

Definitions of CMV infection and disease in transplant patients for use in clinical trials

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Brief summary: This is an update of existing definitions of CMV disease for use in clinical trials. The main changes include a “probable disease” category and the use of NAT in some disease category

Abstract

Cytomegalovirus (CMV) infection and disease are important causes of morbidity and mortality in transplant recipients. For the purpose of developing consistent reporting of CMV outcomes in clinical trials, definitions of CMV infection and disease were developed and most recently published in 2002. Since then there have been major developments in its diagnosis and management. Therefore, the CMV Drug Development Forum consisting of scientists, clinicians, regulators, and industry representatives has produced an updated version incorporating recent knowledge with the aim to support clinical research and drug development. The main changes compared to previous definitions are the introduction of a “probable disease” category and to incorporate quantitative nucleic acid testing (NAT) in some end-organ disease categories. As the field evolves, the need for updates of these definitions is clear and collaborative efforts between scientists, regulators, and industry can provide a platform for this work.

Introduction:

Cytomegalovirus (CMV) infection and disease are important causes of morbidity and mortality in transplant recipients. For the purpose of developing consistent reporting of CMV outcomes in clinical trials, definitions of CMV infection and disease were initially developed and published as part of the proceedings of the 4th International CMV Conference in Paris in 1993 [1], and these were subsequently updated in 1995 [2] and most recently in 2002 [3]. These definitions have since been used in many published clinical trials.

During the last two decades, major advances have been made regarding the diagnosis and management of CMV in transplant patients. These advances have been made possible through the development of new diagnostic techniques for the detection of the virus and through the performance of prospective clinical trials that evaluated the efficacy and safety of novel antiviral agents. Therefore, the aim of this report from the “CMV Drug Development Forum” (<http://www.hivforum.org/projects/drug-development/cmv>) is to update the published definitions of CMV infection and disease, taking into account the current state of knowledge in this field recognizing that more work needs to be done to standardize CMV DNA quantification across laboratories and centers.

Methodology

The “CMV Drug Development Forum” was created in 2014 and includes US, European, and Canadian experts on transplantation, transplant infectious disease, and clinical virology, regulators from the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), and representatives of the pharmaceutical and

diagnostic industry. The CMV Forum is based on the Forum for Collaborative HIV Research model: a neutral, independent venue for all stakeholders to engage in dialogue and deliberation to advance regulatory science in disease specific areas [4, 5].

From the members of the CMV Forum, a Disease Definitions Working Subgroup was formed which reviewed the previously published document and proposed changes. The main changes were to introduce a “probable disease” category and to incorporate nucleic acid testing (NAT) in some end-organ disease categories. These changes were discussed and approved at a meeting for the entire CMV Forum. The subgroup addressed the received comments and the updated document was presented at a second CMV Forum meeting and issues without consensus were voted upon. The definitions in this report were developed for application to solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients but can also, as appropriate, be applied to other immunocompromised individuals.

CMV disease consists of “end-organ disease” and CMV syndrome. To define “proven CMV end-organ disease”, the presence of appropriate clinical symptoms and/or signs are required together with documentation of CMV in tissue from the relevant organ by histopathology, virus isolation, rapid culture, immunohistochemistry, or DNA hybridization unless there are data supporting that other materials can be accepted as having similar significance. It is recognized that high viral DNA-levels detected with quantitative NAT, such as polymerase chain reaction (PCR), in tissue from the relevant organ likely represent CMV disease and could therefore be accepted as “possible CMV end-organ disease” especially when blood sampled at the same time does not contain CMV DNA. However, due to the lack of studies, viral load cut-off levels have not yet been defined and will need to be assessed when new evidence becomes available.

There is only one clinical condition (CMV retinitis) where the symptoms and/or signs are sufficiently characteristic to allow a diagnosis of proven disease, even without testing for CMV in a tissue sample. The presence of CMV in the blood, together with symptoms and/or signs, is not sufficient for the definition of either proven or probable CMV disease at any other site, with the exception of CMV-syndrome in SOT patients, but can be used for further research in cohort studies as a definition of possible CMV disease. In this situation, methods for exclusion of other causes of the clinical symptoms and/or signs need to be clearly defined. The assessment of the response to anti-CMV therapy might also be considered to increase the likelihood for CMV as the cause of the symptoms and/or signs. However, it is recognized that, unless an antiviral drug with activity solely against CMV is used, other viral infections might also respond to broad spectrum antiviral therapy. Furthermore, from a regulatory perspective, response to therapy should not be used as a study endpoint or element in a composite study endpoint.

The presence of co-pathogens, such as *Aspergillus* species together with typical radiologic signs of *Aspergillus* pneumonia would indicate fungal pneumonia, although a role of CMV cannot be conclusively excluded if the criteria for CMV disease are otherwise met. It is therefore recommended that studies report separately cases where CMV disease is found with or without co-pathogens with details given on the co-pathogens.

Definitions of CMV infection

CMV Infection: “CMV infection” is defined as virus isolation or detection of viral proteins (antigens) or nucleic acid in any body fluid or tissue specimen. It is recommended that both the source of the specimens tested (e.g., plasma, serum, whole

blood, peripheral blood leukocytes (PBL), cerebrospinal fluid (CSF), bronchoalveolar lavage (BAL) fluid, urine, or tissue) and the diagnostic method used be described clearly.

CMV Replication: The term replication can be used to indicate evidence of viral multiplication and is sometimes used instead of CMV infection.

Primary CMV Infection: Primary CMV infection" is defined as the first detection of CMV infection in an individual who has no evidence of CMV exposure before transplantation. It is recognized that severely immunocompromised individuals such as transplant patients might not develop CMV specific antibodies.

Recurrent CMV Infection: "Recurrent infection" is defined as new CMV infection in a patient with previous evidence of CMV infection, which has not had virus detected for an interval of at least 4 weeks during active surveillance. Recurrent infection may result from reactivation of latent virus (endogenous) or reinfection (exogenous). It is recognized that CMV specific antibodies can be passively transferred by blood products or immune globulin administration. For practical purposes, presence or absence of CMV specific antibodies by serology can be used as acceptable estimates of previous CMV exposure to classify patients for entry into clinical trials.

CMV Reinfection. "Reinfection" is defined as detection of a CMV strain that is distinct from the strain that caused the initial infection.

CMV Reactivation. CMV reactivation is likely if the 2 viral strains (prior and current strain) are found to be indistinguishable either by sequencing specific regions of the viral genome or by using a variety of molecular techniques that examine genes known to be polymorphic.

CMV detection in blood

Several specific definitions for CMV detection in blood are recommended. It should be noted that evidence suggests that the detection of virus, antigen, or DNA in blood does not mean that CMV is replicating in blood.

Viremia. “Viremia” is defined as the isolation of CMV by either standard or rapid culture techniques. These techniques are, however, rarely used today for monitoring of transplant recipients

Antigenemia. “Antigenemia” is defined as the detection of CMV pp65 antigen in PBL.

DNAemia. “DNAemia” is defined as the detection of CMV DNA in samples of plasma, serum, whole blood, isolated PBL or in buffy-coat specimens. There are several techniques available for the detection and quantitation of CMV DNAemia. It is strongly recommended that the nucleic acid amplification techniques have been calibrated to a standard calibrator, such as the WHO International Standard for Human CMV [6].

RNAemia. “RNAemia” is defined as the detection of CMV RNA in samples of plasma, serum, whole blood, isolated PBL or in buffy-coat specimens. These techniques are not commonly used for monitoring of transplant patients despite having the theoretical advantage of documenting transcription of the genomic sequence.

Definitions of CMV disease

CMV Pneumonia:

Proven disease requires clinical symptoms and/or signs of pneumonia such as new infiltrates on imaging, hypoxia, tachypnea, and/or dyspnea combined with CMV documented in lung tissue by virus isolation, rapid culture, histopathology, immunohistochemistry, or DNA hybridization techniques.

Probable CMV pneumonia is defined as the detection of CMV by viral isolation, rapid culture of BAL fluid, or the quantitation of CMV DNA in BAL fluid combined with clinical symptoms and/or signs of pneumonia. A definite cut-off for CMV DNA load cannot be established at the present time. The cut-off is likely to vary between different patients, how the BAL procedure and processing is performed, and the assay used for CMV DNA quantitation. Furthermore, CMV DNA levels may vary considerably between patients with varying degrees of severity of CMV pneumonia, which may impact the predictive values of any cut-off. It should be recognized that CMV shedding in the lower respiratory tract does occur and therefore a low CMV DNA load might well represent asymptomatic infection [7]. The likelihood for CMV pneumonia increases with increasing DNA viral load. In one study in HSCT patients, CMV viral load >200-500 IU/ml in BAL fluid was likely (with a positive predictive value of ~50% based on disease prevalence figures of approximately 10% among patients at risk for CMV pneumonia undergoing BAL testing) to represent pneumonia in HSCT recipients (M Boeckh, manuscript in preparation), while lower levels were likely indicating pulmonary shedding. Data from lung transplant patients suggest that the viral load in BAL fluid in patients with CMV pneumonia is approximately 1.5 log₁₀ higher than viral load in patients with detectable CMV DNA in BAL fluid without evidence of CMV pneumonia (a cut-off of 5500 IU/mL had a sensitivity of 91% and a specificity of 75%) (Lodding et al; abstract ID week 2015). On the other hand, a negative CMV DNA test in the BAL fluid has a negative predictive value close to 100% and therefore excludes the possibility of CMV pneumonia. The use of quantitative PCR on biopsies is an evolving field. Presently, these findings could be defined as possible CMV pneumonia.

CMV Gastrointestinal Disease:

Proven disease requires upper and/or lower gastrointestinal (GI)-symptoms **plus** macroscopic mucosal lesions **plus** CMV documented in tissue by histopathology, virus isolation, rapid culture, immunohistochemistry or DNA hybridization techniques.

Studies should give information regarding the presence or absence of gut Graft-vs-Host disease (GVHD) in HSCT recipients.

Probable GI disease requires upper and/or lower GI-symptoms and CMV documented in tissue but without the requirement for macroscopic mucosal lesions. Studies should give information regarding the presence or absence of gut GVHD in HSCT recipients. CMV documented in blood by NAT (e.g., PCR) or antigenemia or CMV documented by PCR from tissue biopsies is not sufficient for the diagnosis of CMV GI-disease. The use of quantitative PCR on gut biopsies is an evolving field. Presently, these findings could be defined as possible GI-disease

CMV hepatitis:

Proven disease requires abnormal liver function tests **plus** CMV documented in tissue by histopathology, immunohistochemistry, virus isolation, rapid culture, or DNA hybridization techniques **plus** the absence of other documented cause of hepatitis.

Probable is not a recommended category for CMV hepatitis. Due to the risk for other confounders such as acute and chronic allograft rejection in liver transplant recipients or GVHD in HSCT recipients, as well as the common occurrence of drug-associated liver dysfunction, a probable CMV hepatitis category is not defined.

CMV retinitis:

Proven disease requires typical ophthalmological signs judged by an ophthalmologist experienced with the diagnosis of CMV retinitis. If the presentation is atypical or an experienced ophthalmologist is not available, it is recommended that the diagnosis is supported by CMV documented in vitreous fluid by NAT (such as PCR).

A **probable** disease category should not be used.

CMV encephalitis and ventriculitis:

Proven disease requires central nervous system (CNS) symptoms **plus** detection of CMV in CNS tissue by virus isolation, rapid culture, immuno-histochemical analysis, *in situ* hybridization, or (preferably) quantitative PCR.

Probable disease requires CNS symptoms **plus** detection of CMV in CSF without visible contamination of blood (“bloody tap”) **plus** abnormal imaging results or evidence of encephalitis on EEG.

Nephritis:

Proven disease is defined by the detection of CMV by virus isolation, rapid culture, immunohistochemical analysis, or *in situ* hybridization in a kidney allograft biopsy specimen obtained from a patient with renal dysfunction together with the identification of histologic features of CMV infection. The detection of CMV in urine by PCR or culture is not sufficient for the diagnosis of CMV nephritis since asymptomatic viral shedding in urine is common.

Cystitis:

Proven disease is defined by the detection of CMV by virus isolation, rapid culture, immunohistochemical analysis, or *in situ* hybridization in a bladder biopsy specimen

obtained from a patient with cystitis together with the identification of conventional histologic features of CMV infection. The detection of CMV in urine by PCR or culture is not sufficient for the diagnosis of CMV cystitis since asymptomatic viral shedding in urine is common.

Myocarditis:

Proven disease is defined by the detection of CMV by virus isolation, rapid culture, immunohistochemical analysis, or in situ hybridization in a heart biopsy specimen obtained from a patient with myocarditis together with the identification of conventional histologic features of CMV infection.

Pancreatitis:

Proven disease is defined as the detection of CMV by virus isolation, rapid culture, immunohistochemical analysis, or in situ hybridization in a pancreatic biopsy specimen obtained from a patient with pancreatitis together with the identification of conventional histologic features of CMV infection.

Other end-organ disease categories:

CMV can also cause disease in other organs, and the definitions of these additional disease categories include the presence of compatible symptoms and signs and documentation of CMV by biopsy by virus isolation, rapid culture, immunohistochemical analysis, or in situ hybridization.

CMV syndrome

CMV syndrome is a disease definition that should only be used in SOT recipients. Since it is impossible to exclude all other causes of the clinical symptomatology described as CMV syndrome, a **proven** category cannot be defined. The definition of **probable** CMV syndrome requires detection of CMV in blood by viral isolation, rapid culture, antigenemia or an NAT together with at least two of the following:

- a. Fever $\geq 38^{\circ}\text{C}$ for at least 2 days
- b. New or increased malaise (toxicity grade 2) or new or increased fatigue (toxicity grade 3) (National Cancer Institute: Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0).
- c. Leukopenia or neutropenia on two separate measurements at least 24 hours apart defined as: a WBC count of $< 3,500/\mu\text{L}$, if the WBC count prior to the development of clinical symptoms was $\geq 4,000/\mu\text{L}$ **or** a WBC decrease of $> 20\%$, if the WBC count prior to the development of clinical symptoms was $< 4,000/\mu\text{L}$. The corresponding neutrophil counts are $< 1,500/\mu\text{L}$ or a decrease of more than 20% if the neutrophil count before the onset of symptoms was below 1,500/ μL
- d. $\geq 5\%$ atypical lymphocytes.
- e. Thrombocytopenia defined as a platelet count of $< 100,000/\mu\text{L}$ if the platelet count prior to the development of clinical symptoms was $\geq 115,000/\text{mL}$ or a decrease of $> 20\%$ if the platelet count prior to the development of clinical symptoms was $< 115,000/\mu\text{L}$
- f. Elevation of hepatic transaminases (ALT or AST) to $2 \times$ upper limit of normal (applicable to non-liver transplant recipients)

Discussion

Several new antiviral agents and vaccines to prevent CMV infection and/or disease are in clinical development [8-11]. In order for meaningful comparison of clinical outcomes, it is important that clinical studies of new agents use common (standardized) definitions regarding trial endpoints. Updating the previous definitions of CMV infection and disease is warranted, since transplant practices and diagnostic techniques have advanced and continued to evolve. It should also be recognized that some “gold standard” techniques never were submitted to critical assessment but have been used in clinical trials based on old and not necessarily well-controlled studies. It is also very unlikely that comparative studies between “old” and “new” diagnostic techniques will be performed since many diagnostic laboratories no longer perform classic virus isolation or rapid culture techniques. We therefore wanted to address these issues by adding a “probable” CMV disease category. It is likely that future studies will include both proven and probable CMV disease definitions in their design but the classification will allow the possibility to find differences in outcome between patients having developed these different disease categories. We are aware that there are situations not covered by these two categories and we therefore describe a couple of instances where “possible” CMV disease can be defined. At this time, however, we do not recommend to include these in clinical trial design until more data is available.

The “gold standard” of CMV end-organ disease for documenting the effects of new agents is difficult to incorporate in current clinical trial designs since it has become increasingly rare [12, 13]. Use of surrogate outcomes, such as viremia, DNAemia, and antigenemia has been suggested by others. However, there is variability between different assays used for detection of CMV. Most current assays detect nucleic acids in a quantitative manner. A major advance during the last decade has been the introduction

of an international standard for CMV DNA quantitation allowing comparison of results from different techniques [6] although a recent report indicates that, while the standard is an improvement, variability between assays remains high [14, 15]. It is therefore strongly encouraged to use assays that have been calibrated to a standard in the clinical trial setting and preferably to use a central laboratory. Another need for future research is to define thresholds for quantitation of CMV from tissue material and this is an area of active investigation. Additional developments are in the field of detecting specific immune responses to CMV but these techniques are not ready at this time for widespread use and incorporation in clinical trial design.

In SOT recipients, the “CMV-syndrome” category will be the most frequently documented type of CMV disease. However, the different clinical symptoms and signs included in the definition are very common in the immunocompromised patients. CMV syndrome is not a precisely defined entity, and therefore future research should focus on a scoring system ultimately establishing a threshold score for this entity.

As the field evolves, the need for updates of these definitions is clear and collaborative efforts between scientists, regulators, and industry can provide a platform for this work.

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Table 1. CMV disease categories and required quality of evidence

Disease	Proven	Probable	Possible
Pneumonia	Yes	Yes	(Yes)
Gastrointestinal disease	Yes	Yes	(Yes)
Hepatitis	Yes	No	No
Retinitis	Yes	No	No
Encephalitis/ventriculitis	Yes	Yes	No
Nephritis	Yes	No	No
Cystitis	Yes	No	No
Myocarditis	Yes	No	No
Pancreatitis	Yes	No	No
Other end-organ diseases	Yes	No	No
Syndrome	No	Yes	No

All three categories require appropriate clinical symptoms and/or signs.