, (C) threshold of >600 IU/mL in the blood of recipients who ceased prophylaxis at 5 mo post-LTx, (D) threshold of >0 copies/mL in the allograft of recipients who ceased prophylaxis at 5 mo post-LTx, (E) threshold of >600 IU/mL in the allograft of recipients who ceased prophylaxis at 5 mo post-LTx, (F) threshold of >0 copies/mL in the blood of all recipients post-LTx, (G) threshold of >600 IU/mL in the blood of all recipients post-LTx, (H) threshold of >0 copies/mL in the allograft of all recipients post-LTx, and (I) threshold of >600 IU/mL in the allograft of all recipients post-LTx. ELISpot-negative recipients are shown in red lines and ELISpot-positive recipients are shown in black lines. To calculate the difference in SFU between R^+ and D^+/R^- individuals, the unpaired *t* test was used. For Kaplan–Meier survival curves, the log-rank test was used to assess the difference between ELISpot-negative and -positive recipients. CMV, cytomegalovirus; ELISpot, enzyme-linked immunosorbent spot; LTx, lung transplantation; PBMC, peripheral blood mononuclear cell; QFN-CMV, QuantiFERON-CMV; SFU, spot-forming unit.

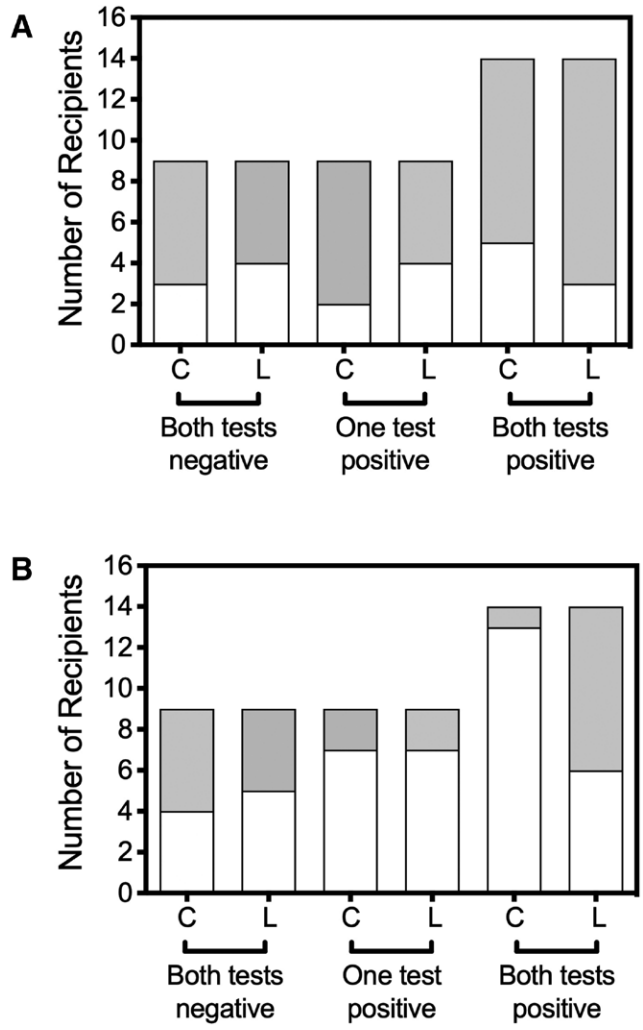

FIGURE 4. Correspondence between a positive QFN-CMV test and ELISpot tests (T-Track) and the presence of >0 IU/mL CMV replication (A) or >600 IU/mL CMV replication (B) in the blood (circulation, C) or BAL (lung, L). No CMV replication is shown as white bars and CMV replication as gray bars. BAL, bronchoalveolar lavage; CMV, cytomegalovirus; ELISpot, enzyme-linked immunosorbent spot; QFN-CMV, QuantiFERON-CMV.

DISCUSSION

In this preliminary study, we compared the commercial CMV immune monitoring modalities, QFN-CMV and T-Track-CMV (ELISpot) for their utility in predicting CMV replication in the first 18 mo after LTx. The QFN-CMV test specifically focuses on assessing the $CD8^+$ T-cell response to viral epitopes derived from pp65, pp50, gB, and IE-1.[17] A negative QFN-CMV test indicating poor anti-CMV CD8 T-cell response has previously been linked to a higher incidence of CMV replication in heart[18] and kidney[19] transplant recipients. Moreover, our previous study (that included some of the patients in the current study) found that the incidence of CMV replication in the blood was reduced in recipients with a positive QFN-CMV assay, proposing an association between the presence of functional antiviral CD8 T cells and protective immunity against CMV replication.[13] However, measuring $CD8^+$ T-cell function in the blood does not provide adequate information as to the level of protective immunity in the lung allograft. Previous studies have shown that when monitoring T-cell immunity over time, fluctuating levels of immunity and a drop in IFN-γ response are observed in some patients who develop viremia and infection in the allograft.[20,21] However, our results, combined with our extended cohort,[13] indicate that the QFN-CMV assay is not predictive of CMV replication in the LTx setting compared with the recipients of other solid organ transplant recipients. It is possible that in some LTx recipients, $CD8^+$ cell-mediated immunity is unable to control CMV infection in the lung because of either higher immunosuppression or higher viral load in the allograft.[22,23] Furthermore, the vast majority of LTx recipients are HLA mismatched. As recipient $CD8^+$ T cells are selected on their ability to recognize self-HLA,[24] it is possible that recipient-derived CD8 T cells are unable to control CMV replication in the HLA-mismatched allograft, where the CMV antigens are presented on donor-derived HLA molecules.

Our results suggest that the ELISpot assay can predict freedom from CMV replication in the blood. Moreover, although the QFN-CMV assay was only able to predict freedom from CMV replication in patients on shorter periods of prophylaxis, the ELISpot assay also predicted freedom from CMV replication when including patients on extended prophylaxis. This is consistent with a previous study of LTx patients in which ELISpot nonresponders were found to have a significantly higher incidence of CMV viremia.[25] However, in contrast (but similar to the QFN-CMV test), the ELISpot assay was a poor predictor of CMV replication in the allograft. It

has previously been shown that CMV replication in the allograft was not associated with ELISpot results.[26,27] Indeed, we showed that the highest number of recipients without CMV replication in the allograft were both QFN-CMV and ELISpot negative. Overall, these results suggest that CMV immunity in circulation does not indicate protection against CMV replication in the lung. It is possible that lymphocytes isolated from BAL samples and used for the QFN-CMV or ELISpot assay may more accurately provide a measure of CMV immunity in the lung allograft and, given the devastating effect of CMV, such studies should be initiated with high priority.

Our current study had several limitations. We used frozen PBMCs instead of PBMCs from blood drawn within 24 h of analysis. The extended prophylaxis received by some patients who were QFN-CMV negative also reduced the CMV infection incidence in that group, which in turn complicated our analysis. Additionally, as our samples were tested at a fixed time point, we were unable to analyze how the recipients' response to the assays changed over time, which would have provided a more dynamic analysis of changes in their CMV immunity. Furthermore, immunosuppression has a significant effect on immune responses and the likelihood of CMV infection. Although our patients typically received similar protocol-guided doses of immunosuppressive medications in the first year posttransplant, with tacrolimus blood levels being followed closely, this may be a contributing factor in this study. Also, we acknowledge that despite our efforts to standardize BAL testing, there can be variability in the BAL sampling that may complicate the result interpretation.

CONCLUSION

In summary, both QFN-CMV and T-Track-CMV (ELISpot) assays were able to predict the development of CMV replication in circulation, and using both tests together may further improve predictive ability. However, there was limited ability for either test to predict CMV replication in the lung allograft. The clinical application of these assays should be further explored using samples of lymphocytes from the lung allograft. A larger, prospective study that assesses multiple time points to examine CMV immunity is needed to fully understand the utility of these tests in predicting local allograft CMV reactivation.

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