INTRODUCTION

Cytomegalovirus (CMV) infection is generally asymptomatic and relatively benign in the immunocompetent population [1]. However, CMV infection in solid organ transplantation (SOT) is significantly associated with increased morbidity and mortality [2,3]. In SOT, CMV is transmitted via a transplanted organ, and the risk of CMV infection is increased if the recipient does not have pre-existing CMV-specific cell-mediated immunity (CMV-CMI) and the graft has a certain amount of lymphoid tissue [4,5]. Long-term immunosuppressive treatment to prevent rejection increases the risk of viral reactivation in seropositive recipients who had previously acquired a latent infection [6].

The worldwide CMV seroprevalence has been estimated to be approximately 83% in the general population [7]. A high seroprevalence of CMV has been reported in Korea, and a recent study estimated the overall CMV immunoglobulin G (IgG) seropositivity rate to be 94% [8]. Therefore, Koreans were generally considered seropositive donors and recipients (D+/R+), who are at intermediate risk for CMV infection and/or latent virus reactivation after trans-
CMV may indirectly affect transplant recipients, in part due to its ability to regulate the immune system [2]. CMV is associated with increased rates of bacteremia, invasive fungal infection, and Epstein-Barr virus-mediated posttransplant lymphoproliferative disorders [10-13]. CMV infection has been reported to be associated with acute rejection and chronic allograft nephropathy in renal recipients [14-17], bronchiolitis obliterans in lung recipients [18], and cardiac allograft vasculopathy [19,20]. Many studies have presented an association between CMV infection and decreased patient survival [16,21].

The incidence of CMV infection or disease in SOT has been reported to be 12%–20% and 6%–30%, respectively [23]. The adjusted incidence rate of CMV disease in Korea, excluding CMV syndrome, was recently reported to be 33.1 (95% confidence interval [CI], 28.8–38.0) per 1,000 person-years in SOT and 5.1 (95% CI, 4.6–6.1) per 1,000 person-years in hematopoietic stem cell transplant (HSCT) recipients. In the SOT cohort, heart transplant recipients had the highest rate of CMV disease, at 104.2 (95% CI, 66.4–163.7) per 1,000 person-years, liver transplant recipients showed the lowest rate, at 11.1 (95% CI, 7.7–16.3) per 1,000 person-years, and renal transplant recipients had an intermediate rate, at 44.3 (95% CI, 37.7–52.1) per 1,000 person-years [24].

The development and implementation of accurate and reliable diagnostic assays for CMV infection are essential for the improvement of early posttransplant CMV prevention and disease management [25]. In this review, we describe the laboratory tests for the diagnosis of CMV infection that are currently widely used in clinical laboratories. Tests for direct virus detection and measurement of host immune response are used in SOT recipients (Table 1). Laboratory tests that directly detect CMV are recommended for diagnosis, surveillance, and monitoring, whereas immune status analysis is utilized for CMV risk assessment and risk factor stratification [26].

### ASSAYS OF VIRUS DETECTION

Laboratory methods for virus detection include molecular assays, antigenemia, culture, and histopathology.

#### Molecular Assay

The commercial quantitative nucleic acid amplification test (QNAT) is the preferred assay for diagnosing CMV infection, guiding preemptive treatment, and monitoring the response to therapy [27]. QNAT is very sensitive, provides a rapid turnaround, and is preferred over antigenemia [26]. Quantitative detection is preferred over qualitative analysis, as CMV QNAT can distinguish CMV replication with a high viral load from latent viral infections with low levels of CMV DNAemia [28,29]. Various commercial assays have been developed to detect and quantify CMV DNA [26]. Commercially available Food and Drug Administration (FDA)-cleared or approved platforms in the USA include the Cobas AmpliPrep/Cobas TaqMan CMV test (Roche, Basel, Switzerland), the Artus CMV RGQ MDX kit (Qiagen, Hilden, Germany), and the Abbott RealTime CMV assay (Abbott, Chicago, IL, USA) [27,30].
There are no commercially available assays for the detection of CMV RNA, although CMV RNA is a specific indicator of CMV replication. CMV DNA may not accurately reflect CMV replication, because highly sensitive QNATs can simply amplify latent viral DNA [2]. Whole blood and plasma are common specimens, but bronchoalveolar lavage (BAL) fluid and cerebrospinal fluid (CSF) are also available [31,32]. CMV DNA in CSF suggests the possibility of central nervous system disease [22]. Another challenging area for the diagnosis of CMV in HSCT and SOT recipients is CMV pneumonia. CMV QNAT in BAL fluid can be used as a less invasive tool for diagnosing CMV pneumonia, especially if performing a transbronchial biopsy would be risky. Although the reported viral load values in BAL fluid vary widely across studies, higher viral loads in BAL fluid compared to asymptomatic shedding correlated with biopsy-proven CMV pneumonia [22,31]. Further studies are warranted to develop a standardized method for collecting BAL fluid, analyzing and reporting the results, and identifying an optimal cut-off applicable in various clinical contexts. If successful, this approach could replace invasive biopsy and viral cultures for the diagnosis of CMV pneumonia in clinical settings [32]. The use of QNAT for intestinal biopsy is an evolving field. CMV QNAT on tissue biopsy specimens alone is insufficient for diagnosing CMV gastrointestinal disease. However, the guideline developed by the CMV Drug Development Forum for clinical trial standardization considers that QNAT of a biopsy specimen in a compatible clinical setting is indicative of possible gastrointestinal disease [22].

Viral load trends over time are directly related to the possibility of severe CMV disease. Higher or rapidly increasing viral loads are associated with the high risk of serious CMV disease [26]. Conversely, reduction of the viral load during antiviral therapy correlates with the clinical resolution of disease [28]. A sustained increase or minimal decrease in the viral load suggests refractory or drug-resistant CMV [33]. The guideline recommends treating patients until a negative threshold is reached, as persistent viremia at the end of antiviral therapy is a risk factor for relapse [2].

A limitation of the CMV QNAT is the absence of applicable viral load thresholds for various clinical indications. The World Health Organization (WHO) international standard for CMV nucleic acid amplification techniques was developed in October 2010 [34]. Implementation of the WHO international standard for calibration has significantly improved the degree of agreement of viral load values between different assays and allows results to be reported in units of IU/mL [27,35]. A multicenter study for intra-assay harmonization of the CMV QNAT on plasma samples has been conducted, but clinically relevant differences in viral load values have been reported between the various assays [36]. Viral load variability among assays calibrated by the WHO international standard occurred due to differences in the assay platform, gene targets, and amplicon size [36,37]. Therefore, the same assay platform should be used for serial CMV surveillance and monitoring using the same type of sample [27,38,39]. It is recommended that transplant centers work with their laboratories to define and validate center-specific viral load thresholds for each clinical application [2,40].

As another method, droplet digital polymerase chain reaction shows potential to reduce the variability of CMV DNA load measurements, but it is not yet widely used [41-43]. Novel strategies are being tried to improve adherence by allowing patients to collect and submit their blood samples for monitoring without visiting hospitals or standard phlebotomy [44]. Dried blood spots, a previously studied method for the diagnosis of congenital CMV, can be used to assess the CMV viral load using finger-stick blood samples [44]. Dried blood spot quantification was validated in 35 SOT recipients [45] and is currently being evaluated in a multicenter randomized controlled trial using mobile device-assisted CMV monitoring in HSCT recipients at high risk of late CMV disease (ClinicalTrials.gov identifier NCT03910478) [44].

**CMV Antigenemia**

CMV pp65 antigenemia is an indirect immunofluorescence-based assay that identifies the pp65 antigen of CMV in peripheral blood leukocytes [46]. This assay uses monoclonal antibodies to detect the pp65 antigen, an immediate early antigen of CMV [26]. This assay has limitations, including a lack of standardization with significant inter-laboratory variability, lack of automation, the need for sufficient leukocytes in the sample (limited to leukopenia), a labor-intensive nature, and subjective interpretation [26]. The use of antigenic assays has declined significantly, and antigenic assays are now being replaced by molecular assays in most transplant laboratories [28]. CMV pp65 antigenemia was comparable to QNAT in previous studies [2,46].
Table 1. Characteristics of cytomegalovirus assays in solid organ transplantation

| Assay                           | Technique                                      | Advantage                                                                 | Limitation                                                                 |
|---------------------------------|------------------------------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------------------|
| **For virus detection**         |                                                |                                                                           |                                                                           |
| CMV QNAT                        | - Detects and quantifies CMV DNA              | - Various commercial assays are available                                | - Lack of universal viral load threshold                                    |
|                                 | - Reporting unit: IU/mL                       | - Rapid and sensitive tool for diagnosis of CMV infection                 | - Lack of standardization despite WHO internal standard material due to various aspects of assay (limit of detection, quantification range, sample type, amplicon size, gene target) |
|                                 |                                                | - Surveillance for preemptive treatment                                   |                                                                           |
|                                 |                                                | - Monitoring the response of antiviral therapy                           |                                                                           |
|                                 |                                                | - Implementation of the WHO internal standard for calibration            |                                                                           |
| Antigenemia                     | - Immunofluorescence-based assay              | - Monitoring CMV infection                                               | - Lack of assay standardization                                             |
|                                 | - Detect CMV pp65 antigen expressed in leukocytes using monoclonal antibody | - Monitoring the response of antiviral therapy                           | - Need for enough leukocytes in sample (limited in neutropenia)             |
|                                 | - Reporting unit: number of pp65 positive cells per number of leukocytes |                                                                           | - Lack of automation                                                       |
|                                 |                                                |                                                                           | - Interpretation is subjective                                              |
|                                 |                                                |                                                                           | - Labor-intensive                                                          |
| **For CMV-specific cell-mediated immunity** |                                                |                                                                           |                                                                           |
| Serology                        | - Usually detects CMV IgG antibodies          | - The risk of CMV infection is determined depending on the CMV serology in donor and transplant candidate | - IgM is not recommended due to false positivity                          |
|                                 |                                                |                                                                           | - The use of serology is limited for diagnosis of CMV infection after transplantation |
| QuantiFERON-CMV                 | - ELISA-based                                  | - Commercial assay                                                       | - HLA class I restricted                                                   |
|                                 | - Measures IFN-γ                               | - Standardized high-throughput assay                                     | - Only measures CD8+ T cells (not CD4+ T cells)                            |
|                                 | - Collecting whole blood into tubes containing the CMV peptide pool | - Can be performed routinely in laboratories                             | - Indeterminate results in immunosuppressed patients                       |
| ELISpot                         | - Measures IFN-γ                               | - Commercial assays are available: T-SPOT.CMV, T-Track CMV               | - Requires PBMC isolation procedure                                         |
|                                 | - Stimulates PBMCs with CMV-overlapping peptides |                                                                           | - Lack of proper cut-offs for positivity                                  |
|                                 | - Reporting unit: spot forming units/ PBMCs    | - Highly sensitive                                                       | - Requires ELISpot reader                                                  |
|                                 |                                                | - Not limited by HLA                                                     | - Unable to differentiate between CD4+ T cells and CD8+ T cell response    |
|                                 |                                                | - Measures both CD4+ T cells and CD8+ T cells                           | - Lack of standardization as many laboratories use in-house methods        |
| Intracellular staining and flow cytometry | - Detects intra-cytoplasmic cytokines produced by stimulation of whole blood or PBMCs with CMV peptides using a fluorochrome antibody | - Simultaneous detection of multiple cytokines and cell surface markers   | - Requires flow cytometer                                                  |
|                                 |                                                | - Can differentiate T-cell phenotypes                                   | - Lack of standardization                                                  |
|                                 |                                                | - Can differentiate between CD4+ T cells and CD8+ T cell response       | - Expensive                                                                |
|                                 |                                                |                                                                           | - Labor-intensive                                                          |
|                                 |                                                |                                                                           | - Limited to research use only                                             |

CMV, cytomegalovirus; QNAT, quantitative nucleic acid amplification test; WHO, World Health Organization; IgG, immunoglobulin G; IgM, immunoglobulin M; ELISA, enzyme-linked immunosorbent assay; IFN-γ, interferon-gamma; HLA, human leukocyte antigen; ELISpot, enzyme-linked immunosorbent spot; PBMC, peripheral blood mononuclear cell.
ASSAYS FOR IMMUNE RESPONSES AFTER CMV INFECTION

Assays for detecting the immune response to CMV in SOT include serology and CMV-specific cell-mediated immune assays [26].

CMV Serology

Pretransplant CMV IgG measurement in the donor and recipient is the most commonly recommended test for predicting and stratifying the risk of CMV infection [47]. Based on CMV IgG results, recipients are classified as high-risk (seropositive donors and seronegative recipients), intermediate-risk (seropositive recipients), or low-risk (seronegative donors and recipients) [26]. Although CMV-seropositive recipients are considered to have a lower risk of CMV infection than CMV-seronegative recipients, the risk of CMV infection after transplantation remains. A recent study found that in seropositive recipients, patients with low pretransplant CMV IgG titers (<20 AU/mL) were more likely to have a CMV infection (hazard ratio, 2.98) after kidney transplantation (KT) than patients with CMV IgG titers greater than 20 AU/mL [48].

Assay for CMV-CMI

The CMV-CMI test was developed to complement conventional CMV viral load assays and provide an opportunity to identify immunocompetent patients capable of controlling viral replication via host immune mechanisms without antiviral therapy [44]. The posttransplant risk of CMV infection can be predicted by pretransplant CMV-CMI assessment. CMV-CMI can be measured during or at the end of CMV prophylaxis to predict the risk of CMV infection, or at the completion of treatment to predict the risk of CMV recurrence or to determine whether secondary prophylaxis is needed [47].

CMV-CMI studies, mostly performed in KT recipients, showed that measured immunity levels correlated with virologic outcomes. Several studies have reported that increased CMV-CMI levels measured by CMV-specific interferon-gamma (IFN-γ) release assays are associated with reduced incidence of CMV infection or disease, lower initial and peak viral load, reduced CMV recurrence, and freedom from CMV events [49-64]. It has been shown that a lack of adequate CMV-specific CD4+ and/or CD8+ T cell immunity is correlated with a higher likelihood of CMV disease, recurrence, and treatment failure [2]. However, to date, well-designed large-scale intervention studies demonstrating the clinical utility of CMV-CMI are still lacking.

Commercially available CMV-CMI assays have the advantages of relative ease of use, standardized approaches, and suitability for comparing results between studies of different populations [27]. Commercially available assays include the QuantiFERON-CMV (CMV-QF) enzyme-linked immunosorbent assay (ELISA; Qiagen), T-SPOT.CMV (Oxford Immunotec, Abingdon, UK) and T-Track CMV (Lophius Biosciences, Regensburg, Germany), which are enzyme-linked immunosorbent spot (ELISpot) assays [44]. CMV-QF, T-SPOT.CMV and T-Track CMV are not FDA-approved, but are CE (Conformité Européenne) marked in Europe [65]. Recently, the novel CMV T Cell Immunity Panel (Viracor Eurofins Inc. Laboratories, Lee's Summit, MO, USA), which measures CMV-specific cellular immunity by intracellular cytokine staining (ICS) and flow cytometry, has become available in the USA [66,67] (Table 2).

QuantiFERON-CMV

CMV-QF measures the IFN-γ response of CD8+ T cells to various human leukocyte antigen (HLA) class I restricted synthetic CMV T cell epitopes. The peptides are designed to target A1, A2, A3, A11, A23, A24, A26, B7, B8, B27, B35, B40, B41, B44, B51, B52, B57, B58, B60, and Cw6 (A30, B13) HLA class I haplotypes covering >98% of the human population (https://www.quantiferon.com/wp-content/uploads/2018/10/L1075110-R05-QF-CMV-ELISA-IFU-CE.pdf). These peptide epitopes include pp65, pp50, IE-1, IE-2, and the glycoprotein B antigens. Whole blood is incubated with CMV peptides for 18–24 hours, and then the supernatant is harvested and the level of IFN-γ is measured based on ELISA. The cut-off recommended by the manufacturer is 0.2 IU/mL [68]. One major limitation of CMV-QF is that the use of HLA class I-restricted CMV epitopes may not reflect the ability of some individuals to recognize and respond to the epitope [69]. About 60% of pretransplant CMV-seropositive recipients were negative for CMV-QF [70]. A recent study performed CMV-CMI in healthy individuals and found 18.3% (13/71) revealed humoral/cellular discordance, showing CMV-seropositivity with CMV-QF negativity [71]. Individuals with inconsistent results had lower levels of CD4+ and CD8+ T cell proliferation in response to CMV lysate stimulation and expressed a lower level of anti-CMV IgG. This suggests that immune response to CMV was highly heterogeneous in healthy subjects [71]. In addition, CMV-QF only measures the CMV-specific CD8+ T cell response. However, CMV-specific CD4+ T cells are
important for generating a pool of memory CD8+ T cells capable of controlling CMV infection [66], and evidence for antiviral properties of CD4+ T cells against CMV has also been reported [72]. Caution is needed in interpreting indeterminate results of CMV-QF, which may occur in up to 38% of HSCT recipients [73]. Indeterminate results may result from improper processing of the samples, but many of them are caused by inadequate T cell responses to the mitogen, which reflects low or dysfunctional T cells [73,74].

**ELISpot Assays**

The ELISpot assay measures IFN-γ production by both CD4+ and CD8+ T cells in response to stimulation by CMV antigens. Production levels are quantified by counting the number of spot forming units per given number of target cells, such as peripheral blood mononuclear cells (PBMCs). Currently commercially available assays are the T-Track CMV and T-SPOT.CMV. For analysis, PBMCs are isolated from whole blood, placed in wells, and stimulated with CMV pp65 and IE-1 antigens (urea-formulated proteins in T-Track CMV, peptides in T-SPOT.CMV). The T-Track CMV assay uses recombinant urea-formulated pp65 and IE-1 antigens that activate a wide range of effector cells such as CD4+, CD8+ T cells, natural killer (NK), and NK T cells [75]. In both assays, after 17–21 hours of incubation, secreted IFN-γ binds to IFN-γ specific capture antibodies in the well, and the binding of enzyme-linked secondary antibody generates insoluble spots for detecting antibody-bound IFN-γ [47]. CMV-QF is easier to perform than ELISpot because it does not require additional procedures and laboratory instruments for PBMC purification [65]. However, ELISpot assays are more sensitive than ELISA-based assays and produce quantitative spot results [76]. A limitation of the ELISpot assay is the inability to differentiate between CD4+ and CD8+ T cells [27]. Moreover, since many laboratories use in-house methods, there are no adequate cut-

---

**Table 2. Characteristics of commercially available CMV-specific cell-mediated immunity assays**

| Assay principle | QuantiFERON-CMV | T-SPOT.CMV | T-Track CMV | CMV T cell immunity panel |
|-----------------|-----------------|-----------|-------------|---------------------------|
| Assay principle | ELISA           | ELISpot   | ELISpot     | Intracellular staining and flow cytometry |
| Product company | Qiagen          | Oxford Immunotec | Lophius Bioscience | Viracor Eurofins |
| Sample          | 3 mL of whole blood | Purified PBMCs from 12 mL of whole blood | Purified PBMCs from 15 mL of whole blood | 10 mL of whole blood |
| PBMCs required  | No              | Yes       | Yes         | Yes                       |
| CMV antigen     | Various HLA class I restricted 21 CMV peptides | pp65, IE-1 | T-activated CMV-specific pp65 and IE-1 | Whole viral lysate, pp65, IE-1 |
| Measurement     | IFN-γ           | IFN-γ-specific spot-forming cells | IFN-γ-specific spot-forming cells | % CMV-specific CD4+ and CD8+ T-cells |
| Cut off for positivity | CMV antigen minus | Nil control ≥0.2 | NA | Either pp65 or IE-1 ≥10 spots | CMV-specific CD4+ or CD8+ responses >0.2 |
| Measuring range (linearity) | Up to 10 IU/mL | NA | 10~1,000 spots | NA |
| Clinical sensitivity | 80.5% (insert) | 93.3% (insert) | 89.6% (insert) | 79%–82% [66] |
| Time to test    | 16-24 hours     | 30–40 hours | 30–40 hours | 3–4 business days from receipt of specimen in USA |
| Quality control | Nil control ≤8.0 Mitogen control | Nil control <10 spots Positive control (mitogen solution containing PHA) >20 spots | Negative control <10 spots Positive control (SEB) >400 spots | Negative control Positive control (SEB) |
| Comment         | CE-marked Not FDA-approved | CE-marked Not FDA-approved | CE-marked Not FDA-approved | Not FDA-approved |

CMV, cytomegalovirus; ELISA, enzyme-linked immunosorbent assay; ELISpot, enzyme-linked immunosorbent spot; PBMC, peripheral blood mononuclear cell; HLA, human leukocyte antigen; IFN-γ, interferon-gamma; NA, not available; PHA, phytohemagglutinin; SEB, Staphylococcus aureus enterotoxin type B; CE, Conformité Européenne; FDA, Food and Drug Administration.
offs and a lack of standardization [77].

**ICS and Flow Cytometry**

ICS using flow cytometry may be a superior method due to its ability to simultaneously measure multiple cell surface molecules and cytokines in real time and provide quantitative characteristics of CMV-specific CD4+ and CD8+ T cells [27]. Whole blood or PBMCs are stimulated with CMV peptides, and then the cells produce cytokines such as IFN-γ, tumor necrosis factor-alpha, and interleukin (IL)-2. Intracellular cytokines of interest are stained with fluorescein-coated antibodies [77]. ICS is the only assay capable of analyzing CD4+ and CD8+ T cell responses separately [66]. However, the ICS assay has several disadvantages, including a lack of standardization, resource intensiveness, a high cost to perform, and the need for expert interpretation [77]. Additionally, there are only a few studies supporting ICS assays, and to date, they have not been widely used in clinical laboratories [78-81]. In a recent multicenter cohort study of 124 CMV-seropositive KT recipients not receiving anti-thymocyte globulin (ATG), ICS was measured before and 15 days after transplantation [81]. Low levels of CMV-CMI (defined as <2.0 CD8+ T cells/mL or <1.0 CD4+ T cells/mL) measured on day 15 were associated with a higher likelihood of CMV events (asymptomatic infection or disease).

The CMV inSIGHT T cell immunity panel from Viracor Eurofins Laboratories was recently commercialized in the USA (https://www.eurofins-viracor.com/clinical/test-menu/30360-cmv-insight-t-cell-immunity/). This assay measures CMV-specific CD4+ and CD8+ T cell responses based on the percentage of cells expressing surface CD69 and IFN-γ after stimulation with CMV epitopes (including whole viral lysate, pp65 vs. IE-1) [82]. Although further studies are needed, this assay has shown good diagnostic performance in predicting CMV events [66,67].

**Factors Influencing CMV-CMI**

It is necessary to review the factors that may affect CMV-CMI in SOT. First, it has been demonstrated that the kinetics of CMV-specific CD4+ T cell responses are different from those of CD8+ T cells [83]. The level of CMV-specific CD4+ T cells significantly decreased at 2 months post-KT compared to the pretransplantation level and gradually increased to the pretransplant level by 12 months. In contrast, it was found that the CMV-specific CD8+ T cell response decreased rapidly within the first 2 weeks post-KT, but returned to the pretransplant levels by 2 months post-KT [83]. Second, posttransplant CMV-specific T cell levels may be affected by induction immunosuppression. Abate et al. [84] reported that ATG treatment had no impact on CMV-specific T cell responses. However, recent prospective studies revealed that the ATG group showed significantly lower cellular immunity than the group of recipients treated by anti-IL 2 receptor antibodies (basiliximab) [49,50]. Calcineurin inhibitors have also been reported to have direct inhibitory effects on CMV-specific T cell reactivity [80,85]. Third, the influence of antiviral prophylaxis on CMV-CMI levels has yet to be conclusively determined [47]. In addition to promising data showing the clinical utility of CMV-CMI assessment for risk stratification, a proportion of SOT recipients (4%-10%) developed late-onset CMV disease [86,87]. This suggests that a higher cut-off for positive CMV-CMI, which can reliably predict protection against CMV disease, may be necessary, or that other aspects of the immune response, such as neutralizing antibodies, should also be considered [88]. A previous study showed that quantifying epithelial cell neutralizing antibodies was useful for identifying liver transplant recipients with lower risk for CMV disease [86].

Although many studies over the past 20 years have consistently evaluated the clinical application of CMV-CMI assays, they have not been routinely incorporated into clinical practice. The reasons are the absence of well-defined thresholds for positivity and negativity, the variability in CMV antigens in a protocol (whole cell lysate vs. peptide pools, pp65 vs. IE-1), and the heterogeneity of study populations (transplanted organ, serostatus of the donor/recipient, use of antiviral prophylaxis vs. preemptive therapy). In addition, most of the data were from observational studies, and there were only a few intervention studies in which treatments were made based on the CMV-CMI results [47].

**Recent Observational Studies of CMV-CMI Assays**

Observational studies have confirmed that CMV-CMI assays can predict the risk of subsequent CMV replication and disease development. Strong evidence indicates that assessing CMI using standardized assays such as CMV-QF and Elispot (T-Track CMV and T-SPOT.CMV) can stratify recipients according to their risk of CMV events [77].

A large, prospective observational study of KT recipients showed that CMV-specific immune assessment using the ELISpot assay can predict protection from CMV infection [49]. In 583 recipients consisting of 260 seronegative recipients with a seropositive donor (D+/R-) and 277 seropositive recipients (R+), the CMV ELISpot assay was per-

www.ekjt.org
formed at prophylaxis discontinuation and once per month for 4 months and at 6 months. CMV events were significantly lower in ELISpot-positive patients (>40 spot-forming units per 2.5 ×10^5 cells for either pp65 or IE-1) vs. negative patients (3.0% vs. 19.5%, P=0.0001). However, the positive predictive value was low (less than 20%), showing poor sensitivity for distinguishing patients at high risk of CMV replication. This study showed the possibility of performing real-time CMV-CMI in a central laboratory, and also confirmed that this assay is applicable in routine clinical practice.

Two recent studies evaluated the role of CMV-CMI in lung transplant patients, who constitute a high-risk group for CMV-related complications. The first Spanish study tested the T-SPOT.CMV assay in 60 CMV seropositive recipients at posttransplant 6 months when prophylaxis was withdrawn [89]. Recipients who developed late-onset CMV DNAemia showed significantly lower ELISpot responses, particularly to the IE-1 antigen, than patients who did not. In the second study, 39 lung transplant candidates underwent CMV-QF prior to transplantation [90]. CMV-QF negativity was associated with a higher likelihood of CMV reactivation after transplantation. This is consistent with previous studies, in which CMV-CMI at the time of transplantation had better predictive value than CMV serostatus for the development of CMV replication.

Recent Interventional Clinical Trials of CMV-CMI Assays
A few interventional clinical studies on CMV-CMI assay have been published [50,63,64,91]. The first interventional trial using CMV-CMI was a pilot nonrandomized uncontrolled trial in 27 SOT recipients with CMV replication and/or disease, in whom a CMV-QF assay was performed after 2–3 weeks of administration of antiviral therapy [64]. Fourteen patients (51.9%) had positive CMV-QF responses at the end of treatment, and the antiviral therapies were discontinued. The remaining 13 patients (48.1%) had negative results and received secondary antiviral prophylaxis for 2 months. Patients with detectable CMV-CMI had a lower rate of CMV relapse than recipients who were CMV-CMI negative and received longer-term antiviral therapy (7.1% vs. 69.2%, P=0.001). This was the first interventional study to demonstrate the validity and safety of real-time CMV-CMI assessments to guide changes in CMV management.

A second randomized controlled trial was conducted to determine the duration of antiviral prophylaxis according to the results of CMV-QF [63]. In this study, lung transplant recipients (n=118) were randomized to receive a fixed duration of prophylaxis (5 months) or a duration determined by the CMV-QF assay, performed at 5, 8 and 11 months posttransplant. The incidence of CMV infection (>600 copies/mL in BAL fluid) was significantly lower in the CMV-QF guided group than the standard of care group (36.6% vs. 58.3%, P=0.03). Of the 80 patients who ceased prophylaxis (36 in the standard-of-care group and 44 in the CMV-QF-directed group), the incidence of severe viremia (>10,000 copies/mL) was lower in recipients with positive CMV-QF than in those with negative CMV-QF (3% vs. 50%, P<0.001). CMV-CMI monitoring allows an individualized approach to CMV prophylaxis and reduces late CMV infection within the lung allograft.

A third trial used pretransplant cell-mediated immune status by means of an ELISpot-CMV to determine the risk for developing posttransplant CMV replication [50]. Using pretransplant T-SPOT.CMV (cut off, 20 spots/3×10^5 PBMCs), patients were divided into two groups: group A (low-risk T-SPOT.CMV, ≥21 spots/3×10^5 PBMCs) and group B (high-risk T-SPOT.CMV, ≤20 spots/3×10^5 PBMCs). Each group was randomized at a 1:1 ratio and divided into a 3-month antiviral drug prophylaxis group (subgroups A1 and B1) or a preemptive treatment group (subgroups A2 and B2). Patients at high risk according to the pretransplant T-SPOT.CMV results showed a higher risk of CMV infections than those at low risk for both the prophylactic (33.3% vs. 4.1%) and preemptive approach (73.3% vs. 44.4%). However, the predicted capacity of CMV-CMI to identify recipients at high risk of CMV infection was only found in patients treated with basiliximab (not receiving T cell-depleting antibodies) for both prophylactic and preemptive therapy. Furthermore, 15-day posttransplant T-SPOT.CMV was a better predictor of CMV infection than CMV-CMI measured before transplantation in basiliximab-treated patients. This study showed that CMV-CMI monitoring may guide decisions regarding the type of CMV preventive strategy in KT.

A randomized clinical trial by Singh et al. [91] compared preemptive therapy versus prophylaxis in 205 CMV seronegative liver transplant recipients from seropositive donors. Patients received either valganciclovir (900 mg) prophylaxis daily for 100 days, or valganciclovir (900 mg) twice a day if viremia was detected during weekly monitoring with CMV QNAT for 100 days. The incidence of symptomatic CMV disease was significantly lower in the preemptive group than in the prophylaxis group (9.0% vs. 19.0%, P=0.04). The incidence of opportunistic infections, rejection, graft loss, and mortality showed no differences.
between the two groups. In this study, CMV pp65 ELISpot responses were stronger after preemptive therapy than prophylaxis for both CD4+ and CD8+ T cells. Among the preemptive recipients, CD8+ T cell responses were significantly higher in patients with preceding viremia than in those without viremia.

Two additional randomized interventional trials have been completed, and we are looking forward to the upcoming results [65]. In one clinical trial (Clinicaltrials.gov identifier NCT03123627), CMV seropositive KT recipients who underwent ATG induction therapy were randomized to receive a fixed period (3 months) of valganciclovir prophylaxis or a period determined by CMV-QF results. Therapy was stopped in case of a positive CMV-QF. A second trial (Clinicaltrials.gov identifier NCT02538172) used an ELISpot (T-Track CMV) and included D+/R- and R+ kidney and liver transplant recipients receiving ATG. The control group received valganciclovir prophylaxis up to posttransplant 90 days, and the prophylaxis period of the intervention group was determined by the T-Track CMV assay. We expect these studies to support a promising role for the CMV-CMI assay in modulating the duration of antiviral prophylaxis.

ASSAYS FOR CMV DRUG MUTATION

Genotyping should be performed to detect specific drug mutations when drug-resistant CMV infection is suspected [2]. A 1-log reduction in the CMV viral load is expected when treated with an appropriate dose after at least 2 weeks. Refractory CMV infection is defined as an increase of >1 log_{10} in CMV DNA levels between baseline and after at least 2 weeks of an appropriate dose of antiviral treatment [33]. Probable refractory infection is defined as CMV DNA levels that persist (at the same level or increasing by less than 1 log_{10}) after at least 2 weeks of antiviral therapy. However, persistent CMV DNA titers less than 1,000 IU/mL, particularly detected but non-quantifiable levels (<137 IU/mL), should not be considered as refractory [33]. Genotyping is possible for viral sequences that are amplified from blood (plasma, whole blood, or leukocytes), body fluids (CSF, BAL fluid, urine, or vitreous humor), or tissue [2]. The sample should have sufficient CMV DNA levels. The results are more reliable when the CMV copy number is greater than 1,000 IU/mL [27]. The accuracy of variant subpopulation detection was lower at 1,000 copies/mL than at 10,000 copies/mL [92]. CMV genes associated with commercially available or novel antiviral agents are UL97 and UL54 for ganciclovir; UL97 and UL27 for maribavir; UL54 for cidofovir and foscarnet; and UL51, UL56, and UL89 for letermovir [33].

CONCLUSION

Advances in diagnostics have been essential for improving our understanding of CMV immunity and its role in disease. The use of sensitive CMV QNAT has become the standard of care as a means to monitor and treat CMV disease in transplant patients. CMV immune monitoring to better identify individuals at high risk for CMV-related complications is an area of continuing high clinical need and interest. With the development of standardized CMV-CMI assays such as CMV-QF and ELISpot, CMV immune monitoring is being integrated into routine clinical care, moving one step closer to personalized medicine.

ACKNOWLEDGMENTS

Conflict of Interest
Eun-Jee Oh is an editorial board member of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflict of interest relevant to this article was reported.

Funding/Support
This study was supported by research grant from the Korean Society for Transplantation (2022-00-02001-001).

ORCID
Hyeyoung Lee  https://orcid.org/0000-0001-8871-5091
Eun-Jee Oh  https://orcid.org/0000-0001-5870-915X

Author Contributions
Conceptualization: EJO. Visualization: HL. Writing–original draft: all authors. Writing–review & editing: all authors.

REFERENCES
1.  Raval AD, Kistler KD, Tang Y, Murata Y, Snydman DR.
Epidemiology, risk factors, and outcomes associated with cytomegalovirus in adult kidney transplant recipients: a systematic literature review of real-world evidence. Transpl Infect Dis 2021;23:e13483.

2. Razonable RR, Humar A. Cytomegalovirus in solid organ transplant recipients: guidelines of the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant 2019;33:e13512.

3. Kim MH, Cho MH, Ahn YH, Bae JM, Moon JS, Kang HG. Concurrent cytomegalovirus enteritis and atypical hemolytic uremic syndrome with gastrointestinal tract involvement: a case report. Korean J Transplant 2021;35:262-7.

4. Kaminski H, Fishman JA. The cell biology of cytomegalovirus: implications for transplantation. Am J Transplant 2016;16:2254-69.

5. Navarro D. Expanding role of cytomegalovirus as a human pathogen. J Med Virol 2016;8:1103-12.

6. Razonable RR, Humar A; AST Infectious Diseases Community of Practice. Cytomegalovirus in solid organ transplantation. Am J Transplant 2013;13 Suppl 4:93-106.

7. Zuhair M, Smit GS, Wallis G, Jabbar F, Smith C, Devleesschauwer B, et al. Estimation of the worldwide seroprevalence of cytomegalovirus: a systematic review and meta-analysis. Rev Med Virol 2019;29:e2034.

8. Choi SR, Kim KR, Kim DS, Kang JM, Kim SJ, Kim JM, et al. Changes in cytomegalovirus seroprevalence in Korea for 21 years: a single center study. Pediatr Infect Vaccine 2018;25:123-31.

9. La Y, Kwon DE, Yoo SG, Lee KH, Han SH, Song YG. Human cytomegalovirus seroprevalence and titres in solid organ transplant recipients and transplant donors in Seoul, South Korea. BMC Infect Dis 2019;19:948.

10. Munoz-Price LS, Slifkin M, Ruthazer R, Poutsiaka DD, Hadley S, Freeman R, et al. The clinical impact of ganciclovir prophylaxis on the occurrence of bactemia in orthotopic liver transplant recipients. Clin Infect Dis 2004;39:1293-9.

11. George MJ, Snydman DR, Werner BG, Griffith J, Falagas ME, Dougherty NN, et al. The independent role of cytomegalovirus as a risk factor for invasive fungal disease in orthotopic liver transplant recipients. Boston Center for Liver Transplantation CMVIG-Study Group. Cytoem, MedImmune, Inc. Gaithersburg, Maryland. Am J Med 1997;103:106-13.

12. Walker RC, Marshall WF, Strickler JG, Wiesner RH, Velosa JA, Habermann TM, et al. Pretransplantation assessment of the risk of lymphoproliferative disorder. Clin Infect Dis 1995;20:1346-53.

13. Park WY, Kim Y, Paek JH, Jin K, Park SB, Han S. Effectiveness of valacyclovir prophylaxis against the occurrence of cytomegalovirus infection in kidney transplant recipients. Korean J Transplant 2020;34:15-23.

14. Helanterä I, Lautenschlager I, Koskinen P. The risk of cytomegalovirus recurrence after kidney transplantation. Transpl Int 2011;24:1170-8.

15. Kliem V, Fricke L, Wollbrink T, Burg M, Radermacher J, Rohde F. Improvement in long-term renal graft survival due to CMV prophylaxis with oral ganciclovir: results of a randomized clinical trial. Am J Transplant 2008;8:975-83.

16. Arthurs SK, Eid AJ, Pedersen RA, Kremers WK, Cosio FG, Patel R, et al. Delayed-onset primary cytomegalovirus disease and the risk of allograft failure and mortality after kidney transplantation. Clin Infect Dis 2008;46:840-6.

17. Witzke O, Hauser IA, Bartels M, Wolf G, Wolters H, Nitschke M, et al. Valganciclovir prophylaxis versus preemptive therapy in cytomegalovirus-positive renal allograft recipients: 1-year results of a randomized clinical trial. Transplantation 2012;93:61-8.

18. Zamora MR. Controversies in lung transplantation: management of cytomegalovirus infections. J Heart Lung Transplant 2002;21:841-9.

19. Potena L, Valantine HA. Cytomegalovirus-associated allograft rejection in heart transplant patients. Curr Opin Infect Dis 2007;20:425-31.

20. Valantine H. Cardiac allograft vasculopathy after heart transplantation: risk factors and management. J Heart Lung Transplant 2004;23(5 Suppl):S187-93.

21. Beam E, Lesnick T, Kremers W, Kennedy CC, Razonable RR. Cytomegalovirus disease is associated with higher all-cause mortality after lung transplantation despite extended antiviral prophylaxis. Clin Transplant 2016;30:270-8.

22. Ljungman P, Boeckh M, Hirsch HH, Josephson F, Lundgren J, Nichols G, et al. Definitions of cytomegalovirus infection and disease in transplant patients for use in clinical trials. Clin Infect Dis 2017;64:87-91.

23. Hakimi Z, Aballéa S, Ferchichi S, Schram M, Odeyemi IA, Tournu M, et al. Burden of cytomegalovirus disease in solid organ transplant recipients: a national matched cohort study in an inpatient setting. Transpl Infect Dis 2017;19:e12732.

24. Han SH, Yoo SG, Do Han K, La Y, Kwon DE, Lee KH. The
incidence and effect of cytomegalovirus disease on mortality in transplant recipients and general population: real-world nationwide cohort data. Int J Med Sci 2021;18:3333-41.

25. Razonable RR, Paya CV, Smith TF. Role of the laboratory in diagnosis and management of cytomegalovirus infection in hematopoietic stem cell and solid-organ transplant recipients. J Clin Microbiol 2002;40:746-52.

26. Razonable RR, Inoue N, Pinninti SG, Bobpapa SB, Lazzarotto T, Gabrielli L, et al. Clinical diagnostic testing for human cytomegalovirus infections. J Infect Dis 2020;221(Suppl 1):S74-85.

27. Kotton CN, Kumar D, Caliendo AM, Huprikar S, Chou S, Danziger-Isakov L, et al. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation 2018;102:900-31.

28. Razonable RR, Hayden RT. Clinical utility of viral load in management of cytomegalovirus infection after solid organ transplantation. Clin Microbiol Rev 2013;26:703-27.

29. Hirsch HH, Lautenschlager I, Pinsky BA, Cardeñoso L, Aslam S, Cobb B, et al. An international multicenter performance analysis of cytomegalovirus load tests. Clin Infect Dis 2013;56:367-73.

30. Food and Drug Administration (FDA). Nucleic acid based tests [Internet]. Silver Spring, MD: FDA; 2021 [cited 2021 Dec 16]. Available from: https://www.fda.gov/medical-devices/vitro-diagnostics/nucleic-acid-based-tests.

31. Beam E, Germer JJ, Lahr B, Yao JD, Limper AH, Binnicker MJ, et al. Cytomegalovirus (CMV) DNA quantification in bronchoalveolar lavage fluid of immunocompromised patients with CMV pneumonia. Clin Transplant 2018;32:e13149.

32. Boeckh M, Stevens-Ayers T, Travi G, Huang ML, Cheng GS, Xie H, et al. Cytomegalovirus (CMV) DNA quantitation in bronchoalveolar lavage fluid from hematopoietic stem cell transplant recipients with CMV pneumonia. J Infect Dis 2017;215:1514-22.

33. Chemaly RF, Chou S, Einsele H, Griffiths P, Avery R, Razonable RR, et al. Definitions of resistant and refractory cytomegalovirus infection and disease in transplant recipients for use in clinical trials. Clin Infect Dis 2019;68:1420-6.

34. Fryer JF, Heath AB, Minor PD; Collaborative Study Group. A collaborative study to establish the 1st WHO International Standard for human cytomegalovirus for nucleic acid amplification technology. Biologicals 2016;44:242-51.

35. Sidoti F, Piralla A, Costa C, Scarasciulli ML, Calvario A, Conaldi PG, et al. Collaborative national multicenter for the identification of conversion factors from copies/mL to international units/mL for the normalization of HCMV DNA load. Diagn Microbiol Infect Dis 2019;95:152-8.

36. Preiksaitis JK, Hayden RT, Tong Y, Pang XL, Fryer JF, Heath AB, et al. Are we there yet? Impact of the first international standard for cytomegalovirus DNA on the harmonization of results reported on plasma samples. Clin Infect Dis 2020;63:583-9.

37. Naegele K, Lautenschlager I, Gosert R, Loginov R, Bir K, Helanterä I, et al. Cytomegalovirus sequence variability, amplicon length, and DNase-sensitive non-encapsidated genomes are obstacles to standardization and commutability of plasma viral load results. J Clin Virol 2018;104:39-47.

38. Ljungman P, de la Camara R, Robin C, Crocchiolo R, Einsele H, Hill JA, et al. Guidelines for the management of cytomegalovirus infection in patients with haematological malignancies and after stem cell transplantation from the 2017 European Conference on Infections in Leukaemia (ECIL 7). Lancet Infect Dis 2019;19:e260-72.

39. Grossi PA, Baldanti F, Andreoni M, Perno CF. CMV infection management in transplant patients in Italy. J Clin Virol 2020;123:104211.

40. Natori Y, Alghamdi A, Tazari M, Miller V, Husain S, Komatsu T, et al. Use of viral load as a surrogate marker in clinical studies of cytomegalovirus in solid organ transplantation: a systematic review and meta-analysis. Clin Infect Dis 2018;66:617-31.

41. Hayden RT, Gu Z, Sam SS, Sun Y, Tang L, Pounds S, et al. Comparative performance of reagents and platforms for quantitation of cytomegalovirus DNA by digital PCR. J Clin Microbiol 2016;54:2602-8.

42. Sedlak RH, Kuypers J, Jerome KR. A multiplexed droplet digital PCR assay performs better than qPCR on inhibition prone samples. Diagn Microbiol Infect Dis 2014;80:285-6.

43. Sedlak RH, Cook L, Cheng A, Magaret A, Jerome KR. Clinical utility of droplet digital PCR for human cytomegalovirus. J Clin Microbiol 2014;52:2844-8.

44. Limaye AP, Babu TM, Boeckh M. Progress and challenges in the prevention, diagnosis, and management of cytomegalovirus infection in transplantation. Clin...
45. Limaye AP, Santo Hayes TK, Huang ML, Magaret A, Boeckh M, Jerome KR. Quantitation of cytomegalovirus DNA load in dried blood spots correlates well with plasma viral load. J Clin Microbiol 2013;51:2360-4.

46. Kwon S, Jung BK, Ko SY, Lee CK, Cho Y. Comparison of quantitation of cytomegalovirus DNA by real-time PCR in whole blood with the cytomegalovirus antigenemia assay. Ann Lab Med 2015;35:99-104.

47. Prakash K, Chandorkar A, Saharia KK. Utility of CMV-specific immune monitoring for the management of CMV in solid organ transplant recipients: a clinical update. Diagnostics (Basel) 2021;11:875.

48. Kirisri S, Vongsakulyanon A, Kantachuvesiri S, Razonable RR, Brumhinit J. Predictors of CMV infection in CMV-seropositive kidney transplant recipients: impact of pretransplant CMV-specific humoral immunity. Open Forum Infect Dis 2021;8:ofab199.

49. Kumar D, Chin-Hong P, Kayler L, Wojciechowski D, Limaye AP, Osama Gaber A, et al. A prospective multicenter observational study of cell-mediated immunity as a predictor for cytomegalovirus infection in kidney transplant recipients. Am J Transplant 2019;19:2505-16.

50. Jarque M, Crespo E, Melilli E, Gutiérrez A, Moreso F, Guirado L, et al. Cellular immunity to predict the risk of cytomegalovirus infection in kidney transplantation: a prospective, interventional, multicenter clinical trial. Clin Infect Dis 2020;71:2375-85.

51. Costa C, Balloco C, Sidoti F, Mantovani S, Rittà M, Piceghello A, et al. Evaluation of CMV-specific cellular immune response by Elispot assay in kidney transplant patients. J Clin Virol 2014;61:523-8.

52. Rittà M, Costa C, Sidoti F, Ballocco C, Ranghino A, Mespina M, et al. Pre-transplant assessment of CMV-specific immune response by Elispot assay in kidney transplant recipients. New Microbiol 2015;38:329-35.

53. Kim SH, Lee HJ, Kim SM, Jung JH, Shin S, Kim YH, et al. Diagnostic usefulness of cytomegalovirus (CMV)-specific T cell immunity in predicting CMV infection after kidney transplantation: a pilot proof-of-concept study. Infect Chemother 2015;47:105-10.

54. Lee H, Park KH, Ryu JH, Choi AR, Yu JH, Lim J, et al. Cytomegalovirus (CMV) immune monitoring with ELISPot and QuantiFERON-CMV assay in seropositive kidney transplant recipients. PLoS One 2017;12:e0189488.

55. Schachtner T, Stein M, Reinke P. CMV-specific T cell monitoring offers superior risk stratification of CMV-seronegative kidney transplant recipients of a CMV-seropositive donor. Transplantation 2017;101:e315-25.

56. De Gracia-Guindo MD, Ruiz-Fuentes MD, Galindo-Sacristán P, Osorio-Moratalla JM, Ruiz-Fuentes N, Rodríguez Granger J, et al. Cytomegalovirus infection monitoring based on interferon gamma release assay in kidney transplantation. Transplant Proc 2018;50:578-80.

57. Gliga S, Korth J, Krawczyk A, Wilde B, Horn PA, Witzke O, et al. T-Track-CMV and QuantiFERON-CMV assays for prediction of protection from CMV reactivation in kidney transplant recipients. J Clin Virol 2018;105:91-6.

58. Chiereghin A, Potena L, Borgese L, Gibertoni D, Squarzon D, Turello G, et al. Monitoring of cytomegalovirus (CMV)-specific cell-mediated immunity in heart transplant recipients: clinical utility of the QuantiFERON-CMV assay for management of posttransplant CMV infection. J Clin Microbiol 2018;56:e01040-17.

59. Sood S, Haifer C, Yu L, Pavlovic J, Gow PJ, Jones RM, et al. Early viral-specific T-cell testing predicts late cytomegalovirus reactivation following liver transplantation. Transpl Infect Dis 2018;20:e12934.

60. Deborska-Materkowska D, Perkowski-Ptasinska A, Sadowska A, Godzowska J, Ciszek M, Serwanska-Swietek M, et al. Diagnostic utility of monitoring cytomegalovirus-specific immunity by QuantiFERON-cytomegalovirus assay in kidney transplant recipients. BMC Infect Dis 2018;18:179.

61. Páez-Vega A, Poyato A, Rodriguez-Benot A, Guirado L, Fortún J, Len O, et al. Analysis of spontaneous resolution of cytomegalovirus replication after transplantation in CMV-seropositive patients with pretransplant CD8+IFNG+ response. Antiviral Res 2018;155:97-105.

62. Thompson G, Boan P, Baumwol J, Chakera A, MacQuillan G, Swaminathan S, et al. Analysis of the QuantiFERON-CMV assay, CMV viraemia and antiviral treatment following solid organ transplantation in Western Australia. Pathology 2018;50:554-61.

63. Westall GP, Cristiano Y, Levvey BJ, Whitford H, Paraskeva MA, Paul E, et al. A randomized study of Quantiferon CMV-directed versus fixed-duration valganciclovir prophylaxis to reduce late CMV after lung transplantation. Transplantation 2019;103:1005-13.

64. Kumar D, Mian M, Singer L, Humar A. An intervention study using cell-mediated immunity to personalize therapy for cytomegalovirus infection after transplantation. Am J Transplant 2017;17:2468-73.
65. Rezahosseini O, Möller DL, Knudsen AD, Sørensen SS, Perch M, Gustafsson F, et al. Use of T cell mediated immune functional assays for adjustment of immunosuppressive or anti-infective agents in solid organ transplant recipients: a systematic review. Front Immunol 2020;11:567715.

66. Rogers R, Saharia K, Chandorkar A, Weiss ZF, Vieira K, Koo S, et al. Clinical experience with a novel assay measuring cytomegalovirus (CMV)-specific CD4+ and CD8+ T-cell immunity by flow cytometry and intracellular cytokine staining to predict clinically significant CMV events. BMC Infect Dis 2020;20:58.

67. Jorgenson MR, Hillis MI, Saddler CM, Smith JA, Parajuli S, Mandelbrot DA. Prediction of cytomegalovirus infection: a single-center experience utilizing a newly available cell-mediated immunity assay by flow cytometry, a risk factor screening tool, and serologically demonstrated immunity. Transpl Infect Dis 2020;22:e13311.

68. Walker S, Fazou C, Crough T, Holdsworth R, Kiely P, Veale M, et al. Ex vivo monitoring of human cytomegalovirus-specific CD8+ T-cell responses using QuantiFERON-CMV. Transpl Infect Dis 2007;9:165-70.

69. Abate D, Saldan A, Mengoli C, Fiscon M, Silvestre C, Fallico L, et al. Comparison of cytomegalovirus (CMV) enzyme-linked immunosorbent spot and CMV quantiferon gamma interferon-releasing assays in assessing risk of CMV infection in kidney transplant recipients. J Clin Microbiol 2013;51:2501-7.

70. Cantisán S, Lara R, Montejo M, Redel J, Rodríguez-Benot A, Gutiérrez-Aroca J, et al. Pretransplant interferon-γ-secretion by CMV-specific CD8+ T cells informs the risk of CMV replication after transplantation. Am J Transplant 2013;13:738-45.

71. Valle-Arroyo J, Aguado R, Páez-Vega A, Pérez AB, González R, Fernández-Ruiz M, et al. Lack of cytomegalovirus (CMV)-specific cell-mediated immune response using QuantiFERON-CMV assay in CMV-seropositive healthy volunteers: fact not artifact. Sci Rep 2020;10:7194.

72. Jackson SE, Sedikides GX, Mason GM, Okecha G, Wills MR. Human cytomegalovirus (HCMV)-specific CD4 + T cells are polyfunctional and can respond to HCMV-infected dendritic cells in vitro. J Virol 2017;91:e02128-16.

73. Tey SK, Kennedy GA, Cromer D, Davenport MP, Walker S, Jones LI, et al. Clinical assessment of anti-viral CD8+ T cell immune monitoring using QuantiFERON-CMV® assay to identify high risk allogeneic hematopoietic stem cell transplant patients with CMV infection complications. PLoS One 2013;8:e74744.

74. Yong MK, Cameron PU, Slavin MA, Cheng AC, Morrissey CO, Bergin K, et al. Low T-cell responses to mitogen stimulation predicts poor survival in recipients of allogeneic hematopoietic stem cell transplantation. Front Immunol 2017;8:1506.

75. Barabas S, Spindler T, Kiener R, Tonar C, Lugner T, Batzilia J, et al. An optimized IFN-γELSpot assay for the sensitive and standardized monitoring of CMV protein-reactive effector cells of cell-mediated immunity. BMC Immunol 2017;18:14.

76. Tanguay S, Killion JJ. Direct comparison of ELISPOT and ELISA-based assays for detection of individual cytokine-secreting cells. Lymphokine Cytokine Res 1994;13:259-63.

77. Yong MK, Lewin SR, Manuel O. Immune monitoring for CMV in transplantation. Curr Infect Dis Rep 2018;20:4.

78. Gerna G, Lilleri D, Formaro F, Comolli G, Lozza L, Campana C, et al. Monitoring of human cytomegalovirus-specific CD4 and CD8 T-cell immunity in patients receiving solid organ transplantation. Am J Transplant 2006;6:2356-64.

79. Gerna G, Lilleri D, Chiesa A, Zelini P, Furione M, Comolli G, et al. Virologic and immunologic monitoring of cytomegalovirus to guide preemptive therapy in solid-organ transplantation. Am J Transplant 2011;11:2463-71.

80. Sester U, Gärtner BC, Wilkens H, Schwaab B, Wössner R, Kindermann I, et al. Differences in CMV-specific T-cell levels and long-term susceptibility to CMV infection after kidney, heart and lung transplantation. Am J Transplant 2005;5:1483-9.

81. Fernández-Ruiz M, Giménez E, Vinuesa V, Ruiz-Merlo T, Parra P, Amat P, et al. Regular monitoring of cytomegalovirus-specific cell-mediated immunity in intermediate-risk kidney transplant recipients: predictive value of the immediate post-transplant assessment. Clin Microbiol Infect 2019;25:381.

82. Lutgen CB, Flebbe-Rehwaldt L, Kleiboeker S, Fausett S, Corde C, Rodgers J, et al. T-cell immunity panel measures CMV-specific CD4 and CD8 T-cell responses. Open Forum Infect Dis 2017;4(Suppl 1):S615.

83. Sester M, Sester U, Gärtner B, Heine G, Girndt M, Mueller-Lantzsch N, et al. Levels of virus-specific CD4 T cells correlate with cytomegalovirus control and predict virus-induced disease after renal transplantation. Transplantation 2001;71:1287-94.

84. Abate D, Saldan A, Fiscon M, Cofano S, Paciolla A, Furian L, et al. Evaluation of cytomegalovirus (CMV-
V)-specific T cell immune reconstitution revealed that baseline antiviral immunity, prophylaxis, or preemptive therapy but not antithymocyte globulin treatment contribute to CMV-specific T cell reconstitution in kidney transplant recipients. J Infect Dis 2010;202:585-94.

85. San-Juan R, Navarro D, García-Reyne A, Montejo M, Muñoz P, Carratala J, et al. Effect of long-term prophylaxis in the development of cytomegalovirus-specific T-cell immunity in D+/R- solid organ transplant recipients. Transpl Infect Dis 2015;17:637-46.

86. Blanco-Lobo P, Cordero E, Martín-Gandul C, Gentil MA, Suárez-Artacho G, Sobrino M, et al. Use of antibodies neutralizing epithelial cell infection to diagnose patients at risk for CMV Disease after transplantation. J Infect 2016;72:597-607.

87. Kumar D, Chernenko S, Moussa G, Cobos I, Manuel O, Preiksaitis J, et al. Cell-mediated immunity to predict cytomegalovirus disease in high-risk solid organ transplant recipients. Am J Transplant 2009;9:1214-22.

88. Navarro D, Fernández-Ruiz M, Aguado JM, Sandonis V, Pérez-Romero P. Going beyond serology for stratifying the risk of CMV infection in transplant recipients. Rev Med Virol 2019;29:e2017.

89. Donadeu L, Revilla-López E, Jarque M, Crespo E, Torija A, Bravo C, et al. CMV-specific cell-mediated immunity predicts a high level of CMV replication after prophylaxis withdrawal in lung transplant recipients. J Infect Dis 2021;224:526-31.

90. Altaf M, Lineburg KE, Crooks P, Rehan S, Matthews KK, Neller MA, et al. Pretransplant cytomegalovirus-specific cellular immunity and risk of viral reactivation following lung transplantation: a prospective cohort study. J Infect Dis 2021;224:312-7.

91. Singh N, Winston DJ, Razonable RR, Lyon GM, Silveira FP, Wagener MM, et al. Effect of preemptive therapy vs antiviral prophylaxis on cytomegalovirus disease in seronegative liver transplant recipients with seropositive donors: a randomized clinical trial. JAMA 2020;323:1378-87.

92. Sahoo MK, Lefterova MI, Yamamoto F, Waggoner JJ, Chou S, Holmes SP, et al. Detection of cytomegalovirus drug resistance mutations by next-generation sequencing. J Clin Microbiol 2013;51:3700-10.