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Use of Viral Load as a Surrogate Marker in Clinical Studies of Cytomegalovirus in Solid Organ Transplantation: A Systematic Review and Meta-analysis

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Symptomatic cytomegalovirus (CMV) disease has been the standard endpoint for clinical trials in organ transplant recipients. Viral load may be a more relevant endpoint due to low frequency of disease. We performed a meta-analysis and systematic review of the literature. We found several lines of evidence to support the validity of viral load as an appropriate surrogate end-point, including the following: (1) viral loads in CMV disease are significantly greater than in asymptomatic viremia (odds ratio, 9.3 95% confidence interval, 4.6–19.3); (2) kinetics of viral replication are strongly associated with progression to disease; (3) pooled incidence of CMV viremia and disease is significantly lower during prophylaxis compared with the full patient follow-up period (viremia incidence: 3.2% vs 34.3%; $P < .001$) (disease incidence: 1.1% vs 13.0%; $P < .001$); (4) treatment of viremia prevented disease; and (5) viral load decline correlated with symptom resolution. Based on the analysis, we conclude that CMV load is an appropriate surrogate endpoint for CMV trials in organ transplant recipients.

Keywords. cytomegalovirus (CMV) viremia; CMV disease; clinical trials; preemptive therapy; prophylaxis.

Cytomegalovirus (CMV) is one of the most common opportunistic infections after solid organ transplantation (SOT) and can produce a spectrum of illness categorized as either viral syndrome or tissue-invasive disease [1]. Viral syndrome typically presents with fever, fatigue, and cytopenias; published guidelines exist for appropriate definitions of CMV disease within particular organ groups for use in clinical trials [2]. The natural history of CMV infection after SOT is complex. A recipient lacking CMV immunoglobulin G antibodies (seronegative) before transplant may be infected from a seropositive donor to cause primary infection (D+/R−). The highest risk of CMV disease occurs after primary infection (D+/R−), followed by either reinfection or reactivation, which are less likely to cause disease [3]. There may be a trend toward more viremia in the D+/R+ group versus the D−/R+ group [1].

There are several laboratory methods to detect CMV, but most centers currently use quantitative viral load testing of whole blood or plasma, which most commonly detects CMV DNA using either a commercially available or in-house assay. Cytomegalovirus prevention strategies include either universal antiviral prophylaxis typically with (val)ganciclovir, or a preemptive strategy that involves regular monitoring of viral load with initiation of antiviral therapy after detection above a certain threshold in order to prevent CMV disease [4–8]. The choice of prevention strategy depends on patient risk factors, including serostatus and type of transplant [1].

In the past, large randomized trials, designed primarily to demonstrate efficacy of antiviral strategies and to obtain regulatory approval for prophylaxis and/or treatment indications, have used symptomatic CMV disease as the primary endpoint. However, more recently this primary endpoint has been questioned for a number of reasons. In current clinical practice, rates of CMV disease are often low, in part due to prolonged prophylaxis, but also due to early detection of viremia and initiation of antiviral therapy before definitive symptoms attributable to CMV are evident [9]. In addition, currently, the most common form of CMV disease after SOT is viral syndrome. Although definitions for viral syndrome exist, it is clear that it represents a spectrum of illness that shares many overlapping features with other infectious and noninfectious etiologies. At a recent forum of content experts, industry, and regulatory advisors, including the US Food and Drug Administration and European Medicines Agency (CMV Forum, a project of the Forum for Collaborative Research University of California, Berkeley), the issue about the potential use of viral load as a surrogate marker in trials of CMV prevention or treatment arose as a major question pertaining to

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development of new clinical trials. The CMV Forum delineated a process by which the utility of CMV load as a potential accepted surrogate endpoint in clinical trials could be more systematically evaluated. The results of that process and systematic review are compiled and presented here as they relate to SOT.

METHODS

Search Methods

A comprehensive search strategy was developed to identify published English-language literature on "cytomegalovirus," "solid organ transplantation," and "viral load." The search strategy was developed by a medical librarian using a combination of database-specific subject headings and text words. Additional keywords were mined from sample articles and generated through input from subject specialists on the team. The search strategy was then customized for each database. No limits for date were applied. Animal-only studies were excluded where applicable, and no study-type filters were applied. Books and conference materials were excluded from Embase results. To ensure sensitivity, the initial strategy in MEDLINE was tested against 7 seminal articles and modified accordingly. The following databases were searched from inception to the date of the search (15 December 2016): Ovid MEDLINE, Ovid MEDLINE In-Process & Other Non-Indexed Citations, Ovid Embase, Cochrane Database of Systematic Reviews, and Cochrane Central Register of Controlled Clinical Trials. A supplementary search was conducted in PubMed for non-MEDLINE records. See the Supplementary Appendix for all search strategies. Two authors (Y. N. and A. A.) independently assessed all studies for risk of bias.

Inclusion and Exclusion Criteria

We included cohort studies or randomized controlled trials of cytomegalovirus, solid organ transplantation, and viral load where the total cohort was >20 cases. We excluded animal studies, those of primarily other pathogens, studies with no use of quantitative polymerase chain reaction (PCR; eg, antigenemia, mRNA, qualitative PCR), review articles /letters without any new data, those not dealing with a solid organ transplant population, and those that did not document blood viral load. From included studies, we collected the following variables: number of subjects, organ transplant types, CMV load, incidence of CMV viremia and disease, CMV prevention strategy (prophylaxis or preemptive), and follow-up period.

Data Analysis

Where relevant, meta-analysis was performed by calculating the mean from the median and quartiles [10] and then standardizing the mean differences (SMD) between 2 groups. The log odds ratios were estimated from SMD [11] and then meta-analysis [12] done using SAS version 9.4 (SAS Institute, Cary, NC) using DerSimonian and Laird's model. Heterogeneity among studies was assessed with I^2 values, which show the variation among studies that is not due to chance. The sensitivity analysis was repeated with fixed and random models with extracted outliers. Publication bias was examined by Egger test and graphed using a funnel plot [13]. Forest plots were based on the log odds ratios and confidence intervals with a value of 1 as the reference. Where relevant, pooled incidence rates were calculated based on the relative risk ratios (event/total) and their confidence interval [14]. We used the exact method of Clopper and Pearson in confidence interval estimation. The pooled values are based on a fixed-effect model for study on CMV disease during prophylaxis and a random-effects model for the other studies. The fixed or random model, for each combined study, was based on assessment of between-study heterogeneity.

RESULTS

Description of Studies

Our strategy resulted in 2506 potential studies. Of these, 1948 were excluded based on review of titles and abstracts because they did not meet eligibility criteria. These included studies that were not in human subjects or not in solid organ transplant recipients. A total of 558 studies underwent full text review, of which 469 were excluded for reasons outlined in Figure 1. This left a total of 89 studies for inclusion in the systematic review, of which a subset of studies were included in each of the meta-analyses performed.

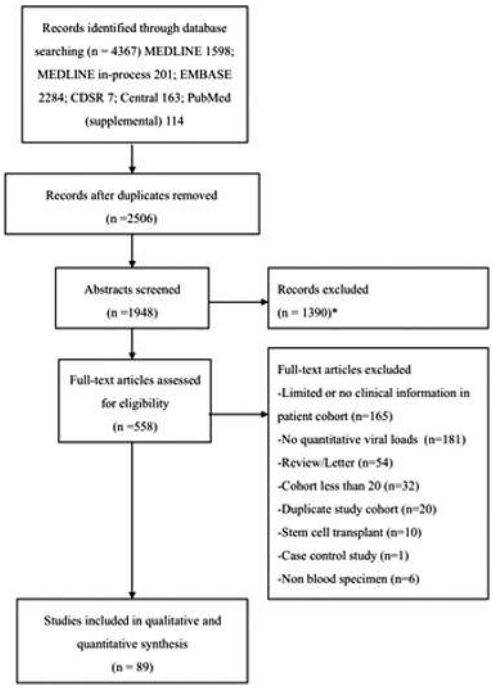

Figure 1. Study selection flow. *The majority of these records were excluded because studies were qualitative, study cohort was <20 subjects, or the studies were primarily of viruses other than cytomegalovirus. Abbreviation: CDSR, Cochrane Database for Systematic Reviews.

Cytomegalovirus Load and Disease Prediction

We first assessed whether viral load was predictive of symptomatic CMV disease. Specifically we ascertained whether differences in viral load were present in patients with asymptomatic viremia versus symptomatic CMV disease (including both viral syndrome and tissue-invasive disease). We used any detectable viral load as the cutoff for positivity when assessing a study. A total of 30 studies that had addressed this question were identified [4, 15–43]. Of these 30 studies, 9 studies were natural history studies [17–19, 32, 34, 38–41]. Ten studies were conducted with COBAS Amplicor viral load assay, whereas the remaining studies used alternative PCR-based assays [4, 26, 29–35, 39, 41]. In a meta-analysis, we included all studies that directly compared these 2 groups (asymptomatic viremia vs CMV disease) (Figure 2A). As a second evaluation, we also conducted a meta-analysis with only studies that used the COBAS Amplicor assay because this was the most common assay used (Figure 2B). Finally, we conducted a third meta-analysis that included only natural history studies (defined as studies in which no prophylaxis or preemptive therapy for asymptomatic CMV viremia is given) (Figure 2C). In all 3 analyses, symptomatic (vs asymptomatic) patients had a substantially and significantly greater viral load (Table 1). In the combined analysis, the mean fold difference in viral load for symptomatic versus asymptomatic patients was 14.9 (95% confidence interval [CI], 6.7–32.5; random-effects model). Based on a sensitivity analysis, there was publication bias for all included studies ($P < .001$). After we removed 3 outliers, we again conducted a meta-analysis, which revealed that mean viral load in CMV disease remained significantly higher than in asymptomatic viremia (odds ratio [OR], 9.3; 95% CI, 4.6–19.3;

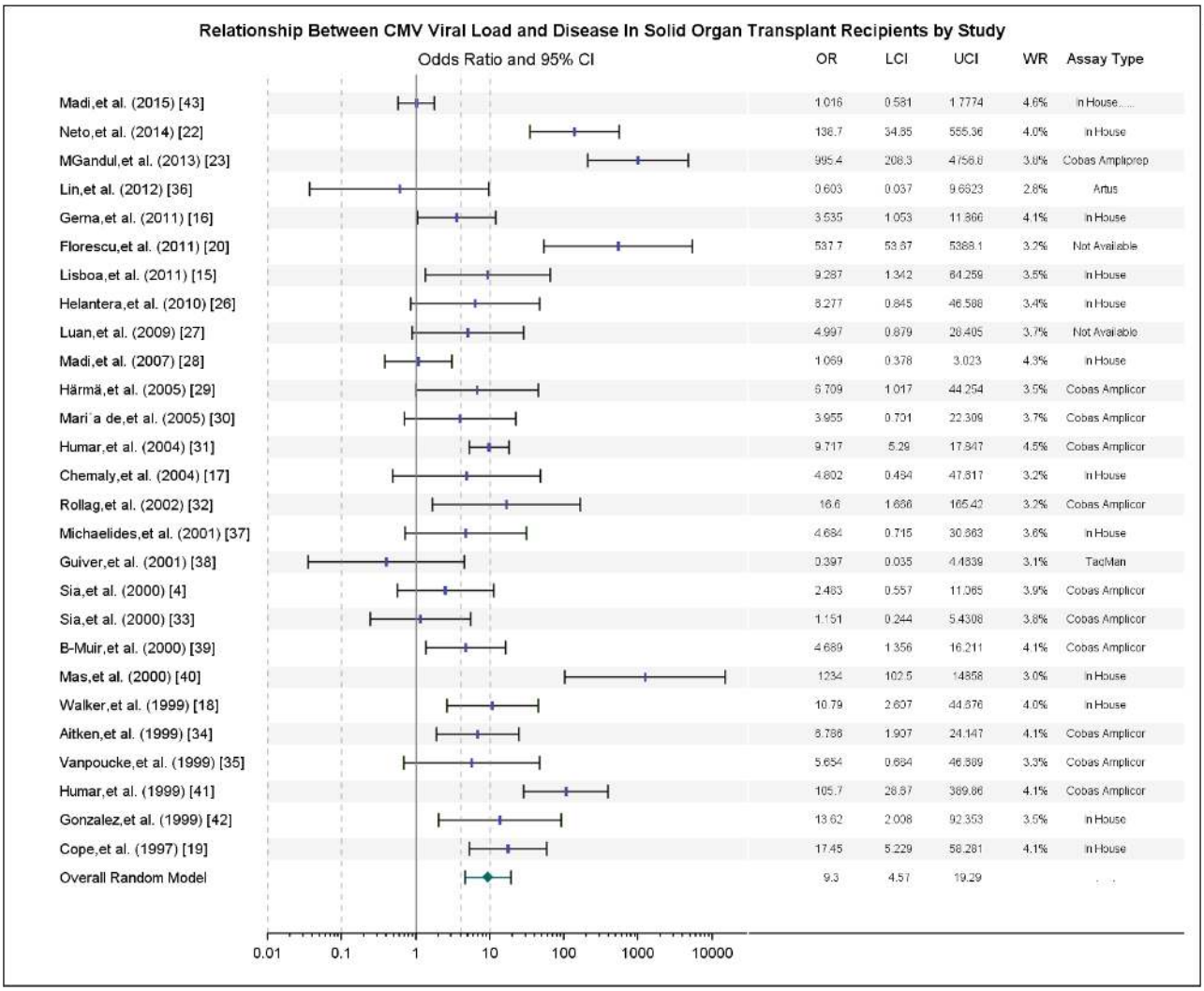

Figure 2. Relationship between cytomegalovirus (CMV) viral load and CMV disease in organ transplant recipients by study. Odds ratio and 95% confidence intervals are shown. *A,* All studies excluding outliers are shown (n = 27). Diamond symbol indicates the result of the DerSimonian-Laird random-effects model. Viral load assay type used in the study is also indicated.

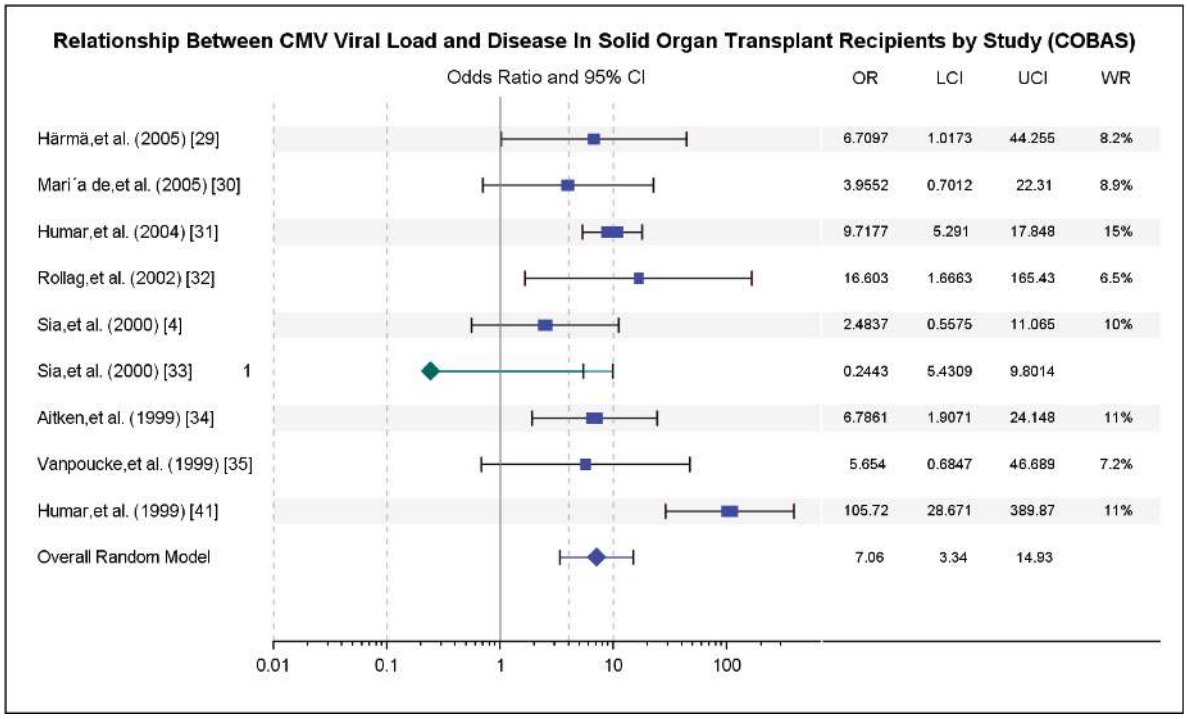

Figure 2. *B,* Relationship between CMV load and disease in studies using the COBAS Amplicor Viral Load Assay (n = 10).

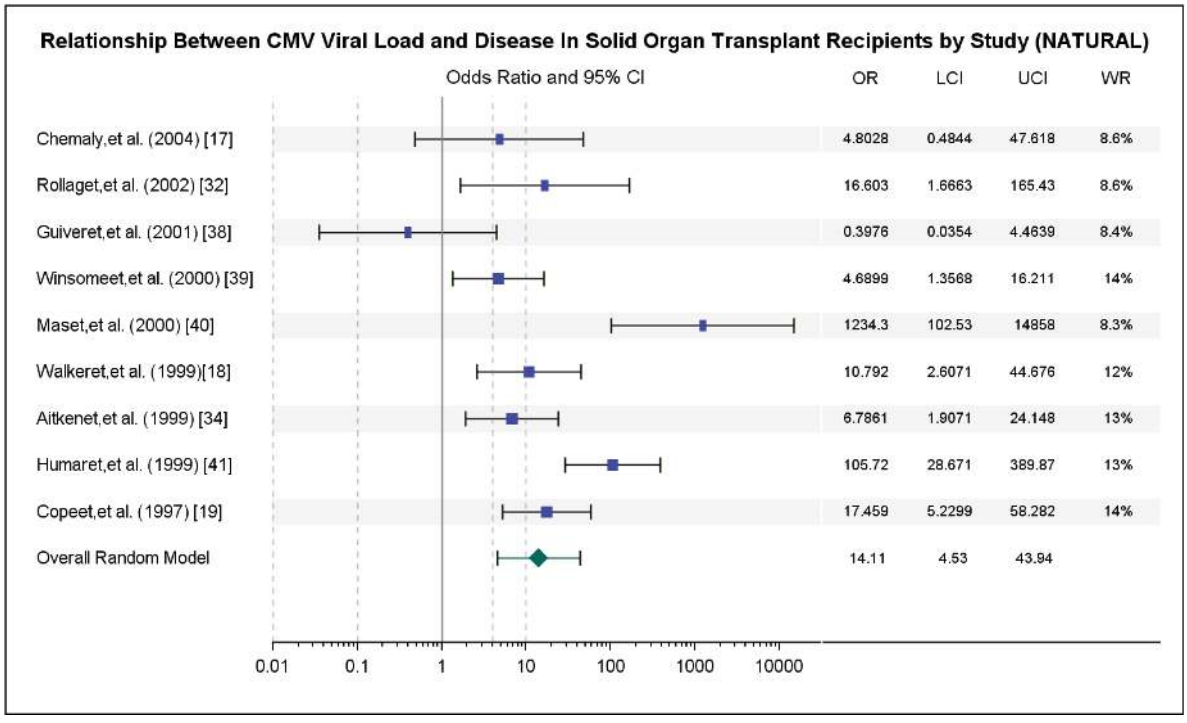

Figure 2. *C,* Relationship between CMV viral load and disease in natural history studies (n = 9). Abbreviations: CI, confidence interval; LCI, lower confidence interval; OR, odds ratio; UCI, upper confidence interval; WR, weight (random).

random-effects model) without publication bias ($P = .10$) (Figure 3). For studies using only the COBAS Amplicor PCR assay, the fold difference was 7.0 (95% CI, 3.3 to 14.9; random-effects model). Finally for natural history studies only, the fold difference was 14.1 (95% CI, 4.5 to 43.9; random-effects model).

Viral Load Kinetics and the Risk of Cytomegalovirus Disease

We evaluated studies that assessed how viral load kinetics (change in viral load over time) influenced the likelihood of CMV disease. The hypothesis was that if viral load is an appropriate surrogate, viral kinetics will influence the likelihood of

Table 1. Summary of Odds Ratios of Asymptomatic Versus Symptomatic Cytomegalovirus Disease in Organ Transplant Recipients

Study type	Number of studies	Model	Pooled OR (95% CI), *P* value	Heterogeneity (I2)	Studies excluded (references)
All included studies	27	REM	9.3 (4.6–19.3), $P < .001$	85.8	Elfadawy et al (2013),[21] Lisboa et al (2012),[15] Gala-Lopez et al (2011)[24]
Studies using COBAS Amplicor Viral Load Assay	10	REM	7.1 (3.3–14.9), $P < .001$	65.0	Helantera et al (2010)[26]
Natural history studies	9	REM	14.1 (4.5–43.9), $P < .001$	77.4	None

Abbreviations: CI, confidence interval; OR, odds ratio; REM, random-effects model.

disease development. Five studies were identified that specifically examined the change in viral load versus the risk of developing CMV disease. The first of the 5 studies showed that the rate of increase in CMV load between the last PCR-negative and first PCR-positive sample was significantly faster in patients with CMV disease ($\log_{10}$ 0.33 versus $\log_{10}$ 0.19 genomes/mL daily; $P < .001$) [44]. In multivariate-regression analyses, both initial CMV load and rate of viral load increase were independent risk factors for CMV disease [44].

A second study showed that the rate of increase in viral replication was strongly associated with progression to CMV disease [45]. A third study in lung transplant recipients,demonstrated that 1 – $\log_{10}$ increases in CMV DNA levels at any time point during the first 6 months after transplant predicted CMV pneumonitis (sensitivity, 67%; specificity, 93%; $P < .01$) [37]. Another study demonstrated a 5- to 10-fold increase in the CMV DNA titers just prior to disease development [46]. Finally, in a study of kidney transplant recipients, an increase in viral load of $\log_{10}$ 0.7 copies per week also distinguished between CMV disease and asymptomatic seropositive recipients with high sensitivity (100%) and specificity (95%) [47]. In summary, all 5 studies suggested that a more rapid viral load increase correlated with the development of CMV disease.

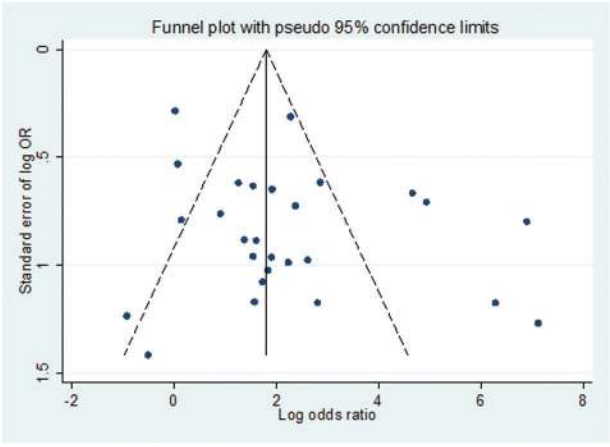

Figure 3. Funnel plot of studies for relationship between cytomegalovirus load and disease (all studies excluding outliers, n = 27). Each dot represents a log odds ratio of each study. Black line represents calculated mean log odds ratio with all studies after excluding 3 outliers. Abbreviation: OR, odds ratio.

Viral Load During and After Antiviral Prophylaxis

We hypothesized that, if viral load was an appropriate surrogate marker, then rates of viremia and disease may be low while a patient was on appropriate prophylaxis and significantly higher after discontinuation of prophylaxis. We identified 44 prophylaxis studies [20, 25–27, 35–37, 48–84]. Of these, 27 studies were identified as prophylaxis studies, whereas 17 studies were identified as prophylaxis versus preemptive therapy studies. The pooled incidence of CMV viremia during prophylaxis was determined for 24 studies for which sufficient data were available. The incidence of CMV viremia during prophylaxis varied from 0% to 18.6%. In the random-effect model, the pooled incidence of CMV viremia while on prophylaxis was 3.2% (95% CI, 2.0%–4.4%) (Figure 4). This contrasted with the incidence of viremia during the entire follow-up period (this includes during and after discontinuation of prophylaxis), which was 5.8% to 73% of patients. After excluding 1 outlier [65], we determined a pooled incidence for viremia of 34.3% (95% CI, 30.0%–38.6%; $P < .001$ vs. pooled incidence during prophylaxis) (Figure 5) (Table 2).

We next analyzed the incidence of CMV disease during prophylaxis based on data from 22 studies. The incidence of CMV disease during prophylaxis varied from 0% to 22.0%. Studies showed good homogeneity ($P = .10$), so a fixed-effect model was used. The pooled incidence was 0.8% (95% CI, 0.4%–1.3%) (Figure 6). In contrast, during the entire follow-up period, CMV disease incidence ranged 0%–36.8% in 41 studies. After excluding 1 outlier [65], we showed that the pooled incidence of disease was 13.0% (95% CI, 10.5%–15.5%; random-effects model; $P < .001$ vs pooled incidence during prophylaxis) (Figure 7). Therefore the incidence of viremia and disease during prophylaxis was overall low, with the majority of viremia and disease occurring after discontinuation of prophylaxis (Table 2).

Therapy of Asymptomatic Viremia and Disease Prevention

We hypothesized that if viral load were an appropriate surrogate marker, then therapy of asymptomatic viremia should prevent progression to CMV disease. This is in fact the basis of preemptive strategies for CMV disease prevention. We identified 32 studies [23, 54–59, 62, 68, 70, 72, 76, 77, 80–83, 85–100] addressing this, of which 17 studies were prophylaxis versus preemptive therapy studies. The incidence of

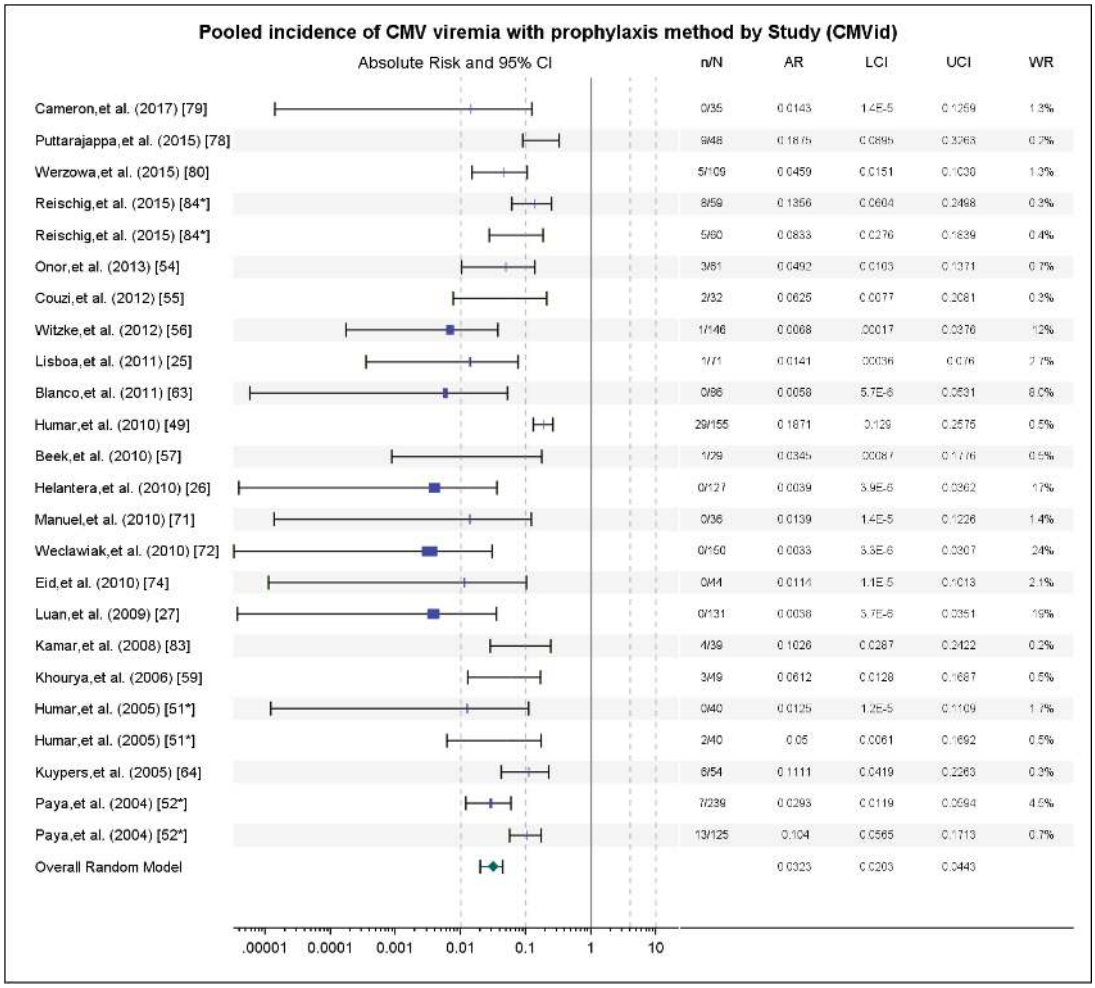

Figure 4. Pooled incidence of cytomegalovirus viremia during antiviral prophylaxis showing the absolute risk and 95% confidence intervals for each study (n = 24). Abbreviations: AR, absolute risk; CI, confidence interval; CMV, cytomegalovirus; LCI, lower confidence interval; N, total number of patients in the study; n, patients with the outcome of interest (cytomegalovirus viremia); UCI, upper confidence interval; WR, weight (random). *Different cohort from same reference.

CMV viremia with a preemptive strategy varied from 5.3% (CMV-seropositive liver transplant recipient) to 91.7% (CMV D+/R− kidney, liver, and heart transplant recipients) with a pooled incidence of CMV viremia of 48.9% (95% CI, 39.6%–58.1%) (Figure 8). The incidence of CMV disease varied from 0% to 26.3% with a pooled incidence of 6.9% (95% CI, 5.2%–8.5%) (Figure 9). The pooled disease incidence was significantly lower than the pooled viremia incidence ($P < .001$) (Table 2).

Viral Load Response Versus Symptom Resolution

We hypothesized that, if viral load is a valid surrogate marker, then clinical response in patients with CMV disease should mirror virologic response. Three studies were identified that provided relevant data [6, 101, 102]. Two studies were from the VICTOR cohort, a large randomized trial comparing intravenous ganciclovir versus oral valganciclovir for the treatment of CMV disease [101, 102]. Both studies demonstrated a correlation between viral load decline and symptom resolution for both CMV tissue-invasive disease and viral syndrome using either the COBAS Amplicor [101] assay or the Roche Taqman international unit–based assay [102]. For example, of 267 patients, 251 had CMV disease resolution by day 49 of treatment. Patients with pretreatment CMV DNA of <18 200 IU/mL had a faster time to disease resolution ($P = .001$), and patients with CMV load suppression at days 7, 14, and 21 had faster times to clinical disease resolution ($P = .005$, <.001, and <.001, respectively) [102]. One additional study assessed 26 patients with biopsy-proven gastrointestinal CMV disease [6]. The median time to negative viral load (22.5 days) was similar to the median reported time to negative follow-up endoscopy (27.0 days).

Therefore, despite a small number of studies, viral load decline during the treatment phase seems to correlate well with symptom resolution, and specifically, achieving viral load negativity correlates strongly with symptom response.

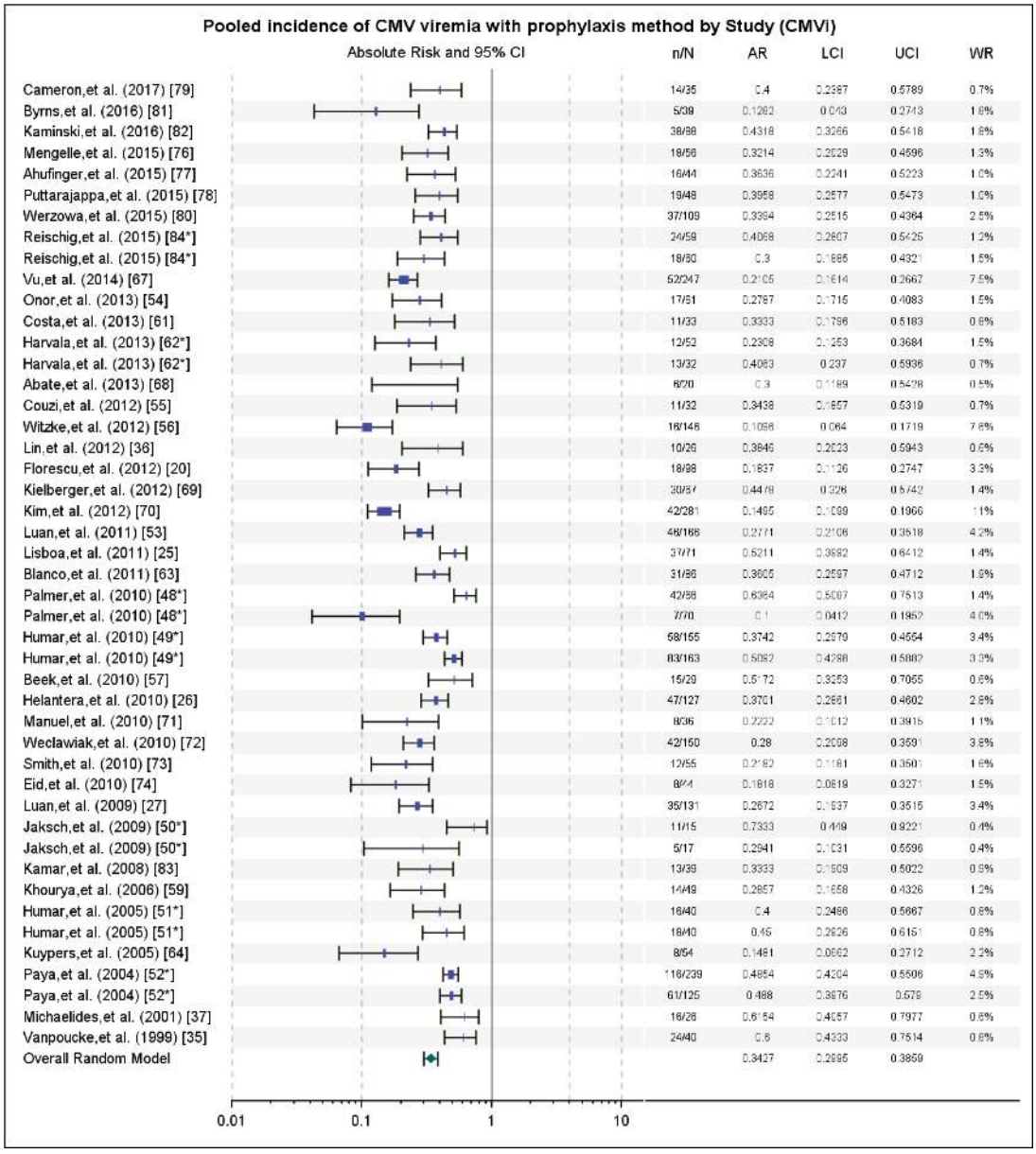

Figure 5. Pooled incidence of cytomegalovirus viremia for the entire follow-up period in studies using antiviral prophylaxis. Abbreviations: AR, absolute risk; CI, confidence interval; CMV, cytomegalovirus; LCI, lower confidence interval; N, total number of patients in the study; n, patients with the outcome of interest (cytomegalovirus viremia); UCI, upper confidence interval; WR, weight (random). *Different cohorts from same reference.

Table 2. Summary of Pooled Incidences of Cytomegalovirus Infection Depending on Prevention Method and Followup Period

Prevention method	Follow-up period[a]	Outcome	No. of studies	Model	Pooled incidence (%) (95% CI)	Heterogeneity (I^2), *P* value
Prophylaxis	During prophylaxis	CMV viremia	24	REM	3.2 (2.0–4.4)	75.5, $P < .001$
	During prophylaxis	CMV disease	24	FEM	0.8 (0.4–1.3)	28.1, $P = .10$
	Entire study period	CMV viremia	46	REM	34.3 (30.0–38.6)	88.5, $P < .001$
	Entire study period	CMV disease	48	REM	13.0 (10.5–15.5)	90.1, $P < .001$
Preemptive	Entire study period	CMV viremia	33	REM	48.9 (39.6–58.1)	98.2, $P < .001$
	Entire study period	CMV disease	27	REM	6.9 (5.2–8.5)	78.6, $P < .001$

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; FEM, fixed-effects model; REM, random-effects model.

[a]Entire study period includes complete study follow-up, including during and after prophylaxis.

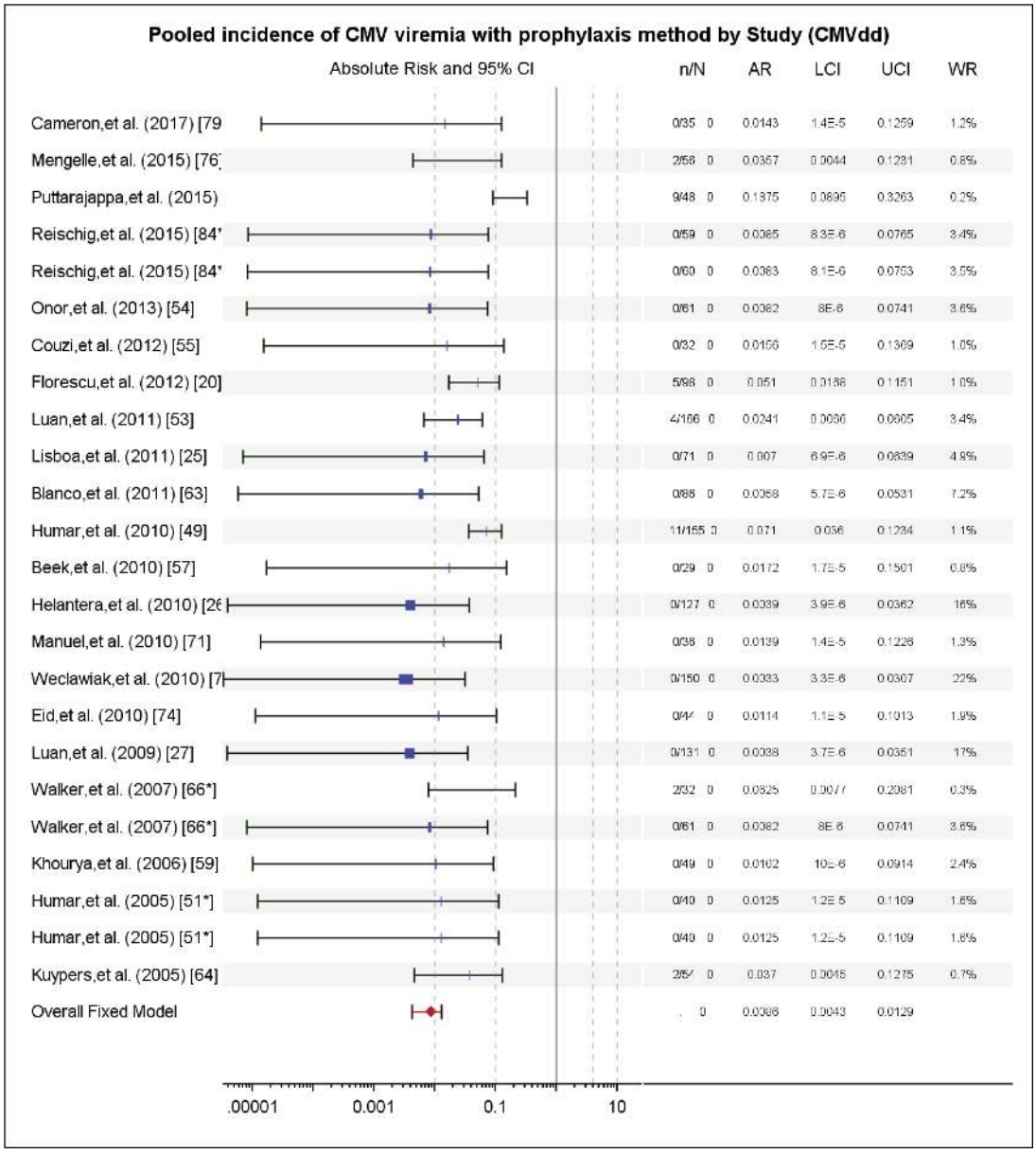

Figure 6. Pooled incidence of cytomegalovirus disease during antiviral prophylaxis. Abbreviations: AR, absolute risk; LCI, lower confidence interval; N, total number of patients in the study; n, patients with the outcome of interest (cytomegalovirus disease); UCI, upper confidence interval; WR, weight (random). *Different cohorts from same reference.

DISCUSSION

We conducted a systematic review and meta-analysis of data related to quantitative viral load testing for CMV and its potential utility as a surrogate marker in clinical trials of CMV after SOT. Viremia is considered a marker of viral lytic cycle activity, which is important for CMV disease development. To better define this, we assessed specific aspects related to different trial designs and indications of antivirals (prophylaxis, preemptive, and treatment). Five predefined and separate areas were examined to arrive at an overall understanding of the potential of viral load testing as a surrogate marker. First, we assessed whether viral load was higher in patients with asymptomatic viremia versus symptomatic CMV disease. We found the fold difference in viral load was much greater in disease versus asymptomatic viremia, (9.3, 7.0, 14.1 with all included studies, trials, studies using only the COBAS Amplicor PCR assay, and natural history studies, respectively). We then assessed whether the rate in change of viral load was predictive of CMV disease. The number of studies was smaller, but each demonstrated a consistent effect relating viral load kinetics to likelihood of disease development. In the third section, we reviewed viremia and disease during prophylaxis versus the entire period of follow-up. The pooled analysis demonstrated low rates of viremia and disease during periods of antiviral prophylaxis compared

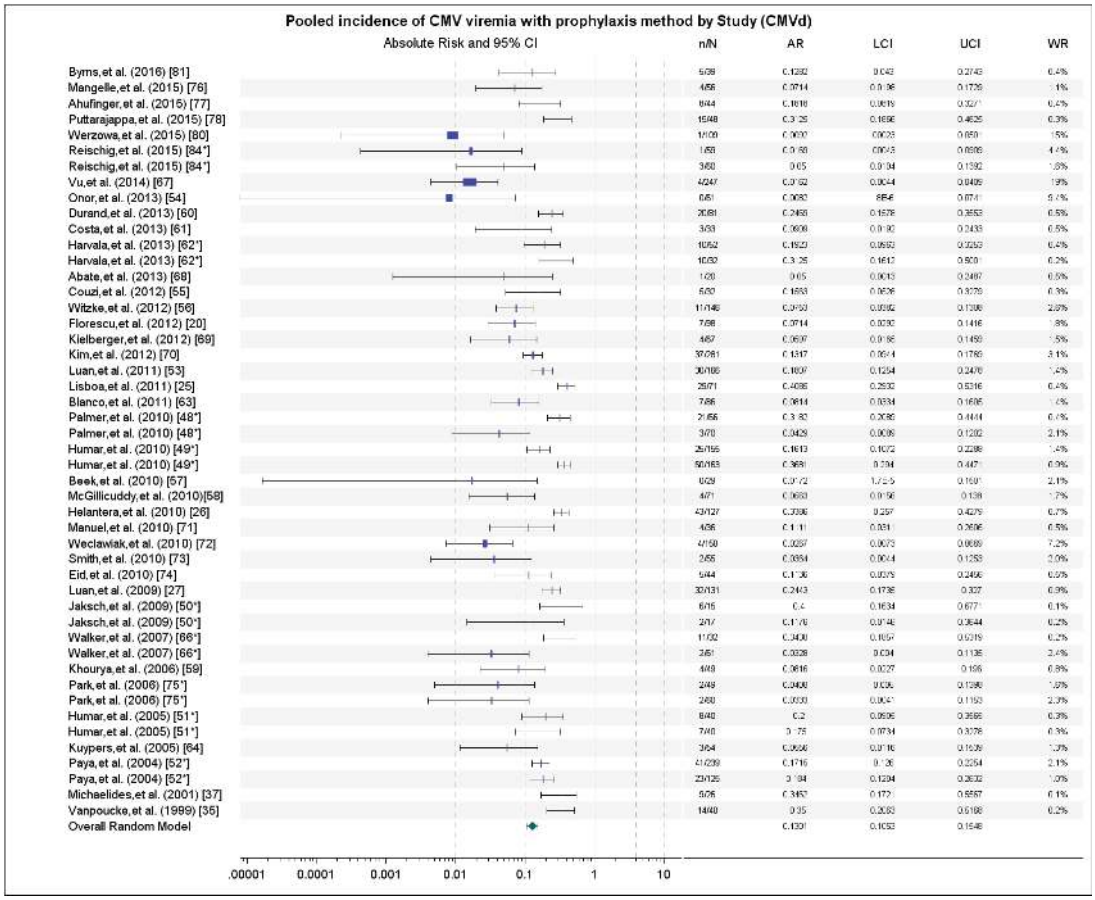

Figure 7. Pooled incidence of cytomegalovirus disease for the entire follow-up period in studies using antiviral prophylaxis. Abbreviations: AR, absolute risk; LCI, lower confidence interval; N, total number of patients in the study; n, patients with the outcome of interest (cytomegalovirus disease); UCI, upper confidence interval; WR, weight (random). *Different cohorts from same reference.

with a significantly higher pooled incidence of viremia and disease in the total follow-up period of the trial. We next reviewed the pooled data on treatment of asymptomatic viremia and the development of CMV disease (preemptive strategy). We observed that the pooled incidence of asymptomatic viremia was 48.9% and the pooled incidence of disease was 6.9%, suggesting that treatment of asymptomatic viremia results in a low incidence of subsequent disease. Finally, we assessed treatment studies, which linked symptom resolution to virologic response. The number of studies was small but strongly indicated that clearance of viremia correlated with symptom resolution.

Although these data in isolation do not provide definitive proof for the utility of viral load as a surrogate endpoint, when taken together, they provide compelling rationale that this may be a reasonable primary outcome in clinical trials. Given the low incidence of CMV disease in most modern clinical settings, coupled with difficulties in the definitive clinical diagnosis of CMV disease after SOT (especially viral syndrome), CMV load may in fact be a preferable and more robust endpoint.

This systematic review and meta-analysis has several limitations for each of the areas analyzed. In all cases, we combined data across studies to estimate effect sizes or associations. However, studies by their nature often include different transplant types, different immunosuppression drugs (eg, different induction agents or rejection episodes), and other factors that may result in heterogeneity of populations. For example, the studies by Lin et al and Guiver et al were less supportive of the link between CMV load and disease likely due to relatively small sample sizes. Analysis-specific limitations were also present. For example, in the analysis of viral load in asymptomatic versus symptomatic patients, viral load assays (including in-house assays), disease definitions, and different prophylactic regimens all likely influence the outcome. To better control for this, we also assessed just studies that used the COBAS Amplicor assay (as the most common viral load assay) and natural history studies. We also did not differentiate viral loads between CMV syndrome and end-organ disease because a very small number of studies addressed these differences. This analysis is also difficult to interpret because some patients labeled

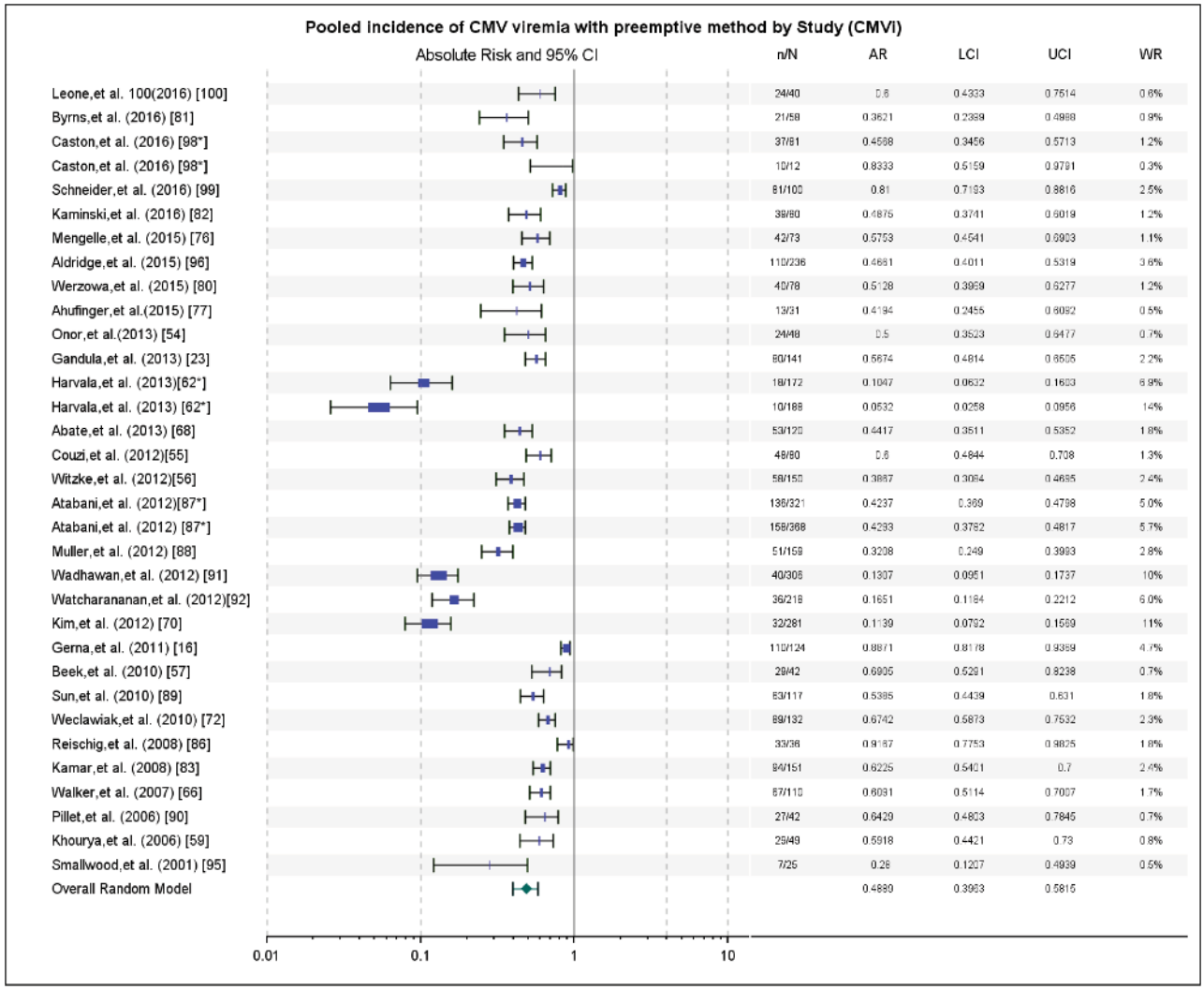

Figure 8. Pooled incidence of cytomegalovirus viremia in studies using preemptive method. Abbreviations: AR, absolute risk; LCI, lower confidence interval; N, total number of patients in the study; n, patients with the outcome of interest (cytomegalovirus disease); UCI, upper confidence interval; WR, weight (random). *Different cohorts from same reference.

as syndrome may have had end-organ disease but did not have a biopsy to prove it. Finally, there are limited pediatric data, and the results presented here may not be generalizable to this population.

Although CMV viremia can be associated with end-organ disease, there are exceptions, including occasional patients who develop CMV disease (especially gastrointestinal disease) with an undetectable viral load [6]. The pathogenesis of end-organ disease in the absence of viremia is uncertain but may reflect local organ-restricted CMV reactivation, something that may behave differently in primary infection versus reactivation or reinfection. Similarly, assessment of viral load in specific organs or tissues such as bronchoalveolar lavage fluid is not included in this analysis. Future clinical trials of candidate CMV drugs or vaccines should therefore attempt to differentiate among primary infection, reinfection, and reactivation and consider that the validity of CMV viremia as a surrogate marker may differ in these different types of infection.

This study was not designed to address the question of optimal viral thresholds, and as noted in the CMV Consensus Guidelines, there are insufficient data for this [1]. The majority of studies analyzed in this review used viral load in copies per milliliter rather than the international standard; however, the use of the international standard is to assist in comparison across laboratories [103], whereas the purpose of this analysis was to assess the potential for surrogacy of viral load regardless of how it is measured.

The findings in this study are consistent with published data in other areas. For example, in human immunodeficiency virus patients, CMV viremia has been shown to be an independent predictor of mortality [104, 105]. In addition,

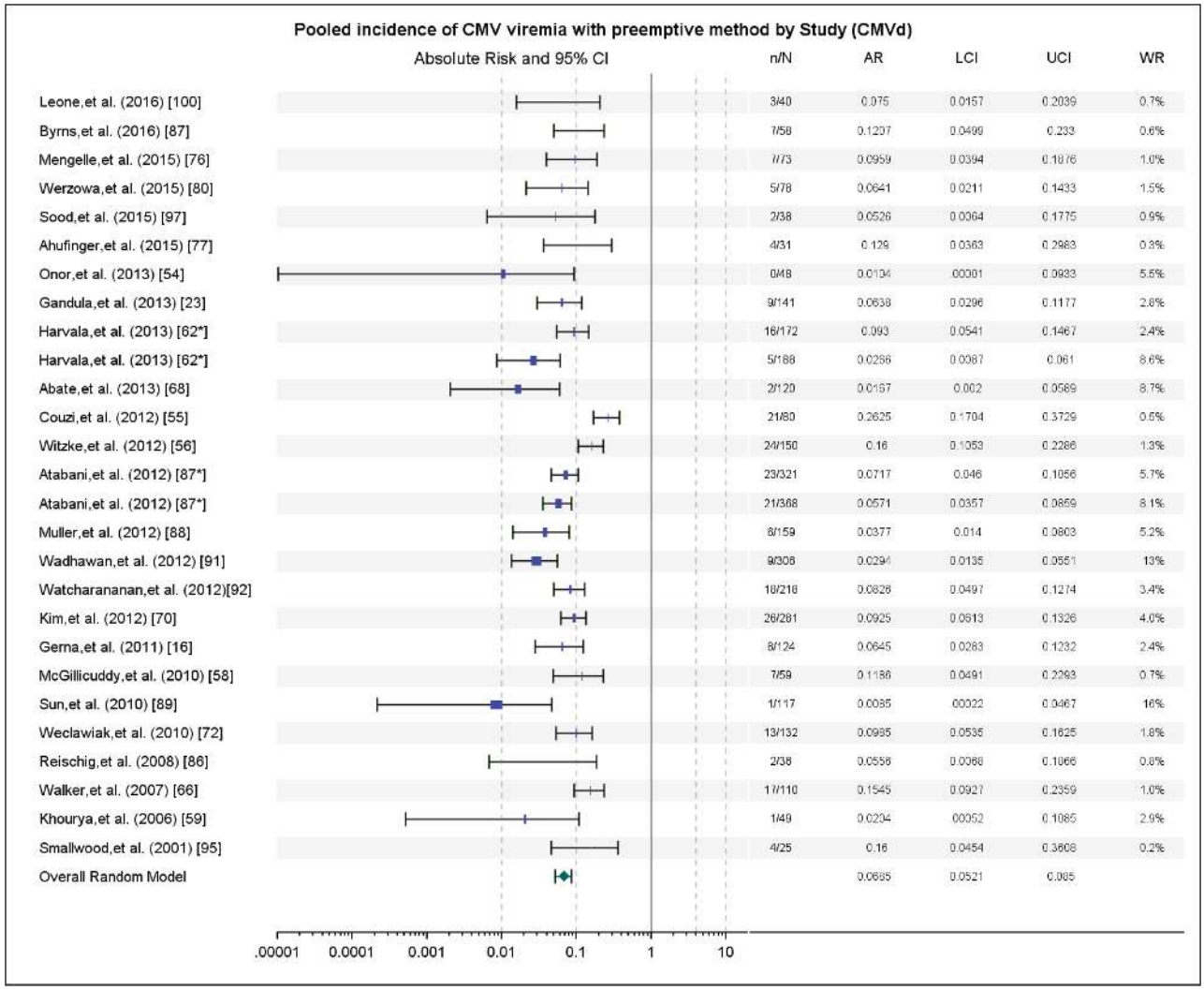

Figure 9. Pooled incidence of cytomegalovirus disease in studies using preemptive method. Abbreviations: AR, absolute risk; LCI, lower confidence interval; N, total number of patients in the study; n, patients with the outcome of interest (cytomegalovirus disease); UCI, upper confidence interval; WR, weight (random). *Different cohorts from same reference.

for other viruses, including hepatitis C virus, hepatitis B virus, and human immunodeficiency virus, viral load is routinely used as a surrogate endpoint in clinical trials. For example, recent trials of direct-acting antivirals for HCV routinely use virologic clearance as their primary endpoint [106, 107].

In conclusion, we performed a systematic review of viral load testing in different types of CMV-related trials in solid organ transplant recipients (prophylaxis, preemptive, and treatment). Based on this systematic review, we conclude that viral load likely predicts clinical endpoints in CMV trials in solid organ transplant recipients and may have some logistic and practical benefits over clinical endpoints. Overall, use of viral load as a surrogate endpoint in clinical trials may help speed up development of new antiviral agents and new diagnostics such as cell-mediated immunity assays.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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