Evaluating Cytomegalovirus (CMV) Cell Mediated Immunity Diagnostic Assays

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INTRODUCTION:

Over the past two decades, cellular immune response to cytomegalovirus (CMV) and other viral pathogens has been an interesting area of research and has now evolved into a clinical application of assays that provide a precise measure of cellular response. Current antiviral preventative strategies have shown improved efficacy, but CMV continues to be one of the more common clinically significant infections in solid organ transplant (SOT) recipients, hematopoietic stem cell transplant (HSCT) recipients, and other immunocompromising conditions like HIV. CMV establishes a lifelong latent infection, which may reactivate in both immunocompetent and immunocompromised individuals. A frequent complication after transplantation, CMV infection may cause a series of direct and indirect effects that lead to increased incidence of graft rejection, opportunistic infections, and decreased allograft and patient survival. CMV reactivations have also been reported to occur frequently in critically ill immunocompetent patients and are associated with prolonged hospitalization or mortality.

KEY POINTS:

- **Cytomegalovirus cell mediated immunity (CMV-CMI) provides a more precise measurement of "protective" immunity, as compared to CMV antibody response.**
- **CMV specific CD4+ T cells are beneficial in conferring long term protection in an immunocompromised population, thus requires independent results for both CD4+ and CD8+ responses.**
- **The monitoring of CMV-specific T cell responses utilizing intracellular cytokine staining may aid in the detection of patients at increased risk of CMV disease after transplantation and may be useful in guiding prophylaxis and preemptive therapies.**

LITERATURE REVIEW OF CMV CELL MEDIATED IMMUNITY TESTING: PART 1 – EVALUATING CELL MEDIATED IMMUNITY METHODS

Several assays have been introduced to assist in measuring CMV cell mediated immunity (CMV-CMI). This information can be helpful in several ways in relation to antiviral therapy treatment decisions. Commercially available assays for CMV-CMI are currently limited, and primarily for research use only in the United States. With vastly different methodologies including ELISA, ELISpot, and intracellular cytokine staining (ICS) by flow cytometry, each assay has unique performance characteristics to measure CMV-CMI. There have been numerous studies to evaluate which assay can provide the desired performance to assess CMV specific immunity. Prior to this, the best diagnostic available for SOT and HSCT has been CMV IgG or IgM serology, and monitoring with CMV qPCR. Testing seroconversion post-transplant for a response in IgG has not been shown to equate CMV immunity, nor is it recommended for the diagnosis of CMV disease.[1]

While all three methods to measure CMV-CMI have the potential to predict viremia and disease, there are advantages and limitations to each, as have been described in the International Consensus Guidelines on the Management of Cytomegalovirus in Solid Organ Transplantation. ICS has the advantage of results same day, identification of CD4+ and CD8+ T Cells, and HLA type is not necessarily required to provide results. Limitations include access to flow cytometry instrumentation and the lack of standardized cutoffs. QuantiFERON-CMV is a kit that can be purchased, though currently not available in the US, with analytical time of approximately 2-3 days. The QuantiFERON assay only provides CD8+ T cell responses, and lymphopenia can impact results. There are also some HLA types that are not covered by the assay. ELISpot measures both CD4+ and

CD8+ responses, however results cannot be differentiated. It also does not require HLA typing, and results are available in 2-3 days. Like ICS, the cutoffs are not standardized.[1]

In particular, ICS aids in the detection of patients at increased risk of CMV disease after transplantation and may be useful in guiding prophylaxis and preemptive therapies in immunocompromised patients. T cell responses, both CD4+ and CD8+, are vital components of CMV immunity. While most published ICS methods are for research use only, Eurofins Viracor's (Viracor) CMV T Cell Immunity Panel (CMV TCIP) was the first commercially available assay for clinical use in the United States. Unlike the ELISA & ELISpot methodologies, Viracor's assay measures and reports individual CD4+ and CD8+ CMV-specific responses. Whole blood is stimulated with CMV antigens and lysates resulting through % IFN-y production. This assay is controlled by a negative and positive mitogen control specific to CD4+ and CD8+. The benefit of measuring both CD4+ and CD8+ T cell response is best illustrated in Gabanti, et. al. figure 1.

Gabanti, et. al studied CMV specific immunity in 39 R+ SOT patients, evaluating CD4+ and CD8+ T cells individually. A total of 39 R+ patients were studied for 1-year post-transplant, and 21 patients maintained CMV CD4+ T-cell numbers above the established cutoff (0.4 cell/µL blood), seven patients had no CMV infection, and 14 had a controlled infection. In the presence of CMV-specific CD8+ only responses, nine controlled the infection temporarily until CD4+ T-cell appearance. Finally, nine had to be treated preemptively due to a viral load greater than the established cut-off in the absence of specific CD4+ T-cells. Over half, 21, of the 39 patients had CD4+ T cell results above the threshold of the assay confirming CD8+ T cells alone did not confer long-lasting immune control of CMV infection in all patients examined. CD4+ T cells have several roles in the cell-mediated immune response and do not seem to be replaced by other functions/cells in the post-transplant period.[2]

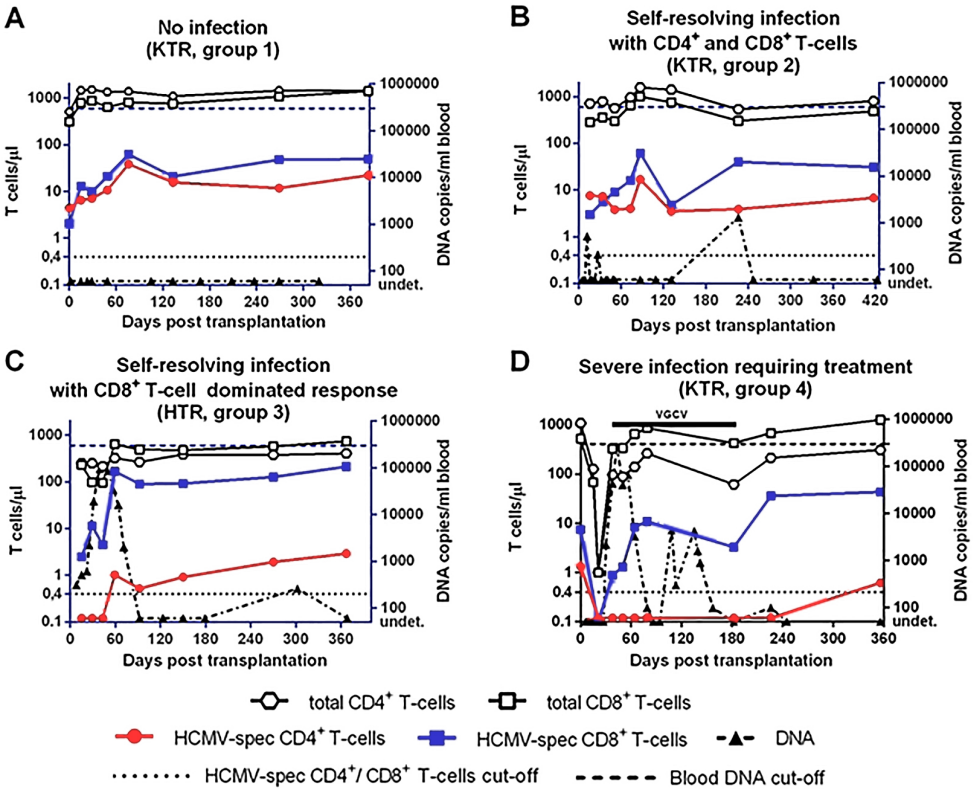

Figure 1. *Kinetics of absolute numbers/ml blood of total and HCMV-specific CD4+ and CD8+ T Cells in four SOTR patients (each representative of one of the four patient groups). Patient A (group 1): no HCMV infection (no viral DNA) is detected and HCMV-specific CD4+ and CD8+ T Cells are consistently above the cut-off (black dotted line corresponding to 0.4 T Cells/ml blood); Patient B (group 2): self-resolving infection in the presence of low viral load and specific CD4+ and CD8+ T Cells consistently above the cut-off; Patient C (group 3): self-resolving infection in the presence of a high viral load peak and a number of HCMV-specific CD8+ T Cells above the cut-off, but in the absence of specific CD4+ T Cells or in the presence of CD4+ T Cells at a level close to the cut-off for the first two-three months after transplantation; Patient D (group 4): uncontrolled infection in the presence of high viral load above the cut-off (requiring antiviral treatment) and absence of specific CD4+ T Cells until 12 months after transplantation. The dashed line indicates the cut-off of viral load to start preemptive therapy. KTR, kidney transplant recipient; HTR, heart transplant recipient; VGCV, valganciclovir.* [2]

QuantiFERON® CMV (QTF-CMV), is an ELISA-based interferon (IFN)-y release assay. The assay begins with collection of whole blood exposed to coated collection tubes with peptides stimulating CD8+-specific epitopes of CMV proteins cells to produce IFN-y. IFN-y release is then measured through ELISA and compared to controls. This method looks singularly at CD8+ CMV-specific response. QTF-CMV is not currently commercially available for clinical use in the United States but has been evaluated in a research setting.

Other assays, T-SPOT® (Oxford Immunotec) and T-Track® (Lophius Biosciences), use the ELISpot platform to assess levels of anti-CMV cell-mediated immunity. Both tests utilize CMV antigens or lysates followed by IFN-γ response through ELISpot as an aggregate response from CD4+ and CD8+. While more sensitive than ELISA techniques to identify those at risk for CMV disease, the aggregate response of CD4+ and CD8+ together may be a limiting factor to differentiate if the patient is fully capable of mounting their own CMV-specific immune response. Furthermore, ELISpot assays are commercially limited, as they are currently only CE marked for the European Union.

LITERATURE REVIEW OF CMV CELL MEDIATED IMMUNITY TESTING: PART 2 - REVIEW OF CELL MEDIATED IMMUNITY DATA

As with many comparisons in the field of immunology, it becomes difficult to compare sensitivity, specificity, PPV and NPV within the various studies to evaluate different methodologies. Some journal articles focus on infection within Donor/Recipient (D+/R-) populations, and others are looking at recurrence in the (D+/R+) population, or a combination of both. Additional questions arise considering clinical application of quantitative versus qualitative results, standardization of cutoffs, specific targets, as well as how the results are reported and quantified (independent vs. aggregate). As cell mediated immunity assays continue to be adopted, best practices evolve, and data is published- we will begin to see further increase in utilization within clinical practice.

In their evaluation of QuanitFERON CMV (QTF-CMV), Ruiz, et. al. published "The QTF-CMV assay at prophylaxis discontinuation exhibited suboptimal accuracy for predicting protective CMV-CMI (sensitivity: 77.4%; specificity: 34.3%; positive predictive value [PPV]: 64.1%; negative predictive value [NPV]: 50.0%), with no differences in 1-year CMV infection rates between patients with negative (nonreactive or indeterminate) or reactive results (45.8% vs 36.1%; P = .244)." "The QTF-CMV assay when assessed as per manufacturer's interpretative criteria, performed poorly to predict protection from CMV infection following discontinuation of [valganciclovir] prophylaxis among ATG-treated CMV-seropositive KT [kidney transplant] recipients."[3]

In the American Journal of Transplantation, Kumar, et. al. evaluated ELISpot to determine if the assay could predict subsequent CMV events. The data showed "In the D+/R− subgroup, no cutoff was significant, although 40 sfu provided the optimal NPV [of 97%]. This represents a population where the majority of patients received 6 months of antiviral prophylaxis. The overall PPV of the test was low." Furthermore, the article noted, "To our knowledge, 2 ELISpot assays have been commercialized: T-SPOT.CMV as used in the current study and T-Track CMV (Lophius Diagnostics, Germany). Although both assays use pp65 and IE-1 for T cell stimulation, the T-SPOT.CMV uses peptide pools, whereas T-Track CMV uses modified whole proteins. Such differences may lead to variations in cutoff values, sensitivity, and specificity."[4]

Table 1. Summary of published results of QuantiFERON and ELISpot related CMV recurrence

Journal Article DOI	Authors	Assay	Sensitivity	Specificity	PPV	NPV
DOI: 10.4110/in.2017.17.5.317	Ji-Soo Kwon, et. al.	QTF-CMV	60% (26-88)	59% (42-75)	29% (11-52)	85% (65-96)
DOI: 10.1111/ajt.15793	Mario Fernandez-Ruiz, et. al	QTF-CMV at prophylaxis discontinuation	77.4%	34.3%	64.1%	50.0%
DOI: 10.4110/in.2017.17.5.317	Ji-Soo Kwon, et. al.	pp65-ELISPOT	90% (56–100)	39% (24–57)	28% (14–47)	94% (70–100)
DOI: 10.1111/ajt.15315	Kumar, Deepali, et. al.	pp65-ELISPOT R+, cutoff >40	N/A	N/A	9.1%	98.6%
DOI: 10.1371/journal.pone.0189488	Hyeyoung Lee, et. al.	pp65-ELISPOT	70% (34.8-93.3)	72.2% (60.4-82.1)	25.9% (16.8-37.8)	94.5% (86.9-97.8)

**Sensitivity, Specificity, PPV and NPV are taken directly from the published papers and not adjusted by prevalence which may make values difficult to compare.*

Recent publications have measured the performance of Viracor's CMV TCIP and the value of reporting both CD4+ and CD8+ CMV specific T cell activity. These studies assess the prediction of CMV adverse events, determining if CMV prophylaxis can be discontinued or continued further and understanding the significance of individual reporting of CD8+ and CD4+ results.

Rogers et al reported "we found a strong correlation between the results of the CMV TCIP, specifically low CMV-specific CD4+ T Cells measured by ICS and FC, and subsequent CMV events. The association between CMV events and CMV-specific CD8+ T Cells did not reach statistical significance, although P-value was 0.06. In patients with repeat CMV-TCIP, CMV-specific CMI became stronger over time, facilitating discontinuation of valganciclovir. Viracor's report provides the first real world data on the predictive value of this commercially available assay that is supportive of its potential clinical utility."[5] Additionally, within Rogers, et al, the %CMV-specific CD4+ cells were significantly lower in patients with CMV events (median 0.13, IQR 0.08–0.3) compared to those without CMV events (0.73, 0.32–2.19, P = 0.002). The % CMV-specific CD8+ cells were also lower in patients with CMV events (0.46, 0.13–1.33) than those without CMV events (0.9, 0.37–3.75), though these results did not reach statistical significance (P = 0.08). Comparable results were also described in a study by Jorgensen, et al, who assessed 25 transplant recipients (mainly kidney), of which 76% were D+/R- and 76% were receiving treatment. The PPV for the assay to predict lack of CMV was 88%. This increased to 93% when patients with anti-viral resistance were removed and 91% in those receiving treatment for CMV. Additionally, they showed a PPV of 92% when using a Recurrence Risk Factors Screening Tool.[6]

Table 2. Summary of published results using Viracor's CMV T Cell Immunity Panel related to CMV recurrence

	Sensitivity	Specificity	PPV	NPV
CD4+ only (>0.22% cutoff) Rogers et al. 2020	79%	75%	85%	67%
CD8+ only (>0.21% cutoff) Rogers et al. 2020	82%	44%	72%	58%
CD4+ and CD8+ (>0.2% cutoff) Jorgensen et al. 2020	88%	67%	88%	67%

POTENTIAL CLINICAL APPLICATIONS:

There is an unmet clinical need to more precisely determine post-transplantation risk for CMV beyond D/R serostatus, with the goal of tailoring CMV prophylaxis duration to an individual's CMV disease specific risk. Prophylaxis duration varies as each center tries to navigate the high cost of antiviral treatments, risk of bone marrow suppression, potential toxicity or development of subsequent viral resistance. Whether a center uses antiviral prophylaxis or preemptive therapy in their SOT or HSCT program, there are many considerations and barriers that can limit use or access to these medications. Many variables need to be assessed including donor and recipient D/R serostatus, treatment of rejection or aGVHD, conditioning regimen, as well as ongoing immunosuppression. These factors all contribute to potential confounders of the protocol duration.

Since T cell responses are critical to CMV immune control, assays to measure T cell responses to CMV antigens may predict future infections. CMV-CMI can even confirm if adequate time is allotted or too long within an antiviral protocol, leading to a discussion on decreasing or extending antiviral therapy. A diagnostic that can measure if a patient is mounting a response to CMV prior to removal of therapy, could prove to be clinically useful. Lilleri, et. al. described after 12 months 85/131 HSCT patients, 76 (90%) seropositive and 9 (21%) seronegative, displayed CMV protective immunity, and 80/85 of these patients were able to self resolve a CMV infection (viral load below the threshold) without preemptive therapy. The remaining five patients received preemptive therapy due to GVHD steroid treatment. Monitoring of CMV specific immune recovery can complement viral load and clinical presentation information to help guide preemptive therapy decisions.[7]

The duration of CMV antiviral prophylaxis protocols are mostly standardized based on risk stratification depending upon presence or absence of CMV IgG, and do not often consider recipients CMV-CMI. CMV-CMI can also assist in clinical management of antivirals by confirming the prophylaxis protocol, decrease in prophylaxis duration, and discontinuation or continuation of antiviral therapy. Premature removal of antiviral prophylaxis can lead to recurrence of CMV, as well as the need to adjust immunosuppression leading to increased risk of transplant rejection. Opposite of premature removal, prolonged use, or overuse of antivirals can potentially lead to antiviral resistance, and effect outcomes associated with CMV viremia. Nonadherence to prophylaxis protocol can lead to increased risks of CMV disease in patients, especially those without CMV-CMI. In some cases, patients

cannot afford the costs associated with these medications, which can lead to discontinuance, with limited notification to responsible clinicians. There are also instances when therapy cost or toxicity influence the decision to discontinue antiviral therapy earlier than the protocol dictates. Cases with clinical issues related to inability to tolerate antiviral prophylaxis or therapy present with co-morbidities such as neutropenia or leukopenia, causing physicians to make critical decisions to either remove or continue antiviral prophylaxis or preemptive therapy. Lastly, readmission due to CMV only increases the costs associated with not knowing whether or not the patient has CMV-CMI. Currently, these decisions are often made with limited diagnostics, and limited knowledge of the potential risks of removing or continuing therapy.

CONCLUSION:

Viracor's CMV T Cell Immunity Panel can provide a comprehensive picture of CMV-specific immunity which assists providers in the risk assessment of CMV events and decision making for treatment plans. Studies have shown that being able to assess the CD4+ and CD8+ CMV-specific response independently allows for a clear picture of CMV risk status compared to aggregate scores or those assessing CD8+ T cells alone. The need for this type of testing adds to the movement toward personalized medicine, especially within the immunosuppressed populations of SOT and HSCT. As the number of studies continue to show positive results for the assessment of individualized immune response to CMV, other viral pathogens are likely to follow.

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