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Early viral-specific T-cell testing predicts late Cytomegalovirus reactivation following Liver Transplantation

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Running title: Predicting late CMV after liver transplant

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Abbreviations:

CMV – Cytomegalovirus

OLT – liver transplantation

QFN-CMV – QuantiFERON CMV

M6 – Month 6

PCR – Polymerase chain reaction

D – Donor serum CMV IgG

R – Recipient serum CMV IgG

ABSTRACT

Introduction

Although antiviral prophylaxis is effective in preventing early cytomegalovirus (CMV) reactivation following liver transplantation (OLT), it predisposes patients to late CMV after prophylaxis has ceased. QuantiFERON–CMV (QFN-CMV, Qiagen, The Netherlands) measures an individual's viral-specific immune response.

Methods

Fifty-nine OLT recipients were prospectively monitored post-OLT in an observational cohort study. QFN-CMV was performed at regular time-points. An absolute QFN-CMV <0.1IU/mL was considered non-reactive.

Results

50/59 (84.7%) had a reactive QFN-CMV by M6. 38/59 (64.4%) had antiviral prophylaxis or treatment before M6, with 31/38 (81.6%) developing a reactive QFN-CMV by 6 months. Over 90% already had a reactive result as early as 3 months post-transplant. 3 patients (5.08%) developed late CMV between 6-12 months (median 251 days) - all had a non-reactive M6 QFN-CMV. 2/3 experienced CMV disease. Non-reactive M6 QFN-CMV was significantly associated with late CMV (OR=54.4, PPV=0.33, NPV=1.00, p=0.003).

Conclusion

Although only 5% of recipients developed late CMV, 2/3 suffered CMV disease. M6 QFN-CMV has an excellent NPV for late CMV, suggesting patients who exhibit a robust ex-vivo immune response at M6 can safely cease CMV monitoring. Furthermore, >90% already express viral-specific immunity as early as 3 months. Conceivably, antiviral prophylaxis could be discontinued early in these patients.

Introduction

Most adults (50-85%) have been infected with Cytomegalovirus (CMV) (1), with a benign primary infection followed by a prolonged dormant phase (2-4). Immunosuppressive agents weaken immune responses and increase susceptibility to CMV reactivation after liver transplantation (OLT) (5). Although most CMV reactivation occurs within the first few months after OLT (early CMV), late CMV is noted to occur after 6 months when most units withdraw CMV prophylaxis or treatment.

Unlike early CMV where risk mitigation protocols exist, late CMV has no such strategies. QuantiFERON–CMV (QFN-CMV, Qiagen, The Netherlands) measures an individual's ex-vivo T-cell response towards CMV. We investigate the potential for QFN-CMV measurements to predict late CMV following OLT.

Methods:

QFN-CMV was evaluated regularly as part of a blinded prospective observational cohort study until 6 months post-OLT (6). In brief, pre-OLT donor (D) and recipient (R) serology were used to stratify patient risk. Prophylaxis was offered to D+/R- high-risk patients or after the use of pulsed steroids for treatment of acute cellular rejection. A pre-emptive strategy was offered for low risk patients (D+/R+, D-/R+) where antiviral treatment (valganciclovir) was commenced only if they developed CMV DNAemia. CMV viral loads were performed weekly or on each outpatient review. Patients with neither donor or recipient serology for CMV (D-/R-) were considered very low risk for CMV and evaluated if symptomatic. These patients were excluded from analysis in this study. CMV DNAemia was defined as a polymerase chain reaction (PCR) viral load greater than 1000 copies/mL on a PCR Taqman (Thermo Fisher Scientific, Massachusetts, USA) assay (7). As per unit protocol, this was considered CMV infection and treatment with valganciclovir at 900mg twice daily (renally adjusted) was commenced. Treatment or prophylaxis was continued until 6 months post-OLT.

QFN-CMV was performed at multiple time-points at participants' clinical reviews as per manufacturer's instructions. The primary cut-off for a non-reactive result (suggestive of insufficient immunity and therefore risk of future CMV) was an absolute value of the QFN-

CMV tube of <0.1 IU/mL, irrespective of the results of the QFN-MIT (positive control) or QFN-nil (negative control) tubes (6).

In this current study, we followed patients (n=59) who had prior CMV exposure (pre-transplant D+ or R+), 6 month (M6) QFN-CMV data, and follow-up until 12 months (Figure 1). Patients were diagnosed and treated for late CMV if they developed DNAemia after 6 months post-transplant after being CMV DNAemia negative at 6 months post-transplant. Fisher's exact test was used to compare groups, while the Mann-Whitney test was employed to compare non-parametric variables. The study was approved by the Austin Ethics Committee, and informed consent was available for all patients prior to study entry.

Results:

Of 75 recruited transplant recipients, 59 were included in this study (Figure 1). There was no significant difference in patient demographics (Table 1). The median age at time of transplantation was 53 years, and 66.1% were male. Immunosuppression at 6-12 months post-transplant constituted a combination of prednisolone, azathioprine or MMF and either cyclosporin on tacrolimus. Nearly half the cohort (n=25, 42.4%) had been weaned off prednisolone, while the remainder had a median dose of 5mg. The majority of recipients (n=50, 84.7%) had a reactive QFN-CMV by M6.

A minority of patients (n=3, 5.08%) developed late CMV (median 251 days post-OLT). Pre-transplant serology was significantly associated with the risk of late CMV (Table 1, p=0.03). Two of the patients were high risk pre-transplant (D+/R-), while one patient had commenced CMV therapy on day 38 post-transplant under a pre-emptive protocol (D-/R+) for detectable CMV DNAemia. After antiviral therapy was ceased at 6 months in these three patients, two developed late CMV disease with very high viral loads (79,970 and 276,380 copies/mL) while one commenced therapy for late CMV DNAemia and a viral load of 1100 copies/mL. All 3 recipients had a non-reactive M6 QFN-CMV, indicating they were at high risk of late CMV following cessation of therapy (Figure 1). A M6 non-reactive QFN-CMV was significantly associated with risk of late CMV with an OR of 54.4 (Figure 2, sensitivity 1.00 (95%CI: 0.29-1.00), specificity 0.89 (95%CI: 0.78-0.96), PPV = 0.33 (95%CI: 0.07-0.70), NPV = 1.00 (95%CI:0.93-1.00) p=0.003). Neither white cell count, aetiology of underlying liver disease or immunosuppression was associated with the risk of late CMV (Table 1, columns 2 and 3). Two

of the three patients also had a negative QFN-CMV as early as 3 months post-transplant, despite being on antiviral therapy at the time.

All cases occurred in patients with prior antiviral therapy, identifying the major at-risk group. Overall, thirty-eight OLT recipients (64.4%) had received antivirals for either prophylaxis or therapy before M6, because they were either high-risk (D+/R- or kidney/liver combined transplant), developed early CMV DNAemia, developed symptomatic herpes simplex infections, or required pulsed steroids for treatment of rejection. Despite the use of antivirals, most (31/38, 81.6%) had still developed a reactive QFN-CMV by M6 when antiviral therapy was ceased, and none of these 31 recipients developed late CMV. Of these 31 patients who had a reactive assay result by M6, over 90% were already reactive as early as three months post-transplant.

Discussion

Despite the lower incidence of early CMV with the use of prophylaxis, late CMV infection that occurs after cessation of prophylaxis has become increasingly problematic (8-10). This may be due to the rapid viral load doubling time (11), or the reduced frequency of monitoring as time progresses following transplantation. The risk of late CMV is amplified in high-risk (D+/R-) patients (9). These patients may not be able to develop protective immunity towards CMV while on prophylaxis during their first 6 months post-transplant, whereas D+/R+ patients at least have a subset of memory T-cells which could conceivably undergo clonal expansion in response to minimal increases in CMV replication. Assessing which patients are at risk of late CMV presents an ongoing clinical dilemma in transplant populations. CMV viral load and serology have poor predictive value for late CMV (11-13), and a method to identify high risk patients who require further treatment or monitoring is needed.

Our results show that a single QFN-CMV performed at the end of prophylaxis offered an excellent NPV for late CMV of 100%. This identifies patients who are not at risk of late CMV and can have CMV PCR monitoring ceased.

Furthermore, over 90% of those patients who developed reactive QFN-CMV assay results by 6 months, had similar results by 3 months post-OLT. Three months may offer the optimal time post-OLT for immune system recovery to facilitate the development of a protective response towards the virus. Although due for prospective clinical evaluation, a QFN-CMV at 3 months

post-OLT could allow early antiviral discontinuation in the majority of patients who would not be at risk of late CMV. This could allow improved drug costs and side-effects.

OLT recipients appear to be at low risk of late CMV, with only 5.4% of this cohort experiencing late reactivation. Furthermore, only one patient who had pre-transplant exposure to CMV (R+) developed late CMV after 6 months, compared with the two recipients who were exposed to CMV through their donor organ (D+/R-). This highlights that patients at high risk of early CMV and require prophylaxis (D+/R-) are also likely to be at greatest risk of late CMV. This may be due to a lack of opportunity for these recipients' immune system to be exposed to and develop immunity towards the virus whilst on antivirals. Of clinical importance, 2 of the 3 patients who developed late CMV did not just develop CMV DNAemia, but actually suffered symptomatic CMV disease with very high viral loads.

International consensus guidelines regarding the management of CMV following solid-organ transplantation have emphasised a potential role for individualised CMV-related immune monitoring to help guide management (14), however only 12% of solid organ transplant units employ a form of CMV specific T-cell monitoring (15). QFN-CMV is simple and available as it is based on the widely available QuantiFERON-gold (Qiagen Ltd, The Netherlands) laboratory platform used to diagnose tuberculosis. It measures *ex vivo* stimulation of host CD8+ T-cells with various viral T-cell epitopes from viral proteins (16), and therefore offers a glimpse into an individual's immune system function directed against CMV.

The two largest prospective trials into QFN-CMV and the incidence of late CMV have demonstrated a potential role for QFN-CMV after cessation of prophylaxis in solid organ transplantation, but have included multiple solid organ transplant and only minimal numbers of OLT recipients (17, 18). Given the varied risk of immunosuppression and viral reactivation in different solid organ transplants, we examined the role of QFN-CMV specifically in OLT recipients.

Similar to the study investigating the risk of early CMV (6), we employed a lower QFN-CMV titre of 0.1 IU/mL, which has previously been suggested by Manuel et al. as more relevant for a transplant population (17). Furthermore, we used an absolute cut-off of 0.1 IU/mL without consideration of the positive and negative controls, which are likely unnecessary for clinical utility of the assay in an immunosuppressed transplant population as we have previously described (6).

There are further limitations to this study. In particular, surveys have demonstrated significant variability in CMV management protocols following liver transplantation. For example, the international consensus guidelines suggest CMV prophylaxis durations of between 3-6 months, along with potential consideration of prophylaxis after steroid therapy, and a variable rate to

commence CMV antiviral therapy in pre-emptive approaches with no defined setpoint to commence therapy. This variabilities in the guidelines is reflected in clinical practice, and the results from this study may not be generalisable to other units or transplant cohorts. This is particularly so, when transplant immunosuppression protocols vary greatly as well.

This study represents the largest number of OLT recipients prospectively evaluated with QFN-CMV for the risk of late CMV, and shows a potential benefit for a single QFN-CMV assay performed at 6 months post-transplant, and potentially earlier. The excellent sensitivity (100%) and specificity (89%) of the assay demonstrate a clear role for QFN-CMV in stratifying recipient risk of late CMV in the OLT setting. We further identify the potential for early antiviral cessation in a majority of individuals who appear to develop sufficient CMV-specific immunity by 3 months post-OLT. Those who remain QFN-CMV non-reactive at this stage, should have continuation of prophylaxis until at least 6 months, at which a repeat QFN-CMV test could be undertaken. Further multicentre, prospective, randomised trials are needed to validate these results and push for a change to standard of care for CMV in the transplant setting.

Disclosure

This investigator-initiated study received research funding by Cellestis Ltd (now purchased by Qiagen, The Netherlands). Qiagen did not have a role in study analysis or write-up. Author L.Y. is a former employee of Cellestis Ltd. Authors KV, AT and LY have received research funding from Cellestis and Qiagen.

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Figure Legends

Figure 1 – Risk of late CMV based on 6 month (M6) QFN-CMV

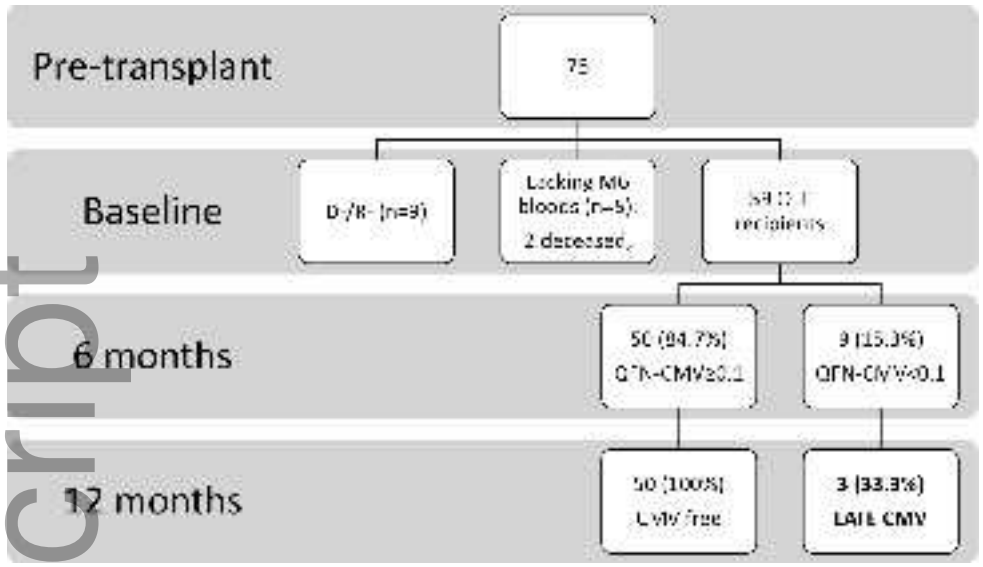
Figure 2 – Late CMV-free survival based on 6 month QFN-CMV

Table 1 - Patient characteristics

Patient Characteristics	N=59	No late CMV (n=56)	Late CMV (n=3)	p
Age (median)	53	53	59	0.50
Gender (M/F)	39/20	37/19	2/1	1.00
Pre-transplant CMV serology				**0.03**
D+/R+, D-/R+	52	51	1	
D+/R-	7	5	2	
Prophylaxis v Pre-emptive				0.08
Prophylaxis group (D+/R-)	7	5	2	
Pre-emptive group exposed to antivirals in first 6 months post-transplant	31	30	1	
Aetiology of Liver Disease				0.74
HCV	24	24		
ETOH	7	7		
NASH	6	5	1	
PSC	5	4	1	
AIH	3	3		
PBC	4	4		
HBV	1	1		
Other	9	8	1	
Immunosuppression at 6 months				
Prednisolone	34	33	1	0.57

Prednisolone dose (median)	5mg	5mg	10mg	0.74
MMF	33	31	2	1.00
AZA	11	10	1	0.47
Cyclosporin	19	18	1	1.00
C2 level (median)	791	700	1151	*
Tacrolimus	40	38	2	1.00
Tacrolimus level (median)	8.65	8.1	9.35	0.65
WCC	3.6	3.6	4.5	0.70
PMN	2.3	2.25	3.3	0.48

* insufficient numbers to allow statistical comparison



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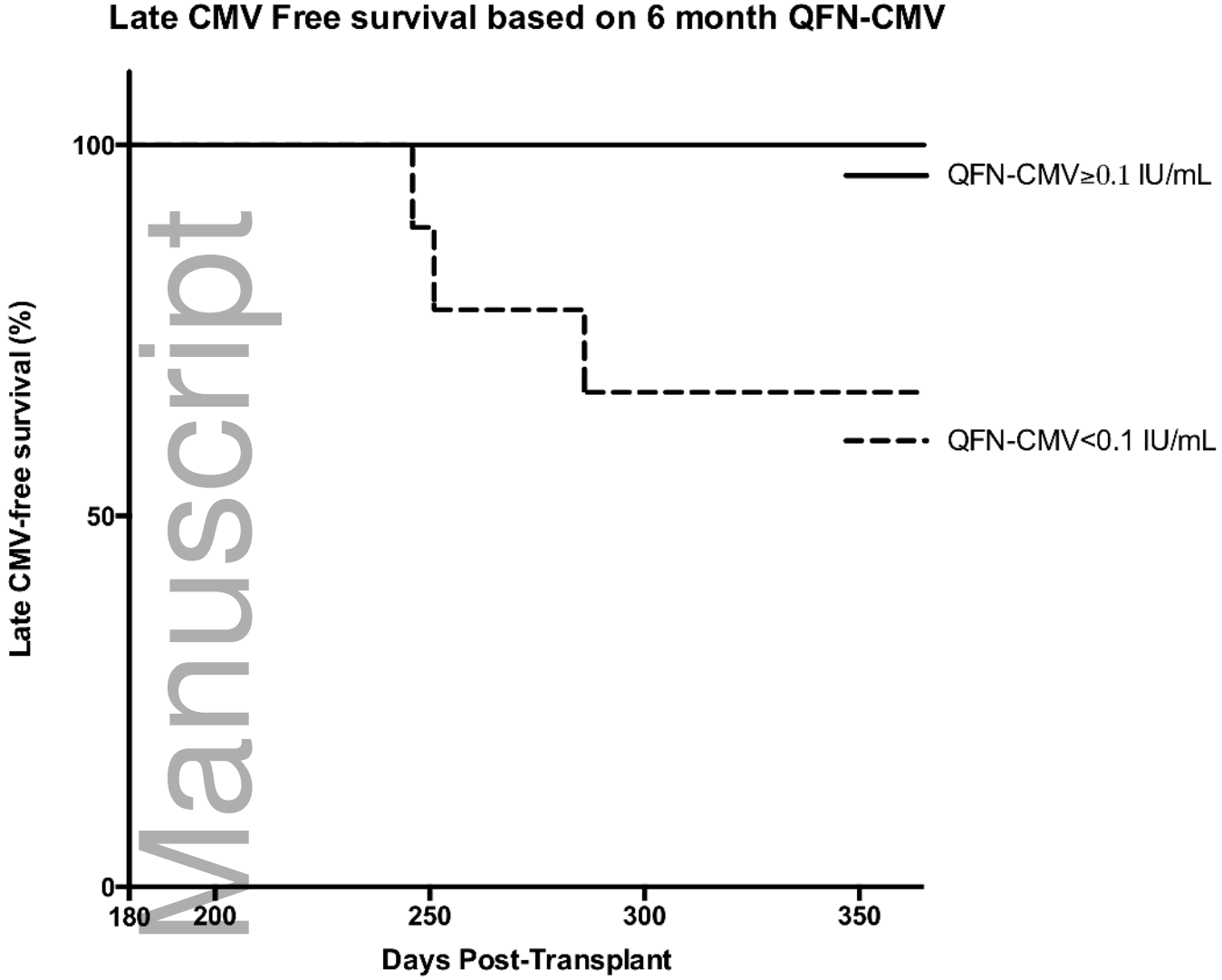

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