Cytomegalovirus Infection and Relative Risk of Cardiovascular Disease (Ischemic Heart Disease, Stroke, and Cardiovascular Death): A Meta-Analysis of Prospective Studies Up to 2016

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Background—Several studies have suggested that cytomegalovirus infection is likely associated with an increased relative risk of cardiovascular disease (CVD); however, the results are inconsistent. We aimed to provide a systematic review and meta-analysis of community-based prospective studies assessing the association between cytomegalovirus infection and relative risk of CVD.

Methods and Results—We searched Medline and EMBASE to retrieve prospective studies that reported risk estimates of the association between cytomegalovirus infection and relative risk of CVD. The search yielded 10 articles including a total of 34 564 participants and 4789 CVD patients. Overall, exposure to cytomegalovirus infection was associated with a 22% (relative risk: 1.22, 95% CI: 1.07−1.38, *P*=0.002) increased relative risk of future CVD. We estimated that 13.4% of CVD incidence could be attributable to cytomegalovirus infection.

Conclusions—In conclusion, cytomegalovirus infection is associated with a significantly increased relative risk of CVD. (***J Am Heart Assoc***. 2017;6:e005025. DOI: 10.1161/JAHA.116.005025.)

Key Words: cardiovascular disease risk factors • cytomegalovirus • infectious disease • meta-analysis • prospective cohort study • virus

T he current study aims to examine the relative risk of cardiovascular disease (CVD) in cytomegalovirus-infected persons by summarizing the currently available prospective evidence.

CVD is the leading cause of morbidity and mortality in the population worldwide. The occurrence of CVD in populations is incompletely explained by traditional cardiovascular risk factors, and the identification of additional risk factors of CVD would have profound implications for the development of new preventative strategies that could improve public health.

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Cytomegalovirus is a DNA virus that belongs to the herpes family of virus.[1] Cytomegalovirus infection is widely distributed in the population.[2] Moreover, previous studies have provided evidence that infection with cytomegalovirus may play a role in the development of atherosclerosis. For example, researchers have detected cytomegalovirus DNA in atherosclerotic plaques,[3–5] and the presence of cytomegalovirus has been correlated with restenosis in patients who have undergone coronary atherectomy or angioplasty.[6,7] Other studies found the level of serum cytomegalovirus DNA was higher in patients with stable coronary artery disease and acute coronary syndrome than in healthy controls[8,9]; cytomegalovirus IgG seropositivity was associated with future risk of stroke after adjusting for other risk factors.[10] Furthermore, there is growing evidence implying an important role of this virus in vascular pathology by introducing slow but persistent inflammation in the vessel wall.[11]

Despite these studies, whether cytomegalovirus infection increases the relative risk of CVD remains uncertain. In the Framingham Heart Study, researchers found that cytomegalovirus IgG seropositivity is not associated with incidence of CVD during 10 years of follow-up.[12] On the contrary, cytomegalovirus IgG seropositivity was associated with a slight excess risk of subsequent myocardial infarction, stroke, or cardiovascular death in HOPE (Heart Outcomes Prevention Evaluation) study patients.[13] While several studies found that

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Clinical Perspective

What Is New?

- Our study suggests that exposure to cytomegalovirus infection is associated with a 22% (relative risk: 1.22, 95% CI: 1.07−1.38, *P*=0.002) increased risk for future development of cardiovascular disease. We estimate that 13.4% of the cardiovascular disease incidence can be attributed to a cytomegalovirus infection.

What Are the Clinical Implications?

- Given the high prevalence and incidence of cytomegalovirus infection and the burden of cardiovascular disease in the population, our research provides an incentive to develop a vaccine for cytomegalovirus as a potential preventive measure for cardiovascular disease.

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cytomegalovirus infection is linked to a higher relative risk of CVD,[14,15] negative results were also reported in other studies.[12,16] Therefore, we conducted a systemic review and a meta-analysis to determine the relationship between cytomegalovirus infection and relative risk of future CVD events. We also performed subgroup analysis by different population features and study characteristics.

Methods

Search Strategy and Study Selection

Two authors (H.W. and J.B.) independently searched Medline and EMBASE for studies reporting the association between cytomegalovirus infection and risk of CVD morbidity and mortality up to October 2016. Our overall search strategy included key words for cytomegalovirus infection (eg, cytomegalovirus, cytomegalovirus infection, cytomegalovirus IgG, cytomegalovirus antibody, and cytomegalovirus seropositive) and CVD (eg, CVD, cardiovascular disease mortality, ischemic heart disease [IHD], coronary heart disease, coronary artery disease, myocardial infarction, stroke, and heart failure). In addition, we searched the reference lists of all retrieved articles and relevant reviews. The eligibility of studies was assessed through a 3-step process. First, 2 independent reviewers performed an initial screening of all titles and abstracts according to the following criteria: (1) original research articles published with English language were included, and other types of articles, including reviews, editors, commentaries and meta-analyses, or published with other languages were excluded; (2) population-based prospective studies reporting the relationship between cytomegalovirus infection and CVD risk were included without age limitation, and articles focused on other exposure or

outcomes, or designed as a retrospective (case−control, cross-sectional, or nested case−control) study were excluded. The full texts of all potentially relevant articles were then reviewed, and studies were included if they met the following criteria: (1) predefined diagnosis criteria for both cytomegalovirus infection and CVD; and (2) reported a risk estimate (by univariate or multivariate statistic) and 95% CI (eg, hazard ratio or relative risk [RR] relating cytomegalovirus infection to subsequent CVD events). Finally, discrepancies were resolved by consensus or consultation with a third reviewer. Also, we assessed agreement between reviewers with the kappa statistics.[17]

Data Extraction and Quality Assessment

Two authors (H.W. and J.B.) independently abstracted the characteristics and risk estimates of the included studies by using a predesigned data abstraction form. The form included questions on the primary authors' name, years of publication, numbers of total participants and CVD patients, years of follow-up, and the characteristics of cytomegalovirus infection and outcome. The methods for the ascertainment of outcomes were carefully reviewed for each included study, and were classified as referencing to secure records (hospital records, autopsy records, death certificates, or telephone interviews) or by International Classification of Diseases codes. To assess risk of bias of individual studies, we performed study quality assessment according to the Newcastle-Ottawa scale, which was recommended by the Cochrane guidelines. The detailed criteria for assessing study quality and the quality score of each included study are presented in Table 1.

Data Synthesis and Analysis

The principal estimate was the RR. The hazard ratio, a type of RR, was directly considered as RR.[22] For each study included, we retrieved the reported RR estimates and the corresponding 95% CIs for the assessed outcomes. If several estimates were reported in the same study, we chose the most adjusted estimate that may reduce the impact of confounding factors. We calculated a pooled RR estimate across all studies by a random-effects model that assumes that individual studies are estimating different association effects. We adopted this model for it is probably the most conservative analysis to account for variance within and between studies and take into account the presence of heterogeneity into their calculations.[23] Heterogeneity between studies was assessed using Cochran Q statistics and I^2 statistics.[24] We considered the result for heterogeneity to be significant at $P<0.10$ (2-sided) for the Q statistics. Heterogeneity was classified as low ($I^2<25\%$), moderate ($I^2<50\%$), or high ($I^2>50\%$). To detect publication bias that may affect the cumulative evidence, we

Table 1. Quality Score Assessment Criteria for the Included Studies

	Score	Gkrania-Klotsas et al, 2012[18]	Haider et al, 2002[12]	Fagerberg et al, 1999[16]	Simanek et al, 2011[19]
Selection					
(1) Representativeness of the exposed cohort					
(a) Truly representative of the individuals exposed to CMV infection in the community	2	2	2	2	2
(b) Somewhat representative of the individuals exposed to CMV infection in the community	1	...	...	...	...
(c) Selected group of users (eg, nurses, volunteers)	0	...	...	...	...
(d) No description of the derivation of the cohort	0	...	...	...	...
(2) Selection of the nonexposed cohort					
(a) Drawn from the same community as the exposed cohort	2	...	...	2	2
(b) Drawn from a different source	1	...	...	...	...
(c) No description of the derivation of the nonexposed cohort	0	...	...	...	...
(3) Ascertainment of exposure					
(a) Laboratory test	2	2	2	2	2
(b) Medical record	1	...	...	...	...
(c) Written self report	0	...	...	...	...
(4) Demonstration that outcome of interest was not present at start of study					
(a) Yes	1	1	1		1
(b) No	0	...	...	0	...
Comparability					
(1) Comparability of cohorts on the basis of the design or analysis					
(a) Study controls for age and any additional factor	2	2	2	2	2
(b) Study controls for any confounding factor	1	...	...	...	...
(c) No adjustment	0	...	...	...	...
Outcome					
(1) Assessment of outcome					
(a) Referencing to secure records	3	...	3	3	...
(b) Record linkage	2	2	...	...	2
(c) Self report	1	...	...	...	...
(d) No description	0	...	...	...	...
(2) Was follow-up long enough for outcomes to occur?					
(a) Yes ($\geq$4 y)	1	1	1	1	1
(b) No	0	...	...	...	...
Total score	13	12	13	12	12
Quality level*		High	High	Medium	High

	Score	Roberts et al, 2010[14]	Spyridopoulos et al, 2016[20]	Corrado et al, 2006[21]	Smieja et al, 2003[13]	Elkind et al, 2010[10]
Selection						
(1) Representativeness of the exposed cohort						
(a) Truly representative of the individuals exposed to CMV infection in the community	2	2	2	2	2	2

Continued

Table 1. Continued

|	Score	Roberts et al, 2010[14]	Spyridopoulos et al, 2016[20]	Corrado et al, 2006[21]	Smieja et al, 2003[13]	Elkind et al, 2010[10]
(b) Somewhat representative of the individuals exposed to CMV infection in the community	1	…	…	…	…	…
(c) Selected group of users (eg, nurses, volunteers)	0	…	…	…	…	…
(d) No description of the derivation of the cohort	0	…	…	…	…	…
(2) Selection of the nonexposed cohort						
(a) Drawn from the same community as the exposed cohort	2	2	2	2	2	2
(b) Drawn from a different source	1	…	…	…	…	…
(c) No description of the derivation of the nonexposed cohort	0	…	…	…	…	…
(3) Ascertainment of exposure						
(a) Laboratory test	2	2	2	2	2	2
(b) Medical record	1	…	…	…	…	…
(c) Written self report	0	…	…	…	…	…
(4) Demonstration that outcome of interest was not present at start of study						
(a) Yes	1	1	1	1	1	1
(b) No	0	…	…	…	…	…
Comparability						
(1) Comparability of cohorts on the basis of the design or analysis						
(a) Study controls for age and any additional factor	2	2	…	2	2	2
(b) Study controls for any confounding factor	1	…	1	…	…	…
(c) No adjustment	0	…	…	…	…	…
Outcome						
(1) Assessment of outcome						
(a) Referencing to secure records	3	…	3	3	…	3
(b) Record linkage	2	2	…	…	2	…
(c) Self report	1	…	…	…	…	…
(d) No description	0	…	…	…	…	…
(2) Was follow-up long enough for outcomes to occur?						
(a) Yes (≥4 y)	1	1	1	1	1	1
(b) No	0	…	…	…	…	…
Total score	13	12	12	13	12	13
Quality level*		High	High	High	Medium	High

CMV indicates cytomegalovirus.

*Quality level was defined as low (≤7), medium (8–10), or high (≥11) according to quality score.

visually examined the asymmetry of funnel plots in which the log estimates were plotted against their standard errors. Furthermore, we also employed an Egger regression test to calculate *P* values for quantifying publication bias. To assess the influence of each individual study on the pooled estimate, we performed sensitivity analysis by omitting 1 study at a time and recalculating the pooled RRs of the remaining studies. In addition, we carried out multiple subgroup analyses by different study characteristics. Finally, the overall quality of the evidence at each outcome level was assessed by the GRADE approach.[25] Analyses were performed with Stata Version 12.0 (StataCorp LP, College Station, TX).

We calculated absolute risk differences associated with cytomegalovirus infection by multiplying the background incidence rate of CVDs in the general population with (estimated RR−1), in which the RR was derived from this meta-analysis. Population-attributable risk was calculated based on the following equation: Population-attributable risk %=100×Pe (RR−1)/(Pe [RR−1]+1), for which Pe is the prevalence of the exposure (cytomegalovirus seropositivity) in
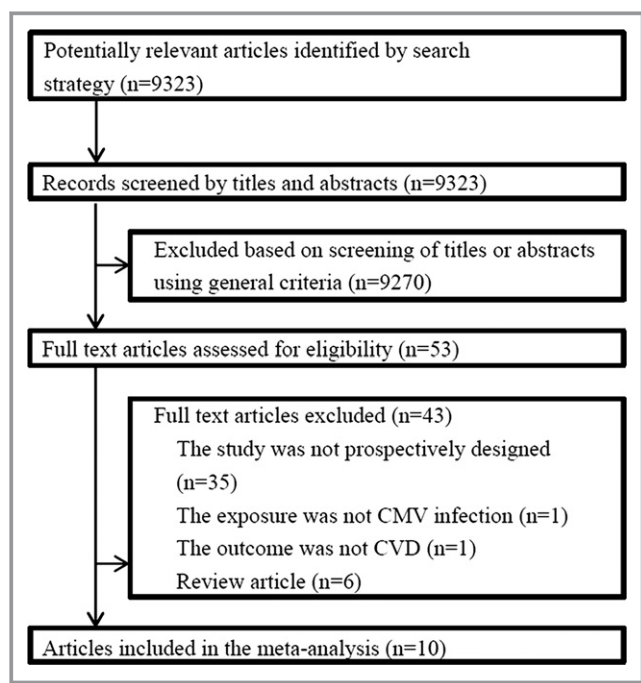
Figure 1. Flowchart on the selection of eligible studies. CMV indicates cytomegalovirus; CVD, cardiovascular disease.

the population and the RR was derived from this meta-analysis. Ethical approval was not required.

Results

Literature Search

Our literature search yielded 9323 articles. After the initial screening of titles and abstracts, a total of 9270 articles were excluded, leaving 53 articles for retrieval. Full text assessment of these articles resulted in 10 eligible articles that met our inclusion criteria, including a total of 34 564 participants and 4789 CVD patients. The inter-reviewer reliability for the study selection was almost perfect (κ=0.97). The procedure for identifying the studies is illustrated in Figure 1.

Description of Studies

Study-specific characteristics are shown in Table 2. Population characteristics are shown in Table 3. The studies were conducted in the United States,[10,12,14,19] United Kingdom,[15,18,20] Canada,[13] Sweden,[16] and Italy.[21] Nine studies included men and women,[10,12–15,18–21] and the other 1 study included only men.[16]

The ascertainment of cytomegalovirus infection varied across studies. All studies ascertained cytomegalovirus infection by serum cytomegalovirus IgG levels with various laboratory assays. Cytomegalovirus infection was defined as being positive for cytomegalovirus IgG antibody in 9 studies.[10,12,13,15,16,18–21] The other one defined exposure as the highest quartile of cytomegalovirus IgG titer.[14]

The method of outcome ascertainment varied across studies. Five studies ascertained CVD by referencing to secure records,[10,12,13,16,21] while the other 5 studies identified CVD events through International Classification of Diseases codes.[14,15,18–20] The range of time to follow-up for the cohort studies was between 4.5 and 18 years.

The quality of studies included in meta-analyses was assessed by applying the Newcastle-Ottawa scale for cohort studies. Table 1 lists details of how the criteria were applied to the included studies reporting cytomegalovirus infection and CVD risk as well as the scores assigned to each included study. Overall the level was adequate, with 3 of the 10 studies scoring 13[10,12,21] and 7 scoring 12.[13–16,18–20] Lower-scoring studies had identified CVD events through International Classification of Diseases codes,[13–15,18,19] had not demonstrated that outcome of interest was not present at start of study,[16] or had not fully controlled for confounding factors.[20] All studies had drawn noncases from the same population as cases, had adequate follow-up time (≥4 years) between exposure assessment and outcomes, and also reported that outcomes were ascertained by medical records or record linkage.

Systematic Review of Evidence

Of the 10 estimates, 4 reported that cytomegalovirus infection was associated with a significantly increased risk of CVD,[13–15,20] 4 that cytomegalovirus infection was associated with a nonsignificantly increased CVD risk,[10,18,19,21] and 2 that cytomegalovirus infection was associated with a nonsignificantly decreased CVD risk.[12,16] No study included in this systematic review reported that cytomegalovirus infection was associated with a significantly decreased risk of CVD.

Meta-Analysis

Figure 2 displays the results of the meta-analysis of the 10 studies. On pooling the retrieved measures of association, we found that prior cytomegalovirus infection was associated with a 22% increase in the relative risk of CVD (RR, 1.22; 95% CI 1.07−1.38; P=0.002), with evidence of moderate heterogeneity between studies (I^2=44.8%, Q=16.3, P=0.061). By pooling together the reported prevalence of cytomegalovirus infection of the included studies, we estimated that 70.1% of all individuals in the population are infected by cytomegalovirus. Using the risk estimate from our meta-analysis, we estimated that 13.4% (95% CI, 12.0−14.5%) of CVD events could be attributable to cytomegalovirus infection.

Table 2. Study Characteristics of the Included Studies

Author	Country	Cohort Name	Follow-up	Total Participants	Total CVD Patients	CVD Type	Adjustment
Gkrania-Klotsas et al, 2012[18]	UK	Cancer–Norfolk Cohort	12	11 022	1356	IHD, cardiovascular death	Age, sex, smoking, SBP, DBP, LDL, HDL, triglycerides, prevalent diabetes mellitus, family history, educational level, occupation, Townsend index, use of antihypertensives, statins, or glucose-control medications, alcohol use, body mass index, C-reactive protein
Haider et al, 2002[12]	US	Framingham Heart Study cohort	10	1187	199	IHD, stroke, cardiovascular death	Age, sex, body mass index, total cholesterol and HDL, diabetes mellitus, smoking, and hypertension
Fagerberg et al, 1999[16]	Sweden	NR	6.5	152	58	IHD, stroke, cardiovascular death	Smoking and the presence of previous CVD and for group allocation in the underlying multiple risk factor intervention study
Simanek et al, 2011[19]	US	National Health and Nutrition Examination Survey	13.8	14 153	1542	Cardiovascular death	Age, sex, race/ethnicity, country of origin, education level, body mass index (kg/m^2), smoking status, diabetes mellitus status, and C-reactive protein level
Smieja et al, 2003[13]	Canada	Heart Outcomes Prevention Evaluation	4.5	3168	905	IHD, stroke, cardiovascular death	Age, sex, smoking, ramipril, diabetes mellitus, hypertension, and hypercholesterolemia
Savva et al, 2013[15]	UK	ERSC Healthy Aging Study	18	511	138	Cardiovascular death	Date of birth and sex
Roberts et al, 2010[14]	US	Sacramento Area Latino Study on Aging	9	1329	220	Cardiovascular death	Age, sex, and education, myocardial infarction, congestive heart failure, stroke, dementia, liver/renal disease, diabetes mellitus, malignancy, and leukemia or lymphoma
Elkind et al, 2010[10]	US	The Northern Manhattan Study Mitchell	7.6	1625	67	Stroke	Age, sex, race/ethnicity, high school education, systolic blood pressure, HDL, LDL, blood glucose level, moderate alcohol use, cigarette smoking status, waist circumference, physical activity, and coronary artery disease
Spyridopoulos et al, 2016[20]	UK	The Newcastle 85+ study	6	749	184	Cardiovascular death	Sex
Corrado et al, 2006[21]	Italy	NR	5	668	120	IHD, stroke, cardiovascular death	Age, male sex, obesity, hypertension, diabetes mellitus, smoking habit, family history of CAD, and dyslipidemia

CAD indicates coronary artery disease; CVD, cardiovascular disease; DBP, diastolic blood pressure; ERSC Economic and Social Research Council; HDL, high-density lipoprotein; IHD, ischemic heart disease; LDL, low-density lipoprotein; NR, not reported; SBP, systolic blood pressure.

Begger funnel plots (Figure 3) did not show obvious asymmetry, and Egger test did not support the existence of publication bias (t=1.34, *P*=0.217). A sensitivity analysis of omitting 1 study in each turn showed that none of the individual studies influenced the pooled RR qualitatively (Figure 4). According to the GRADE guideline, the quality of evidence was leveled as low.

Subgroup Analyses

Figure 5 displays the results for subgroup analyses by different outcomes. Cardiovascular mortality results were available from 6 studies[14–16,19,20,26] with a pooled RR of 1.30 (95% CI, 1.03–1.66; *P*=0.029) from a random-effect model (Figure 3). A high-level heterogeneity was found with an

**Table 3.** Population Characteristics of the Included Studies

Author	Age	Male Sex (%)	BMI	CMV%	Obesity (%)	Smoke (%)	Diabetes Mellitus (%)	Hypertension (%)	Dyslipidemia (%)	Family History of CAD (%)	Previous CV Events (%)
Gkrania-Klotsas et al, 2012[18]	58.5	43.9	NR	58.4	NR	52.7	2.8	NR	NR	36.0	0.0
Haider et al, 2002[12]	69.0	38.0	26.7	68.8	NR	22.0	8.0	61.0	NR	NR	0.0
Fagerberg et al, 1999[16]	65.7	100.0	26.7	84.6	NR	36.2	6.9	NR	NR	NR	29.2
Simanek et al, 2011[19]	47.8	47.8	NR	66.7	23.5	55.7	6.0	NR	NR	NR	NR
Smieja et al, 2003[13]	66.0	73.3	28.0	25.0	NR	14.2	38.5	46.8	NR	NR	87.8
Savva et al, 2013[15]	74.1	49.0	NR	70.0	NR	67.5	NR	NR	NR	NR	NR
Roberts et al, 2010[14]	70.6	40.0	NR	70.4	NR	NR	47.0	67.1	NR	NR	NR
Elkind et al, 2010[10]	68.4	35.1	NR	85.4	NR	16.8	21.0	73.5	60.0	NR	20.9
Spyridopoulos et al, 2016[20]	85+	38.5	24.3	85.6	NR	NR	13.6	57.1	NR	NR	53.6
Corrado et al, 2006[21]	59.5	48.8	NR	33.7	16.6	NR	24.0	55.2	75.2	57.2	NR

BMI indicates body mass index; CAD, coronary artery disease; CMV, cytomegalovirus; CV, cardiovascular; NR, not reported.

I^2=68.7% (Cochrane Q statistic=15.95, *P*=0.007). Most of the studies found an RR above 1.00 except 1 study.[16] According to the GRADE guideline, the quality of evidence was leveled as very low because of serious imprecision and inconsistency.

IHD (including coronary artery disease, coronary heart disease, and myocardial infarction) risk results were available from 3 studies.[13,16,18] Of the 3 estimates, 2 reported that cytomegalovirus infection was associated with slightly increased relative risk of IHD,[13,18] and 1 that cytomegalovirus infection was associated with moderately decreased relative risk of IHD.[16] Evidence synthesis for IHD reported a slightly increased RR in those with cytomegalovirus infection (RR, 1.16; 95% CI, 0.95–1.42; *P*=0.145). According to the GRADE guideline, the quality of evidence was leveled as very low because of serious imprecision.
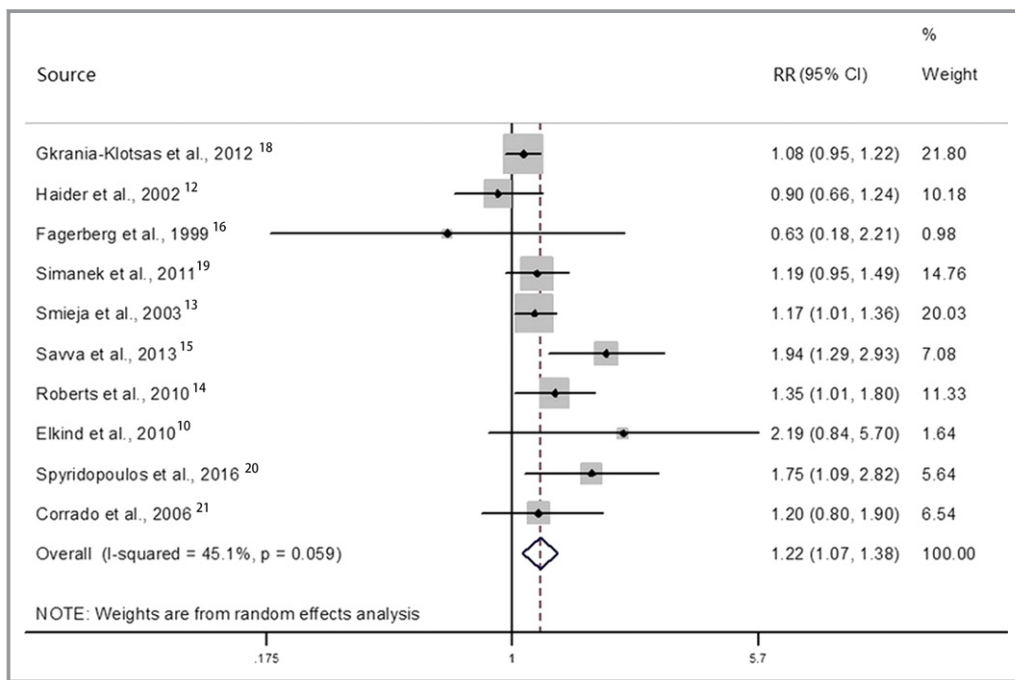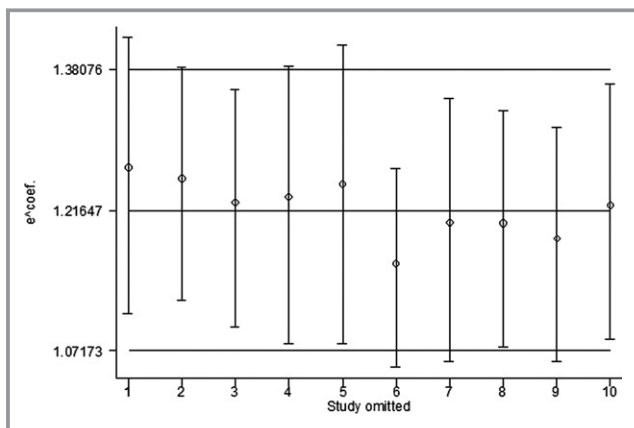

Figure 2. Association between CMV infection and risk of CVDs. Relative risks (RRs) in the individual studies are indicated by the data markers (shaded boxes around the data markers reflect the statistical weight of the study); 95% CIs are indicated by the error bars. The pooled-effect estimate with its 95% CI is depicted as a diamond. CMV indicates cytomegalovirus; CVDs, cardiovascular diseases.
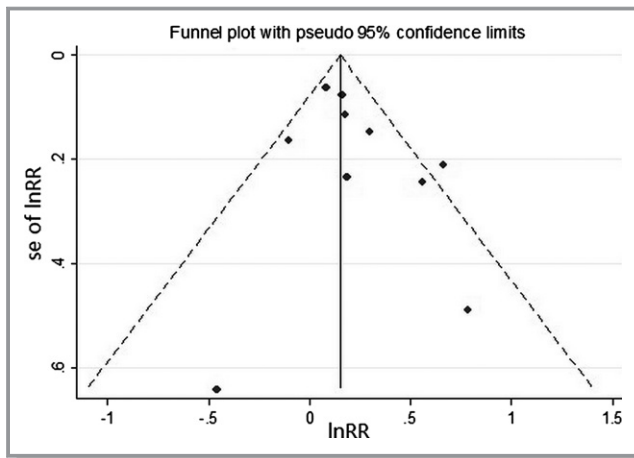
Figure 3. Funnel plots for bias assessment. Each point indicates an individual study. Funnel plots did not show obvious asymmetry. lnRR indicates natural logarithm of relative risk; se of lnRR, standard error of natural logarithm of relative risk.

Three estimates were available for stroke risk.[10,13,16] Two reported that cytomegalovirus infection was associated with slightly or moderately increased RR of stroke,[10,16] and 1 that cytomegalovirus infection was associated with slightly decreased risk of stroke.[13] Pooled estimate indicated a slightly increased stroke risk in those with cytomegalovirus infection (RR, 1.16; 95% CI, 0.67–2.01; *P*=0.145). According to the GRADE guideline, the quality of evidence was leveled as very low because of serious imprecision.

In addition, we also performed subgroup analyses according to population characteristics (Table 4). All subgroups indicated that cytomegalovirus infection was associated with increased RR of CVD, which suggested consistency across population characteristics.

Figure 4. Sensitivity analysis by omitting 1 study at a time. The figure indicates that none of the individual studies influenced the pooled RR qualitatively. RR indicates relative risk.
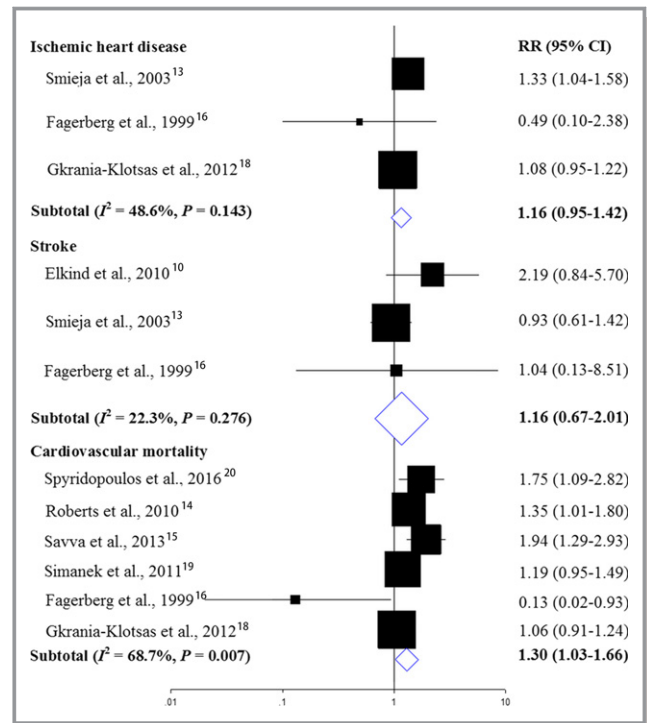
Figure 5. Associations between CMV infection and relative risk of IHD, stroke, and cardiovascular mortality. Relative risks (RRs) in the individual studies are indicated by the data markers. The size of the data markers indicates the weight of the study. The diamond data markers indicate the pooled RRs. CMV indicates cytomegalovirus; IHD, ischemic heart disease.

Discussion

To our knowledge, this meta-analysis is the largest review, including nearly 35 000 individuals and 5000 CVD patients, assessing the relationship between cytomegalovirus infection and risk of future CVD events. We found that cytomegalovirus seropositivity was associated with a 22% increase in the risk of CVDs (RR, 1.22; 95% CI 1.07–1.38; *P*=0.002). Furthermore, cytomegalovirus infection contributes to 13.4% of the epidemic of CVD events in the general population.

Although the current study has focused on cytomegalovirus, a large body of published work has suggested that various bacterial and viral infections might be associated with increased risk of CVD. Substantial evidence has been gathered to support the effect of *Chlamydia pneumonia* and influenza in CVD,[27,28] and the current study provides further evidence supporting the hypothesis that infectious pathogens including cytomegalovirus contribute to the epidemic of CVD in the general population.

Our study found significantly increased risk of mortality in cytomegalovirus-infected patients, but the results were nonsignificant for IHD and stroke. It should be noted that the sample sizes did not reach optimal size in IHD and stroke subgroups. According to GRADE guidelines, the results were

Table 4. Results for Subgroup Analyses

Subgroup	Included Study	Total Participants	RR (95% CI)	P Value
Mean age (y)				
≥70	Ref.[14,15,20]	2589	1.58 (1.26–1.99)	<0.001
<70	Ref.[10,12,13,16,18,19,21]	31 975	1.12 (1.03–1.21)	0.010
Male percentage				
≥40%	Ref.[13–16,18,19,21]	3561	1.20 (1.07–1.35)	0.002
<40%	Ref.[10,12,20]	31 003	1.38 (0.78–2.43)	0.268
Follow-up (y)				
≥10	Ref.[12,15,18,19]	26 874	1.17 (0.95–1.46)	0.147
<10	Ref.[10,13,14,16,20,21]	7690	1.26 (1.09–1.45)	0.001
Total participants				
≥1000	Ref.[10,12–14,18,19]	32 484	1.14 (1.03–1.26)	0.010
<1000	Ref.[15,16,20,21]	2080	1.51 (1.09–2.09)	0.014

RR indicates relative risk.

subjected to serious imprecision, and the quality of evidence was therefore classified as very low. Future studies may improve the quality of evidence. There are several potential explanations for the observed association between cytomegalovirus seropositivity and increased risk of CVD events. The most important was its role in thrombosis. Cytomegalovirus can directly infect cells of the vessel wall, where they could persist in a latent state or replicate at a low level.[29] It was shown that cytomegalovirus infection of endothelial cells causes the appearance of procoagulant activity on these cells.[30] In addition, there was data suggesting that cytomegalovirus can directly interact with prothrombinase proteins and substitute for synthetic procoagulant phospholipid vesicles to catalyze the generation of thrombin.[31] Furthermore, as the infection persists, it is well suited to induce proinflammatory cytokines, including tumor necrosis factor-α and IL-6, which are independently associated with CVD. Also, a recent study suggests that a substantial proportion of the relation between cytomegalovirus and mortality was mediated by circulating tumor necrosis factor-α and IL-6 levels. The high cytomegalovirus antibody levels probably reflect more frequent cytomegalovirus reactivation and higher levels of replication, leading to an increase in the proinflammatory cytokines tumor necrosis factor-α and IL-6 and enhanced vascular damage and plaque instability.[14]

There are several strengths of the current study. Our meta-analysis was based on several prospectively designed, population-based cohort studies. The combined sample size was large, and the follow-up period was long enough. We combined the estimates from the fully adjusted models of each included study in our analyses to reduce the impact of confounding. Despite these strengths, there were also several limitations that should be noted. First, it should be noted that seropositivity represents prior infection. This is insufficient to assess the viral infection activity or acute infection. Future studies should base infection on quantitative DNA to detect ongoing infection as active infection might increase one's risk for CVD. Second, the included studies were limited to those designed as a population-based prospective study and published in English. Therefore, our results may potentially be exposed to publication and language bias. Nonetheless, the included studies had generally satisfactory designs, methods, and outcomes, and all the included studies were of high quality. Furthermore, both funnel plot and Egger test indicated there was no evidence of publication bias. Third, most of the studies that were included were carried out in Europe and the United States, and this limits the direct generalization of our findings. Future high-quality prospective studies conducted on Asian and African populations are required to confirm our findings. Fourth, in spite of the large number of participants, the individual participant data were not available, and we were not able to explore the dose–response relationship between cytomegalovirus infection and CVD risk, or seek for interaction between cytomegalovirus infection and other risk factors. Also, event numbers were low for some outcomes (IHD and stroke); therefore, the power of subgroup analyses was limited. Nonetheless, the consistency of the evidence overall supports a real association between cytomegalovirus infection and CVD risk. Fifth, while our study demonstrated that cytomegalovirus infection may predispose to CVD events, chemoprophylaxis would not be feasible because of the common asymptomatic course of the infection, and there are no effective vaccines available currently. Finally, use of International Classification of Diseases codes to ascertain outcomes in several studies is another limitation of the data.

In conclusion, this meta-analysis provides strong evidence that cytomegalovirus infection is a significant risk factor for

CVD. Given the high prevalence and incidence of cytomega-lovirus infection and CVD in the general population, our research provides an impetus to develop a childhood vaccine as part of the fight against CVD.

Author Contributions

All authors contributed to data collection and wrote the manuscript. Hu and Liu drafted the study protocol. Wang, Peng, Bai, and Huang extracted data. Wang and Peng performed the analyses. Wang, Peng, and Bai drafted the paper. All authors critically reviewed the paper. Wang and Liu had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Wang and Liu are the guarantors of the paper. All authors approved the current manuscript to be published, attested that they contributed substantially to the current work, and disclosed that there was no writing assistance.

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Disclosures

None.

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