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 Fourth 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 2 decades, major advances have been made regarding the diagnosis and management of CMV in transplant patients. These advances have 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 and 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 diagnostics industries. 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 quantitative nucleic acid testing 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.

**Keywords.** CMV; stem cell transplantation; organ transplantation; clinical trials.
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, immuno-histochemistry, 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 (Table 1).

There is only 1 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.

Table 1. Cytomegalovirus Disease Categories and Required Quality of Evidence

<table>
<thead>
<tr>
<th>Disease</th>
<th>Proven</th>
<th>Probable</th>
<th>Possible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Gastrointestinal disease</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Retinitis</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Encephalitis/ventriculitis</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Nephritis</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cystitis</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Other end-organ diseases</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

All 3 categories require appropriate clinical symptoms and/or signs.

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 recipients 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 who 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 PBLs.

**DNAemia**

DNAemia is defined as the detection of CMV DNA in samples of plasma, serum, whole blood, or isolated PBLs, or in buffycoat 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 World Health Organization International Standard for Human CMV [6].

**RNAemia**

RNAemia is defined as the detection of CMV RNA in samples of plasma, serum, whole blood, or isolated PBLs, or in buffycoat 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 and according to how the BAL procedure and processing are 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 approximately 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, unpublished data), 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 the 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, IDWeek 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 (eg, 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 disease 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 be 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, immunohistochemical 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 electroencephalography.

Nephratitis
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 nephratitis as 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 as 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, immunohistochemistry, or DNA hybridization in biopsy material.

CMV Syndrome
CMV syndrome is a disease definition that should only be used in SOT recipients. Because 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 NAT together with at least 2 of the following:

1. Fever &ge;38°C for at least 2 days.
2. New or increased malaise (toxicity grade 2) or new or increased fatigue (toxicity grade 3) (National Cancer Institute: Common Terminology Criteria for Adverse Events, version 4.0).
3. Leukopenia or neutropenia on 2 separate measurements at least 24 hours apart, defined as a white blood cell (WBC) count of &lt;3500 cells/µL, if the WBC count prior to the development of clinical symptoms was &gt;4000 cells/µL, or a WBC decrease of &gt;20%, if the WBC count prior to the development of clinical symptoms was &lt;4000 cells/µL. The corresponding neutrophil counts are &lt;1500 cells/µL or a decrease of &gt;20% if the neutrophil count before the onset of symptoms was &lt;1500 cells/µL.
4. Greater than or equal to 5% atypical lymphocytes.
5. Thrombocytopenia defined as a platelet count of &lt;100 000 cells/µL if the platelet count prior to the development of clinical symptoms was &gt;115 000 cells/µL or a decrease of &gt;20% if the platelet count prior to the development of clinical symptoms was &lt;115 000 cells/µL.
6. Elevation of hepatic aminotransferases (alanine aminotransferase or aspartate aminotransferase) to 2 times the 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]. To achieve 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, as transplant practices and diagnostic techniques have advanced and continue 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 2 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 are 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 as 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, although 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 immunocompromised patients. CMV syndrome is not a precisely defined entity; therefore, future research should focus on a scoring system that ultimately establishes 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.

**Supplementary Data**

Supplementary materials are available at http://academic.oup.com/cid. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

**Notes**

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**References**