AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

COMMENTARY

Immune Monitoring of Infectious Complications in Transplant Patients: an Important Step towards Improved Clinical Management

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ABSTRACT Immune reconstitution following organ transplantation is absolutely critical in preventing infectious complications. However, understanding the kinetics of immune reconstitution and its potential impact on the clinical management of transplant patients remains a significant challenge. Over the last decade, various platform technologies have emerged which have provided important insights into the immune reconstitution kinetics in transplant patients. However, many of these technologies are too complicated and cumbersome to implement in a clinical setting. In this issue of the *Journal of Clinical Microbiology*, Chiereghin et al. (J. Clin. Microbiol. 56:e01040-17, 2018, https://doi.org/10.1128/JCM.01040-17) report the results of their evaluation of the QuantiFERON-CMV (QFN-CMV) assay to assess human cytomegalovirus (CMV)-specific $CD8^+$ T-cell immunity in heart transplant recipients as a prognostic tool. These studies showed that patients with absence of global immune reactivity in the QFN-CMV assay were at a higher risk of developing CMV after discontinuing antiviral prophylaxis. Furthermore, failure to reconstitute CMV-specific immunity after resolution of the first episode of viremia was associated with viral relapse. These observations, along with other recent clinical studies utilizing the QFN-CMV assay, demonstrate that systematic monitoring of antiviral immunity can be successfully used as a prognostic tool and also to guide changes to the clinical management of transplant patients.

Over the last decade, there has been a remarkable improvement in the clinical management of transplant patients (1). Posttransplant infectious complications remain one of the major challenges for transplant physicians (1, 2). Human cytomegalovirus (CMV) infection is among the major complications which cause significant morbidity and mortality (2–4). CMV can have an impact on the engrafted organ through various direct and indirect mechanisms. The direct effects include symptomatic end organ disease and clinical complications such as colitis and pneumonia, while indirect effects include acute or chronic graft rejection mediated through immune inflammation (5). Traditionally, the immunocompetence of transplant patients is based on the monitoring of immunosuppressive drug therapy (5, 6). This monitoring is highly restrictive and does not take into consideration many other clinical variables which may modify the immune response of the patient and alter the patient's susceptibility to specific pathogens. There is now an increasing emphasis on developing targeted immune monitoring strategies which will allow stratification of patients according to the risk of developing infectious complications and also guide modifications of immunosuppressive and antiviral therapies (7, 8).

While prophylactic antiviral therapy is routinely used as a preventive strategy for CMV complications, there is increasing evidence that the surveillance of CMV reactivation in combination with preemptive antiviral therapy upon subclinical reactivation can

Accepted manuscript posted online 17 January 2018

Citation Khanna R. 2018. Immune monitoring of infectious complications in transplant patients: an important step towards improved clinical management. J Clin Microbiol 56:e02009-17. https://doi.org/10.1128/JCM.02009-17.

Editor Yi-Wei Tang, Memorial Sloan Kettering Cancer Center

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For the article discussed, see https://doi.org/10.1128/JCM.01040-17.

be also effectively used in preventing clinical disease (9–11). It is important that transplant patients with effective antiviral immunity can spontaneously clear subclinical CMV reactivation without requiring antiviral therapy. The impairment of antiviral T-cell immunity significantly increases the risk of viral recrudescence and chronic CMV persistence and/or end organ disease (12). Thus, immunological monitoring can allow the adjustment of clinical management and treatment options based on each patient's risk factors. As we improve immune monitoring technologies through formal clinical assessment processes, it is likely that transplant recipients who show strong antiviral immunity and clinical evidence of viral recrudescence may be managed without preemptive therapy or offered a shorter course of antiviral drugs. On the other hand, transplant recipients who fail to show robust CMV-specific immune reconstitution may require more-rigorous virological monitoring and strategically designed preemptive antiviral therapy. Furthermore, immune and virological monitoring may also allow the identification of high-risk patients who are likely to benefit from adoptive T-cell therapy rather than from therapy using antiviral drugs.

Extensive studies on the cell-mediated immune regulation of CMV have revealed that immunodominant T-cell responses are directed toward a large array of viral antigens (12–14). These antigens are encoded at different stages of the viral life cycle and include structural, early, and late proteins. While early studies suggested that T-cell responses are primarily directed toward pp65 and IE-1 antigens, subsequent in-depth analysis revealed that other proteins, including pp28, pp50, pp150, IE-2, gH, and gB, are also frequently recognized by virus-specific T cells. These observations provided an important platform for the development of next-generation immune monitoring technologies, which provide a more comprehensive overview of T-cell immunity. The QuantiFERON-CMV (QFN-CMV) assay was originally developed by Walker and colleagues in 2007 and utilizes whole blood to assess antiviral T-cell immunity by quantitating the IFN-γ levels in the plasma after *in vitro* stimulation of the CMV-specific $CD8^+$ T cells (15). For this assay, the peripheral blood sample is collected in three tubes (1 ml each) containing HLA class I-restricted CMV peptide epitopes or phytohemagglutinin or anticoagulant alone. Twenty-two $CD8^+$ T-cell epitopes from pp65, IE-1, IE-2, pp28, pp50, and gB proteins restricted through 20 HLA class I alleles are included in the assay. These epitopes cover >98% of the human population (16). The QFN-CMV assay is the only functional antigen-specific T-cell monitoring assay that is licensed for use as an *in vitro* diagnostic assay for transplant patients. A number of prospective studies have assessed the potential use of QFN-CMV assay in different clinical settings (see summary in Table 1) (8, 15, 17–35). These settings include pretransplant risk assessment, the prediction of late onset of CMV disease after discontinuation of antiviral prophylaxis in high-risk (R^-/D^+) transplant recipients, and the identification of patients who may spontaneously clear CMV infection without any drug intervention or biomarkers for predicting relapse of CMV replication or disease.

Chiereghin and colleagues (17) have extended these observations and assessed the clinical utility of the QFN-CMV assay in heart transplant patients who were managed with either antiviral preemptive therapy or prophylactic therapy. Forty-four adult heart transplant patients (34 males and 10 females) were recruited between May 2009 and February 2014. Of these, 17 patients received oral valganciclovir as prophylactic therapy (range, 7 to 94 days; mean, 58 days), while 27 patients were managed preemptively. It is important that all recipients recruited for this study were seropositive prior to transplantation. Any transplant recipients with CMV DNA levels higher than 4,600 IU/ml in whole blood or showing a rapid increase in blood viral load were treated with oral valganciclovir at 900 mg twice daily (renal function adjusted). Antiviral therapy was stopped following a negative viral DNA test result in two consecutive blood samples. The authors assessed CMV-specific immunity by using QFN-CMV assay on blood samples collected at five fixed posttransplant time points. These included 15 days and 1, 3, 6, and 12 months posttransplant. To assess the relationship between immune reconstitution and the incidence of CMV recrudescence, QFN-CMV assessments carried out at the time point closest to that of the suspension of prophylaxis (1 or 3 months)

TABLE 1 Summary of select clinical studies in solid organ transplant recipients assessing clinical utility of QFN-CMV assay

Transplant recipient cohort	CMV serological status of transplant recipients	QFN-CMV assay time point(s)	Clinical study conclusion	Reference
SOT[a] (heart/lung and kidney) ($n = 25$)	$D^+/R^+ = 17$, $D^+/R^- = 8$	Various	All seropositive transplant recipients showed positive reactivity in QFN-CMV assay, while seronegative recipients showed negative reactivity	15
SOT (lung) ($n = 39$)	$D^+/R^+ = 18$, $D^+/R^- = 8$, $D^-/R^+ = 6$, $D^-/R^- = 7$	0.5, 1, 2, 3, 6, 9, 12, and 18 mo posttransplant	QFN-CMV assay accurately tracks the development of *de novo* CMV immunity; a striking decrease was seen in the QFN-CMV reactivity prior to the episode of CMV reactivation	35
SOT (kidney, pancreas, lung, heart, liver, and other) ($n = 108$)	$D^+/R^+ = 39$, $D^-/R^+ = 34$, $D^+/R^- = 35$	Monthly for 4 mo after completion of prophylaxis	Monitoring of CMV T cell immunity using QFN-CMV assay may be useful for predicting late-onset CMV disease	34
SOT (kidney) ($n = 14$)	$D^-/R^+ = 1$, $D^+/R^+ = 11$, $D^+/R^- = 2$	Various	QFN-CMV assay is a sensitive and specific test to detect a virus-specific T-cell response; this assay, in combination with viral DNA load estimates, may prove to be useful to stratify patients at risk of CMV disease	33
SOT (kidney, lung, heart, liver, and combined) ($n = 37$)	D^+/R^+ and $D^-/R^+ = 30$, $D^+/R^- = 7$	Monitoring initiated at the onset of CMV viremia	Monitoring of CMV T cell immunity using QFN-CMV assay after the onset of CMV viremia may be useful to predict progression vs spontaneous viral clearance, thereby helping guide in determining the best antiviral therapy and refining current preemptive strategies	8
SOT (lung) ($n = 67$)	$D^-/R^+ = 11$, $D^+/R^+ = 28$, $D^+/R^- = 17$, $D^-/R^- = 11$	Monitoring monthly for 1 year	A standardized measurement of $CD8^+$ T cell immunity using QFN-CMV assay might contribute to monitoring the immune status of lung transplant recipients	27
SOT (kidney, pancreas, lung, heart, liver, and other) ($n = 127$)	$D^+/R^- = 127$	3–6 mo (at completion of prophylaxis) and 1 and 2 mo after completion of prophylaxis	QFN-CMV assay may be useful to predict if patients are at low, intermediate, or high risk for the development of subsequent CMV disease after prophylaxis	28
SOT (lung and kidney) ($n = 113$ [55 evaluated])	$D^+R^+ = 33$, $D^-R^+ = 11$, $D^+R^- = 8$, $D^-R^- = 3$	Pretransplant and posttransplant	Monitoring of CMV T cell immunity using QFN-CMV assay prior to transplantation is useful in informing the risk of posttransplant CMV replication in SOT recipients	36
SOT (lung, liver, kidney) ($n = 114$)	$R^+ = 114$, $R^- = 27$	Various	QFN-CMV assay assessment is recommended for non-HLA A1- and HLA A2-seropositive transplant recipients	37
SOT (liver, lung, kidney) ($n = 68$)	$D^+/R^- = 68$	Not specified	Transplant recipients with positive reactivity in QFN-CMV assay had a higher percentage of late-differentiated $CD8^+$ T cells than patients lacking this response	24
SOT (kidney) ($n = 25$)	$D^+/R^+ = 13$, $D^+/R^- = 9$, $D^-/R^- = 1$, $D^-/R^+ = 2$	4.38 ± 2.73 mo posttransplant	An indeterminate result of QFN-CMV assay seems to be related to impaired immunity; the QFN-CMV assay appears to be useful in identifying the transplant recipients with increased risk of infectious complications who may benefit from immunosuppression reduction and maintenance of antiviral prophylaxis	23
SOT (kidney) ($n = 124$)	$D^+/R^+ = 124$	Pretransplant and 1 mo and 3 mo posttransplant	QFN-CMV assay reactivity is not associated with DNAemia	18
SOT (kidney and lung) ($n = 55$)	$D^+/R^+ = 33$, $D^+/R^- = 8$, $D^-/R^+ = 11$, $D^-/R^- = 3$	Pretransplant and 3 or 6 mo and 12 mo posttransplant	D^-/R^- recipients remained nonreactive in QFN-CMV assay both at pretransplant and posttransplant; D^+/R^- recipients showed lower reactivity in QFN-CMV assay than D^+/R^+ or D^-/R^+ patients	22
SOT (kidney, liver, lung, and combined) ($n = 27$)	$D^+/R^- = 12$, $R^+ = 13$, $D^-/R^- = 1$, unknown = 1	Every 2 wks until 3 mo after completion of prophylaxis	QFN-CMV assay can be used to guide changes to the management of CMV infection	20

[a]SOT, solid organ transplant.

and prior to the onset of CMV infection (for both prophylaxis and preemptive groups) were taken into consideration. Furthermore, the authors also evaluated the potential impact of immune reconstitution, as assessed by QFN-CMV assay, on viral control and relapse.

One of the most interesting aspects of this study was the use of the QFN-CMV assay to assess the kinetics of immune reconstitution in heart transplant patients who were

managed with preemptive or prophylactic therapy. While the two groups of patients showed similar patterns of immune reconstitution during the early stages of follow-up, a significantly higher proportion of preemptively treated recipients showed positive reactivity in the QFN-CMV assay at 12 months posttransplant. Furthermore, a higher proportion of patients who were offered prophylactic therapy showed an indeterminate result in the QFN-CMV assay (an indicator of poor reconstitution of global immunity) at 1 and 3 months posttransplant. While prophylactic therapy is highly effective in preventing CMV recrudescence or disease (especially in seronegative recipients), the development of late CMV infection (and drug resistance) after discontinuation of prophylaxis remains a significant challenge. The prolonged use of prophylactic antiviral therapy often delays immune reconstitution and the development of a CMV-specific effector T-cell response. Indeed, the data presented by Chiereghin and colleagues showed that the preemptively managed patients showed faster immune reconstitution than the patients treated with prophylactic therapy (17). Earlier onset of CMV infection in the preemptively managed group allowed their immune systems to recall/prime the T-cell immunity, which resulted in rapid immune reconstitution. Further analysis of immune reconstitution kinetics clearly showed that patients who failed to develop anti-CMV immunity at the completion of antiviral prophylaxis showed a higher incidence of subsequent CMV infection than those who had positive reactivity in the QFN-CMV assay. These authors argue that the QFN-CMV assay should be performed at the completion of antiviral prophylaxis to identify high-risk patients who might have failed to reconstitute antiviral immunity and would benefit from either closer virological monitoring or the reduction of immunosuppression and/or maintenance of longer antiviral prophylaxis. This strategy can be used to reduce unnecessary use of antiviral drugs and may also prevent future development of antiviral drug resistance. Chiereghin and colleagues also showed that the QFN-CMV assay can be successfully used to identify patients who may not be able to control CMV replication spontaneously and who could thus benefit from appropriate antiviral interventions (17).

In summary, we have now reached a stage where immune monitoring strategies need to be included in the standard process of clinical management of transplant recipients. There are number of major roadblocks impeding the implementation of the QFN-CMV assay in clinical settings. First, almost all clinical studies conducted to date were limited to single clinical centers. There is an urgent need to run multicenter international studies which include patients with diverse baseline risk profiles to allow the formal assessment of the QFN-CMV assay. Indeed, an ongoing multicenter study led by Atul Humar, University Health Network, Toronto, Canada (ClinicalTrials registration no. NCT02784756), is assessing the potential use of the QFN-CMV assay in guiding the duration of primary CMV prophylaxis in solid-organ transplant patients. Another issue which needs to be addressed is the frequency of testing. Immune responses to CMV are highly dynamic, especially in transplant recipients. Antiviral drugs and/or immunosuppressive therapy can alter the immune profile and thus have an impact on the transplant recipient's risk of developing CMV disease. It will be important to consider repeat assessments coincident with viral monitoring and to develop a predictive score which is based on both viral load and the QFN-CMV assay results. This will allow a more robust diagnostic strategy and better clinical management capability.

ACKNOWLEDGMENT

I am listed as the inventor for the CMV peptide epitopes used in the QuantiFERON-CMV assay. I have also acted as a consultant for Cellestis during the development of the QuantiFERON-CMV assay.

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