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MINIREVIEW

Role of the Laboratory in Diagnosis and Management of Cytomegalovirus Infection in Hematopoietic Stem Cell and Solid-Organ Transplant Recipients

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Solid-organ and hematopoietic stem cell transplantation have become vital in the treatment of many illnesses that were previously considered fatal. In 1999, a total of 21,516 solid-organ transplant procedures were performed in the United States (Scientific registry, United Network for Organ Sharing). The use of immunosuppressive agents is essential in maintaining allograft function following organ transplantation. However, the use of these agents predisposes the transplant recipients to opportunistic infections (40).

One of the most common complications following organ transplantation is infection with cytomegalovirus (CMV), a ubiquitous member of the β-herpesvirus family. CMV infection occurs in the majority of humans, mainly during the first two decades of life. The main host defense against CMV is cell-mediated immunity; however, virus-specific antibodies may also modify the disease caused by this virus. Following primary infection, CMV is maintained in a latent state by integration within the host cell chromosome or by persistent low-level viral replication that is adequately controlled by a functioning immune system. Consequently, the dysfunction of the immune system will allow for increased levels of CMV replication; this is further amplified by circumstances that result in viral reactivation from latency, many of which are present following transplantation (1, 7, 33, 39, 40). The deleterious effects of CMV in transplant recipients result from direct viral invasion of various organ systems (resulting in, e.g., pneumonitis, enterocolitis, encephalitis, or retinitis) following dissemination of the virus in the blood (viremia). The most serious forms of CMV disease occur in CMV-seronegative recipients of solid organs obtained from CMV-infected donors (i.e., persons whose CMV serostatus is defined as CMV D+/R−, where D+ and R− denote donor positive and recipient negative, respectively) and in CMV-seropositive hematopoietic stem cell transplant recipients (39). Additionally, CMV influences allograft dysfunction, accelerates graft coronary artery atherosclerosis, and increases opportunistic infections (33). Hence, CMV leads to increased resource utilization and total transplantation cost. Indeed, the impact of CMV on transplantation outcomes is so enormous that it is considered the most important infectious cause of posttransplant morbidity and mortality.

There have been considerable efforts in improving posttransplant CMV disease prevention and management. Currently the treatment of CMV disease solid-organ transplant recipients consists of the administration of intravenous ganciclovir (5 mg/kg of body weight every 12 h, adjusted based on renal function) for 2 to 4 weeks. As will be discussed, the duration of treatment may be tailored as a function of the level of viral replication. In addition, CMV immunoglobulin or polyvalent immunoglobulin is used in hematopoietic stem cell transplant recipients. The strategies aimed at CMV prevention include the use of universal prophylaxis (directed to all transplant recipients or to predisposed individuals) and the use of preemptive therapy (guided by the detection of CMV viremia prior to onset of disease) (2, 11, 20, 32). With the widespread use of currently available antiviral agents (e.g., ganciclovir and valacyclovir), whether universally or preemptively, there has been a decrease in incidence of CMV disease during the early posttransplant period (11, 20). However, CMV disease still occurs after the completion of oral antiviral prophylaxis, particularly among solid-organ transplant recipients who develop allograft rejection (39) and was observed among hematopoietic stem cell transplant recipients who developed graft-versus-host disease or who required prolonged antiviral prophylaxis during the early posttransplant period (29).

The success of CMV prevention during the early posttransplant period and the improvement of CMV disease management are partly attributed to the advancement in diagnostic virology. The development and the widespread implementation of sensitive, specific, and reliable diagnostic assays for CMV detection has been essential in achieving these goals. Moreover, the introduction of viral load quantification has greatly advanced the clinical utility of diagnostic virology. In this review, we will evaluate the clinical applications of the laboratory methods for the diagnosis of CMV infection, with particular emphasis on the recent availability of quantitative assays and the continued utility of conventional assays (Table 1). In addition, we will discuss future directions in the field of CMV diagnosis and assess their potential impact on CMV disease prevention and management following transplantation.

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TABLE 1. Laboratory methods for the diagnosis of CMV infection[a]

Method type, assay type, and assay	Principle	Sample processing and equipments	Turnaround time	Results and clinical utility	Advantages	Disadvantages
Nonmolecular						
Serology	Antibody (IgG or IgM) detection	Serology facility	6 h	IgG detection indicates prior CMV infection; IgM detection implies acute or recent infection	Prognostic evaluation of patients prior to transplant; screening posttransplantation for evidence of seroconversion	Requires acute and convalescent phase sera
Virus cultures						
Tube culture	Viral replication	Recovery of PMN within a few hours; cell culture facility; light microscopy	2 to 4 weeks	Characteristic CPE	Specific for CMV infection; virus would be available for phenotypic susceptibility testing	Long processing time; low sensitivity; rapid loss of CMV activity; Very slow CPE
Shell vial assay	Viral replication	Recovery of PMN within a few hours; cell culture facility; immunofluorescence detection	16 to 48 h	Infectious foci detected by monoclonal antibody directed to immediate-early antigen (72 kDa) of CMV	Specific for CMV infection; more sensitive and more rapid than conventional tube cultures	Relatively low sensitivity compared to molecular methods; rapid decrease of CMV activity in clinical specimens; Risk of cell toxicity with blood samples; not suited for large number of samples
Antigenemia	pp65 antigen	Recovery of PMN within 4 to 6 h; Cytospin; light microscopy or immunofluorescence	6 h	Number of CMV-infected cells per total cells evaluated; early detection of CMV replication	Rapid diagnosis of CMV; can be utilized by medium-size laboratories; quantification used as a guide for preemptive therapy	Subjective interpretation of results; requires rapid processing; role in assessing antiviral response is debated
Molecular						
COBAS AMPLICOR CMV MONITOR	DNA	Plasma sample recommended by manufacturer; COBAS AMPLICOR instrument; reagents supplied by manufacturer	4 h	Reported in numbers of CMV copies per milliliter of plasma (lowest limit, 400 copies/ml); detection of CMV infection; monitor CMV DNA decline; surrogate marker for antiviral drug resistance	Highly sensitive and specific; detects even low levels of CMV DNA; could be used to monitor therapeutic response; rapid turnaround time	
LightCycler	DNA	Various blood components; LightCycler instrument; nucleic acid extraction kit (e.g., IsoQuick)	1 to 2 h (45 min for PCR amplification and detection)	Viral genomic copies per PCR input; detection of CMV infection even at low levels; monitor CMV DNA decline; surrogate marker for antiviral drug resistance	Highly sensitive and specific; detects even low levels of CMV DNA; could be used to monitor therapeutic response; rapid turnaround time; closed amplification and detection to prevent carry-over amplicon contamination	
NucliSens pp67	mRNA	Whole blood is recommended by manufacturer; sample needs to be processed within 24 h (or in lysis buffer at −80°C indefinitely)	6 h	Qualitative assay; early detection of CMV replication (lowest limit of detection, approximately 700 RNA molecules)	Specific for viral replication; clinical utility for preemptive treatment still under investigation	Qualitative assay; less sensitive than DNA assays; clinical utility for preemptive treatment is under investigation
Digene assay	DNA-RNA hybrid	Whole blood; delayed processing possible	6 h	Number of CMV copies per milliliter (lowest limit of detection, 7×10^2 copies per ml of whole blood)	Specific for viral replication; rapid procedure; simple processing of samples; clinical utility under investigation	Clinical utility for preemptive treatment still under investigation

[a] CPE, cytopathic effects.

NONMOLECULAR METHODS

Serology. Humoral response to CMV infection is manifested by the production of CMV-specific antibodies. Immunoglobulin M (IgM) antibody against CMV occurs early (e.g., within 2 to 4 weeks following primary infection), and IgG antibody production occurs soon thereafter; both can be detected by a variety of methods (15, 17).

In the field of transplantation, CMV serology is not indicated to diagnose CMV disease, as CMV infection is widely prevalent and the majority of adults are thus seropositive (IgG) for CMV. Furthermore, the time lag between primary infection and IgM production, the persistence of IgM antibody in some healthy individuals, and the failure of some transplant recipients (e.g., hematopoietic stem cell recipients) to produce IgM antibody significantly decrease the clinical utility of serology in diagnosing CMV disease (15, 17).

Nevertheless, CMV serology remains an integral part in the clinical management of transplant patients, identifying the organ donors and recipients that have been previously infected. This is pertinent during the initial evaluation of patients in order to assess their risk of posttransplant CMV reactivation and disease. For example, CMV D+/R− solid-organ transplant patients and CMV-seropositive hematopoietic stem cell recipients are at highest risk for developing CMV disease. Serology is also useful in screening blood products so that CMV D−/R− patients do not receive blood products from CMV-seropositive blood donors. Furthermore, serologic tests will identify CMV D+/R− patients who developed asymptomatic primary CMV infection during the posttransplant period (39); this is particularly relevant since the demonstration of asymptomatic CMV seroconversion at the end of antiviral prophylaxis identifies CMV D+/R− patients who do not require additional antiviral prophylaxis in order to prevent the occurrence of late-onset CMV disease (39).

Detection of cytomegalovirus. CMV infection following transplantation is characterized by its initial reactivation from latency and increased viral replication within various lymphoid-rich organs, with subsequent dissemination through the bloodstream to distant organs. Thus, the diagnostic assays currently utilized in the management of posttransplant CMV infection rely on the isolation and/or detection of CMV in blood and in target organs. These assays include the recovery of the virus using cell culture techniques or the demonstration of viral nucleic acid (e.g., by PCR assay) and viral proteins (e.g., by antigenemia assay) in clinical specimens. Depending on the organ involved, the clinical specimens for CMV detection could include tissue (liver, kidney, intestine, lung, or brain) or body fluids and secretions (e.g., blood, bronchial washings, or cerebrospinal fluid). The detection of CMV in the urine (viruria) usually represents viral shedding and has poor correlation with CMV disease (32). However, it can be the first evidence of CMV replication in CMV D+/R− transplant recipients (1). In contrast, CMV viremia serves as a marker of active viral replication in target organs or of impending disseminated disease (1). Compared to its different primary components (e.g., plasma, peripheral blood leukocytes [PBL], and peripheral blood mononuclear cells [PBMC]), whole blood was found to be superior to plasma for quantitation of CMV DNA by PCR assay, while PBL fractions were equivalent to PBMC (37).

(i) Viral cultures. Recovery of replicating CMV by cell culture (conventional tube and shell vial assay) has traditionally been the standard method for the diagnosis of CMV infection. Almost 20 years ago, an innovation of the rapid shell vial assay, the use of monoclonal antibody directed to early antigens of replicating viruses, reduced the turnaround time to 16 h, compared to days and sometimes weeks using conventional tube cell cultures (12, 30, 34, 41).

The detection of CMV in cell cultures has a high correlation with CMV disease; nevertheless, the low sensitivity of this technology limits its value in guiding preemptive prevention protocols which require the detection of lower levels of CMV replication (1). The leukocyte fraction of blood provides optimal sensitivity for the isolation of CMV in cell cultures; however, patients such as hematopoietic stem cell transplant recipients are frequently neutropenic for a considerable period following their transplant. In addition, the assays (especially conventional tube cell cultures) are cumbersome and time-consuming, which limits their utility as rapid laboratory tests for confirming the diagnosis of both the typical and the nonspecific presentations of CMV infection.

Despite these disadvantages, shell vial culture assay remains clinically useful in centers where molecular techniques for CMV detection or antigenemia detection (see below) are not available. The culture-based technology has also been useful for antiviral phenotypic susceptibility testing and in assessing the clinical significance of genotypic mutations (46) (see below).

(ii) Antigenemia assays. The antigenemia assay is a rapid quantitative method that detects CMV antigens by directly immunostaining polymorphonuclear leukocytes (PMN) from blood specimens with monoclonal antibodies directed against the CMV lower-matrix protein pp65 (UL83). Quantitative results are expressed as the number of CMV-infected PMN per number of cells evaluated.

Antigenemia assays do not rely on the presence of replicating virus and are more sensitive than the conventional and shell vial cell cultures (47). The antigenemia assay is highly specific for CMV; however, the result is better interpreted in a quantitative manner in order to predict and diagnose CMV disease (e.g., a higher degree of antigenemia is correlated with active disease). The clinically relevant threshold of the number of infected PMN differs among the different patient populations. Thresholds of more than 10 positive cells per 2×10^5 cells and of $\geq$1 to 2 positive cells per 2×10^5 cells have been suggested to guide preemptive therapy in solid-organ and hematopoietic stem cell transplant recipients, respectively (13). Many centers have adopted the use of these assays as aids in guiding the administration of preemptive CMV therapy (3, 47). However, they confer lower sensitivity than techniques based on molecular amplification and have inherent variability resulting from the lack of standardization. In addition, the clinical utility of these assays in assessing antiviral therapy response is debated (3, 14).

There are several clinical limitations to the use of antigenemia assays, including (i) the need for immediate processing of samples after collection, (ii) the nonstandardized technical aspects of sample processing, (iii) the time consuming and cumbersome nature of the procedure, and (iv) the subjective nature of quantification (e.g., results are highly dependent on the experience of the operator). Furthermore, the detection of

antigenemia is only applicable to blood specimens and it requires an adequate number of PMN (i.e., absolute count of 0.2 $\times 10^9$ neutrophils/l); this may not always be possible among neutropenic hematopietic stem cell transplant patients. The lack of standardization among laboratories also limits the applicability and reproducibility of the results.

MOLECULAR METHODS

Nucleic acid amplification. The widespread application of PCR technology for the detection of viral nucleic acid (DNA or RNA) is the most important innovation in laboratory diagnosis and clinical management of posttransplant CMV infection.

"Home-brew" or in house CMV PCR testing with conventional thermocycling instruments that are programmed for nucleic acid amplification for 40 to 45 cycles followed by gel electrophoresis of the PCR-amplified products and probe hybridization techniques is used by many centers. The assay is labor intensive and the turnaround time is not significantly reduced compared to that for shell vial culture or antigenemia assay. The home-brew PCR assay is highly sensitive but has a low predictive value, and the results are reported qualitatively as either positive or negative (6, 31) or quantitatively (25). There is a wide variability of the techniques utilized by different centers (e.g., differences in primers, concentrations of reagents, and cycling parameters) so that the results are not widely reproducible among different laboratories and centers (6, 25, 27, 31).

The recent availability of automated PCR instruments that involve rapid thermocycling formats with the capacity for real-time quantification of viral genomes has significantly changed the practice of CMV diagnosis. The COBAS AMPLICOR CMV MONITOR assay (Roche Diagnostics, Pleasanton, Calif.) was designed to detect CMV DNA by amplifying a segment of the CMV DNA polymerase gene UL54 within a turnaround time of approximately 3 to 4 h (37, 42). The assay is standardized, offering the advantage of reproducibility of results among many centers. With the LightCycler system (Roche Molecular Biochemicals, Indianapolis, Ind.) results can be obtained rapidly (within 30 to 40 min); the system offers automation of PCR by precise air-controlled temperature cycling and provides continuous monitoring of amplicon development by a fluorometer (fluorescence resonance energy transfer) in a closed system (36). When clinical samples collected during 19 episodes of CMV infection were analyzed using the LightCycler system, the results obtained closely correlated with those obtained with the COBAS AMPLICOR CMV MONITOR assay (36). In addition, the LightCycler assay can analyze more samples per run (32 compared to 24) more quickly (240 min compared to 460 min) than the COBAS AMPLICOR CMV MONITOR assay (36); this may offer an advantage to high-volume laboratories that process a large number of clinical samples.

The quantification of CMV viral load has assisted clinicians in accurately diagnosing and managing posttransplant CMV disease (25, 43). Not all patients with CMV reactivation develop clinical disease. It is the degree and the rate of CMV replication that predict impending CMV disease and, thus, need for specific treatment (9, 25). In addition, the level (virus load) of CMV DNA in blood specimens at the end of therapy predicts relapsing CMV infection (43). Preliminary data using the COBAS AMPLICOR CMV MONITOR assay indicates that the threshold of viral load (around 1,000 to 5,000 copies per ml of plasma in solid-organ transplant recipients and around 400 copies/ml of plasma in hematopoietic stem cell transplant recipients) predicts the likelihood of CMV disease, if untreated (13). However, there is need for additional studies to validate the optimal threshold. The wide variability in the laboratory techniques of different centers for detection of CMV DNA (in such things as samples [source, processing, and target volume], reagents [primers, probes, and master mix concentrations], patient characteristics [solid-organ or hematopoietic stem cell transplant recipients], the use and level of immunosuppressive agents, the use of antiviral prophylaxis regimens, and the presence of viral coinfections [human herpesvirus {HHV}-6 and -7]) (2, 7, 8, 26, 32, 37, 39, 40) could account for this lack of standard and defined threshold value. However, it is generally accepted that higher CMV DNA copy levels (25, 43) or an increasing trend in viral loads (9) predict clinical progression to disease or clinical relapse.

The NucliSens assay (Organon Teknika Diagnostics, Boxtel, The Netherlands), an isothermal nucleic acid amplification reaction assay, detects the presence of CMV late-mRNA pp67. The presence of mRNA pp67 indicates active viral replication, and its detection is a marker for active CMV infection (35, 48). Nevertheless, early experience suggests that this assay is less sensitive than DNA amplification assays and antigenemia assays for detection of CMV infection. The lower sensitivity of the assay may result in failure to detect or predict CMV disease in all patients; in one study, the assay did not detect the mRNA transcripts in 4 of 11 patients who developed CMV disease (35).

Nucleic acid hydridization. The Digene Hybrid Capture CMV DNA assay (Digene Corporation, Silver Spring, Md.) is a rapid, qualitative, signal-amplified solution hybridization assay that utilizes RNA probes that bind to the DNA target followed by antibodies directed to RNA-DNA hybrids, as well as a sensitive chemoluminiscence detection system. In a multicenter study that included solid-organ and hematopoietic stem cell transplant recipients (22), this assay was found to be more sensitive that cell culture assays and to have a sensitivity and specificity similar to those of the antigenemia assay. The Digene Hybrid Capture assay has fewer technical variables than the antigenemia test. However, because of the assay's qualitative nature, the clinical significance of a positive result is unclear, as the assay may be detecting subclinical CMV replication that may not evolve into clinical disease (i.e., high sensitivity and low specificity). The utility of the assay in predicting the occurrence of CMV disease and its utility in monitoring the response to antiviral therapy is currently being investigated (22).

ANTIVIRAL SUSCEPTIBILITY TESTING

The three antiviral drugs that are currently licensed for use in the prevention and treatment of CMV are ganciclovir (and its valine ester, valganciclovir), foscarnet, and cidofovir. Other experimental drugs may also be useful for treatment of CMV infections resistant to standard agents (24). Ganciclovir is a prodrug that is monophosphorylated into ganciclovir 5′-mono-

phoshate in CMV-infected cells by virus-encoded thymidine kinase UL97, then di- and triphosphorylated by the host cellular kinases. The active triphosphorylated form of ganciclovir inhibits DNA polymerase by competing with deoxyguanosine triphosphate, thereby terminating viral replication. Foscarnet is a pyrophosphate analogue that directly inhibits viral DNA polymerase by interfering with the release of pyrophosphate during a substrate incorporation event. Cidofovir is a nucleotide analogue that requires phosphorylation by the cellular enzymes in order to achieve its active form. Unlike ganciclovir, neither foscarnet nor cidofovir requires the virus-encoded thymidine kinase for activation.

Antiviral drug resistance in CMV is an emerging problem in transplant recipients (8, 10). Studies of transplant recipients and of patients infected with human immunodeficiency virus suggest that mutations in the viral DNA polymerase UL54 (target of antiviral drugs) and in the thymidine kinase UL97 (phosphotransferase; phosphorylates ganciclovir into active form) confer antiviral drug resistance in CMV. The currently available methods for antiviral susceptibility testing rely on the suppression of virus growth in the presence of serial concentrations of antiviral drugs (phenotypic assays) or the determination of specific mutations that has been associated to confer resistance (genotypic assays).

Phenotypic assays. Phenotypic methods assess the concentration of the drug that inhibits virus replication. Typically, the level of virus is plotted against the concentration of the drug that causes 50% inhibition of the virus in cell cultures. The phenotypic methods that have been employed include plaque reduction assay (inhibition of viral replication), enzyme-linked immunosorbent assays (inhibition of protein synthesis), flow cytometric fluorescence-activated cell sorter, and DNA hybridization assays (inhibition of viral DNA synthesis) (23, 44).

The plaque reduction assay is the standard method of antiviral susceptibility testing for CMV. The test is burdensome and lacks standardization; it requires the recovery of the virus in cell cultures followed by several passages to attain the necessary viral titers for the performance of the assay. Benchmark analysis of several strains of CMV has shown wide variability in results. Typically, these assays require at least 4 weeks to obtain results (18).

Genotypic assays. The significant problems with use of phenotypic assays and the recognition that specific mutations in the UL54 and UL97 genes of CMV are associated with antiviral drug resistance have led to the development of molecular methods for the detection of the CMV mutants.

UL97 encodes for the thymidine kinase that is essential for the initial phosphorylation of ganciclovir into its active form. Accordingly, the functional consequence of these mutations is the inadequate intracellular phosphorylation of ganciclovir into the ganciclovir monophosphate form (10, 19, 45), thus leading to ganciclovir resistance. Since cidofovir and foscarnet do not require thymidine kinase, UL97 mutations do not confer resistance to these agents. Analysis of the UL97 sequences of phenotypically ganciclovir-resistant clinical strains demonstrated mutations and deletions in this region; several of the more common point mutations occur at codons 460 (V460, I460), 520 (Q520), and 591 to 607 (e.g., V594 and S595) (4, 5, 19). Our group demonstrated that deletion in codons 595 to 603 confer clinical resistance to ganciclovir (28). These mutations can be detected by direct sequencing of the PCR products (combination of PCR-based amplification and sequencing for rapid diagnosis of ganciclovir-resistant CMV strains) (28). It has also been recently demonstrated that molecular amplification of the portion of the UL97 gene encoding the C-terminal half of the enzyme followed by two sequencing reactions provided rapid identification of all presently known sites of ganciclovir resistance in this gene (21).

Mutations in UL54 CMV DNA polymerase gene, the main target of all three antiviral drugs, could result in the resistance to any or all of the three drugs. For example, mutations at codons 375 to 540 confer ganciclovir and cidofovir cross-resistance, mutations at codons 756 to 809 confer ganciclovir and foscarnet cross-resistance, and mutations at codons 981 to 987 appear to confer simultaneous mutations to the three drugs. Most UL54 mutations are accompanied by UL97 mutations; strains with double UL97 and UL54 mutations are believed to be highly resistant to ganciclovir with possible cross-resistance to cidofovir and/or foscarnet (16).

The rapid thermocycling utilized by the automated PCR methods may help us attain the goal of real-time antiviral susceptibility testing. For example, the melting curve analysis of the LightCycler assay can detect nucleotide differences in the amplified products and the probe (thus, mutations or deletions) by a shift in the peak melting curve (38). While the LightCycler assay may not detect the exact point mutation, it could serve as a screening method before gene sequencing can be performed. If these applications were to be confirmed, it would be possible to analyze the susceptibility of CMV to various drugs within few hours of specimen collection, compared to the current turnaround time of several weeks with the use of phenotypic methods.

Clinical applications of antiviral susceptibility testing. Antiviral drug resistance in CMV is now an emerging concern in transplantation. Thus, antiviral susceptibility testing will be a common occurrence in the field of transplantation during the upcoming years. Currently, the conventional cell culture-based (phenotypic) methods are not rapid or standardized enough to be of immediate clinical utility in CMV disease management. Thus, surrogate markers such as the failure of the viral load to decrease during antiviral treatment are used as indirect measures of antiviral resistance.

Genotypic assays are easily performed with modern molecular methods such as PCR and sequencing of amplified products. Nevertheless, these methods need optimization and clinical validation. For example, there are mutations and deletions in UL97 and UL54 genes that do not correlate with phenotypic resistance. Extensive research should determine whether a specific mutation in the genome confers low-level or high-level resistance or does not confer any resistance to the antiviral drugs at all.

CONCLUSIONS AND FUTURE DIRECTIONS

The practice of CMV prevention and disease management in hematopoietic and solid-organ transplant patients has clearly evolved during the past decade. In the past, shell vial cell culture detection of CMV has provided rapid (16 h postinoculation) and specific laboratory results, but the assay lacks sensitivity needed to institute early preemptive therapy. Re-

cently, the real-time PCR has provided rapid result reporting (in 4 hs) and the capability for producing quantitative levels of CMV DNA that are useful for predicting disease and monitoring response to antiviral therapy and can serve as surrogate markers for antiviral resistance and clinical relapse in these patients. Serologic determinations continue to be of value for determining antibody status of organ donors and recipients regarding risk for acquiring CMV disease in the posttransplantation course.

Future directions should be focused on the implementation of automated instrumentation to achieve standardized and reproducible laboratory test results applicable for meaningful comparisons between laboratories. Test variables such as optimal specimens of whole blood or its components (plasma, PBL, and PBMC), clinically relevant cutoff, or threshold, levels of CMV DNA, the potential interactions during viral coinfections, and the antiviral susceptibilities (assayed by genotypic analysis) of CMV strains need to be determined according to data-based analysis.

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REFERENCES

1. **Badley, A. D., R. Patel, D. F. Portela, W. S. Harmsen, T. F. Smith, D. M. Ilstrup, J. L. Steers, R. H. Wiesner, and C. V. Paya.** 1996. Prognostic significance and risk factors of untreated cytomegalovirus viremia in liver transplant recipients. J. Infect. Dis. **173:**446–449.
2. **Badley, A. D., E. C. Seaberg, M. K. Porayko, R. H. Wiesner, M. R. Keating, M. P. Wilhelm, R. C. Walker, R. Patel, W. F. Marshall, M. DeBernardi, R. Zetterman, J. L. Steers, and C. V. Paya.** 1997. Prophylaxis of cytomegalovirus infection in liver transplantation: a randomized trial comparing a combination of ganciclovir and acyclovir to acyclovir. NIDDK Liver Transplantation Database. Transplantation **64:**66–73.
3. **Boeckh, M., T. A. Gooley, D. Myerson, T. Cunningham, G. Schoch, and R. A. Bowden.** 1996. Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study. Blood. **88:**4063–4071.
4. **Chou, S., A. Erice, M. C. Jordan, G. M. Vercellotti, K. R. Michels, C. L. Talarico, S. C. Stanat, and K. K. Biron.** 1995. Analysis of the UL97 phosphotransferase coding sequence in clinical cytomegalovirus isolates and identification of mutations conferring ganciclovir resistance. J. Infect. Dis. **171:**576–583.
5. **Chou, S., S. Guentzel, K. R. Michels, R. C. Miner, and W. L. Drew.** 1995. Frequency of UL97 phosphotransferase mutations related to ganciclovir resistance in clinical cytomegalovirus isolates. J. Infect. Dis. **172:**239–242.
6. **Delgado, R., C. Lumbreras, C. Alba, M. A. Pedraza, J. R. Otero, R. Gomez, E. Moreno, A. R. Noriega, and C. V. Paya.** 1992. Low predictive value of polymerase chain reaction for diagnosis of cytomegalovirus disease in liver transplant recipients. J. Clin. Microbiol. **30:**1876–1878.
7. **Dockrell, D. H., J. Prada, M. F. Jones, R. Patel, A. D. Badley, W. S. Harmsen, D. M. Ilstrup, R. H. Wiesner, R. A. Krom, T. F. Smith, and C. V. Paya.** 1997. Seroconversion to human herpesvirus 6 following liver transplantation is a marker of cytomegalovirus disease. J. Infect. Dis. **176:**1135–1140.
8. **Emery, V. C., and P. D. Griffiths.** 2000. Prediction of cytomegalovirus load and resistance patterns after antiviral chemotherapy. Proc. Natl. Acad. Sci. USA **97:**8039–8044.
9. **Emery, V. C., C. A. Sabin, A. V. Cope, D. Gor, A. F. Hassan-Walker, and P. D. Griffiths.** 2000. Application of viral-load kinetics to identify patients who develop cytomegalovirus disease after transplantation. Lancet **355:** 2032–2036.
10. **Erice, A.** 1999. Resistance of human cytomegalovirus to antiviral drugs. Clin. Microbiol. Rev. **12:**286–297.
11. **Gane, E., F. Saliba, G. J. Valdecasas, J. O'Grady, M. D. Pescovitz, S. Lyman, and C. A. Robinson.** 1997. Randomised trial of efficacy and safety of oral ganciclovir in the prevention of cytomegalovirus disease in liver-transplant recipients. The Oral Ganciclovir International Transplantation Study Group. Lancet **350:**1729–1733. (Erratum **351:**454, 1998.)
12. **Gleaves, C. A., T. F. Smith, E. A. Shuster, and G. R. Pearson.** 1984. Rapid detection of cytomegalovirus in MRC-5 cells inoculated with urine specimens by using low-speed centrifugation and monoclonal antibody to an early antigen. J. Clin. Microbiol. **19:**917–919.
13. **Griffiths, P. D., and R. J. Whitley (ed.).** 11 November 2000, posting date. Recommendations from the IHMF Management Strategies Workshop and 8th Annual Meeting of the IHMF. The challenge of CMV infection and disease in transplantation. [Online.] Cambridge Medical Publications, Worthing, United Kingdom. http://www.ihmf.org.
14. **Grossi, P., S. Kusne, C. Rinaldo, K. St George, M. Magnone, J. Rakela, J. Fung, and T. E. Starzl.** 1996. Guidance of ganciclovir therapy with pp65 antigenemia in cytomegalovirus-free recipients of livers from seropositive donors. Transplantation **61:**1659–1660.
15. **Halling, V. W., G. T. Maine, C. M. Groettum, J. A. Wilson, J. Spesard, S. Brojanac, B. Combs, D. Wachta, C. Holas, T. Wilson, D. Wang, S. Tomazic-Allen, T. Lazzarotto, M. P. Landini, S. Jevremovic-Simovic, R. H. Wiesner, C. V. Paya, and T. F. Smith.** 2001. Clinical evaluation of a new recombinant antigen-based cytomegalovirus immunoglobulin M immunoassay in liver transplant recipients. Transplantation **71:**395–397.
16. **Jabs, D. A., B. K. Martin, M. S. Forman, J. P. Dunn, J. L. Davis, D. V. Weinberg, K. K. Biron, and F. Baldanti.** 2001. Mutations conferring ganciclovir resistance in a cohort of patients with acquired immunodeficiency syndrome and cytomegalovirus retinitis. J. Infect. Dis. **183:**333–337.
17. **Landini, M. P., T. Lazzarotto, G. T. Maine, A. Ripalti, and R. Flanders.** 1995. Recombinant mono- and polyantigens to detect cytomegalovirus-specific immunoglobulin M in human sera by enzyme immunoassay. J. Clin. Microbiol. **33:**2535–2542.
18. **Landry, M. L., S. Stanat, K. Biron, D. Brambilla, W. Britt, J. Jokela, S. Chou, W. L. Drew, A. Erice, B. Gilliam, N. Lurain, J. Manischewitz, R. Miner, M. Nokta, P. Reichelderfer, S. Spector, A. Weinberg, B. Yen-Lieberman, and C. Crumpacker.** 2000. A standardized plaque reduction assay for determination of drug susceptibilities of cytomegalovirus clinical isolates. Antimicrob. Agents Chemother. **44:**688–692.
19. **Littler, E., A. D. Stuart, and M. S. Chee.** 1992. Human cytomegalovirus UL97 open reading frame encodes a protein that phosphorylates the antiviral nucleoside analogue ganciclovir. Nature **358:**160–162.
20. **Lowance, D., H. H. Neumayer, C. M. Legendre, J. P. Squifflet, J. Kovarik, P. J. Brennan, D. Norman, R. Mendez, M. R. Keating, G. L. Coggon, A. Crisp, and I. C. Lee.** 1999. Valacyclovir for the prevention of cytomegalovirus disease after renal transplantation. International Valacyclovir Cytomegalovirus Prophylaxis Transplantation Study Group. N. Engl. J. Med. **340:** 1462–1470.
21. **Lurain, N. S., A. Weinberg, C. S. Crumpacker, and S. Chou.** 2001. Sequencing of cytomegalovirus UL97 gene for genotypic antiviral resistance testing. Antimicrob. Agents Chemother. **45:**2775–2780.
22. **Mazzulli, T., L. W. Drew, B. Yen-Lieberman, D. Jekic-McMullen, D. J. Kohn, C. Isada, G. Moussa, R. Chua, and S. Walmsley.** 1999. Multicenter comparison of the digene hybrid capture CMV DNA assay (version 2.0), the pp65 antigenemia assay, and cell culture for detection of cytomegalovirus viremia. J. Clin. Microbiol. **37:**958–963.
23. **McSharry, J. J., N. S. Lurain, G. L. Drusano, A. L. Landay, M. Notka, M. R. O'Gorman, A. Weinberg, H. M. Shapiro, P. S. Reichelderfer, and C. S. Crumpacker.** 1998. Rapid ganciclovir susceptibility assay using flow cytometry for human cytomegalovirus clinical isolates. Antimicrob. Agents Chemother. **42:**2326–2331.
24. **McSharry, J. J., A. McDonough, B. Olson, S. Hallenberger, J. Reefschlaeger, W. Bender, and G. L. Drusano.** 2001. Susceptibilities of human cytomegalovirus clinical isolates to bay38–4766, bay43–9695, and ganciclovir. Antimicrob. Agents Chemother. **45:**2925–2927.
25. **Mendez, J., M. Espy, T. F. Smith, J. Wilson, R. Wiesner, and C. V. Paya.** 1998. Clinical significance of viral load in the diagnosis of cytomegalovirus disease after liver transplantation. Transplantation **65:**1477–1481.
26. **Mendez, J. C., D. H. Dockrell, M. J. Espy, T. F. Smith, J. A. Wilson, W. S. Harmsen, D. Ilstrup, and C. V. Paya.** 2001. Human beta-herpesvirus interactions in solid organ transplant recipients. J. Infect. Dis. **183:**179–184.
27. **Mendez, J. C., M. J. Espy, T. F. Smith, J. A. Wilson, and C. V. Paya.** 1998. Evaluation of PCR primers for early diagnosis of cytomegalovirus infection following liver transplantation. J. Clin. Microbiol. **36:**526–530.
28. **Mendez, J. C., I. G. Sia, K. R. Tau, M. J. Espy, T. F. Smith, S. Chou, and C. V. Paya.** 1999. Novel mutation in the CMV UL97 gene associated with resistance to ganciclovir therapy. Transplantation **67:**755–757.
29. **Nguyen, Q., R. Champlin, S. Giralt, K. Rolston, I. Raad, K. Jacobson, C. Ippoliti, D. Hecht, J. Tarrand, M. Luna, and E. Whimbey.** 1999. Late cytomegalovirus pneumonia in adult allogeneic blood and marrow transplant recipients. Clin. Infect. Dis. **28:**618–623.
30. **Patel, R., D. W. Klein, M. J. Espy, W. S. Harmsen, D. M. Ilstrup, C. V. Paya, and T. F. Smith.** 1995. Optimization of detection of cytomegalovirus viremia in transplantation recipients by shell vial assay. J. Clin. Microbiol. **33:**2984–2986.
31. **Patel, R., T. F. Smith, M. Espy, R. H. Wiesner, R. A. Krom, D. Portela, and C. V. Paya.** 1994. Detection of cytomegalovirus DNA in sera of liver transplant recipients. J. Clin. Microbiol. **32:**1431–1434.
32. **Patel, R., D. R. Snydman, R. H. Rubin, M. Ho, M. Pescovitz, M. Martin, and C. V. Paya.** 1996. Cytomegalovirus prophylaxis in solid organ transplant recipients. Transplantation **61:**1279–1289.

33. **Paya, C. V.** 1999. Indirect effects of CMV in the solid organ transplant patient. Transpl. Infect. Dis. **1:**8–12.
34. **Paya, C. V., A. D. Wold, and T. F. Smith.** 1987. Detection of cytomegalovirus infections in specimens other than urine by the shell vial assay and conventional tube cell cultures. J. Clin. Microbiol. **25:**755–757.
35. **Pellegrin, I., I. Garrigue, D. Ekouevi, L. Couzi, P. Merville, P. Merel, G. Chene, M. H. Schrive, P. Trimoulet, M. E. Lafon, and H. Fleury.** 2000. New molecular assays to predict occurrence of cytomegalovirus disease in renal transplant recipients. J. Infect. Dis. **182:**36–42.
36. **Razonable, R. R., R. A. Brown, M. J. Espy, A. Rivero, W. Kremers, J. Wilson, C. Groettum, T. F. Smith, and C. V. Paya.** 2001. Comparative quantitation of cytomegalovirus (CMV) DNA in solid organ transplant recipients with CMV infection by using two high-throughput automated systems. J. Clin. Microbiol. **12:**4472–4476.
37. **Razonable, R. R., R. A. Brown, J. A. Wilson, C. Groettum, W. Kremers, M. Espy, T. F. Smith, and C. V. Paya.** The clinical utility of various blood compartments for cytomegalovirus (CMV) DNA quantitation in transplant recipients. Transplantation, in press.
38. **Razonable, R. R., C. Fanning, R. A. Brown, M. J. Espy, A. Rivero, J. A. Wilson, W. Kremers, T. F. Smith, and C. V. Paya.** 2002. Selective reactivation of human herpesivirus 6 variant A occurs in critically ill non-immunocompromised hosts. J. Infect. Dis. **185:**110–113.
39. **Razonable, R. R., A. Rivero, A. Rodriguez, J. A. Wilson, J. Daniels, G. Jenkins, T. Larson, W. C. Hellinger, J. R. Spivey, and C. V. Paya.** 2001. Allograft rejection predicts the occurrence of late-onset cytomegalovirus (CMV) disease among CMV-mismatched solid organ transplant recipients receiving prophylaxis with oral ganciclovir. J. Infect. Dis. **184:**1461–1464.
40. **Sarmiento, J. M., D. H. Dockrell, T. R. Schwab, S. R. Munn, and C. V. Paya.** 2000. Mycophenolate mofetil increases cytomegalovirus invasive organ disease in renal transplant patients. Clin Transplant. **14:**136–138.
41. **Shuster, E. A., J. S. Beneke, G. E. Tegtmeier, G. R. Pearson, C. A. Gleaves, A. D. Wold, and T. F. Smith.** 1985. Monoclonal antibody for rapid laboratory detection of cytomegalovirus infections: characterization and diagnostic application. Mayo Clin Proc. **60:**577–585.
42. **Sia, I. G., J. A. Wilson, M. J. Espy, C. V. Paya, and T. F. Smith.** 2000. Evaluation of the COBAS AMPLICOR CMV MONITOR test for detection of viral DNA in specimens taken from patients after liver transplantation. J. Clin. Microbiol. **38:**600–606.
43. **Sia, I. G., J. A. Wilson, C. M. Groettum, M. J. Espy, T. F. Smith, and C. V. Paya.** 2000. Cytomegalovirus (CMV) DNA load predicts relapsing CMV infection after solid organ transplantation. J. Infect. Dis. **181:**717–720.
44. **Stanat, S. C., J. E. Reardon, A. Erice, M. C. Jordan, W. L. Drew, and K. K. Biron.** 1991. Ganciclovir-resistant cytomegalovirus clinical isolates: mode of resistance to ganciclovir. Antimicrob. Agents Chemother. **35:**2191–2197.
45. **Sullivan, V., C. L. Talarico, S. C. Stanat, M. Davis, D. M. Coen, and K. K. Biron.** 1992. A protein kinase homologue controls phosphorylation of ganciclovir in human cytomegalovirus-infected cells. Nature **358:**162–164.
46. **Telenti, A., and T. F. Smith.** 1989. Screening with a shell vial assay for antiviral activity against cytomegalovirus. Diagn. Microbiol. Infect. Dis. **12:**5–8.
47. **van den Berg, A. P., I. J. Klompmaker, E. B. Haagsma, A. Scholten-Sampson, C. M. Bijleveld, J. Schirm, M. van der Giessen, M. J. Slooff, and T. H. The.** 1991. Antigenemia in the diagnosis and monitoring of active cytomegalovirus infection after liver transplantation. J. Infect. Dis. **164:**265–270.
48. **Witt, D. J., M. Kemper, A. Stead, P. Sillekens, C. C. Ginocchio, M. J. Espy, C. V. Paya, T. F. Smith, F. Roeles, and A. M. Caliendo.** 2000. Analytical performance and clinical utility of a nucleic acid sequence- based amplification assay for detection of cytomegalovirus infection. J. Clin. Microbiol. **38:**3994–3999.