Systematic Review with Meta-analysis

Homocysteine-lowering therapy does not lead to reduction in cardiovascular outcomes in chronic kidney disease patients: a meta-analysis of randomised, controlled trials

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Abstract

The efficacy of homocysteine (Hcy)-lowering therapy in reducing the risk of CVD among patients with chronic kidney disease (CKD) remains controversial. We performed a meta-analysis to determine whether pooling the data from the few small randomised, controlled trials that address this topic would improve the statistical power of the analysis and resolve some of the inconsistencies in the results. Randomised, controlled clinical trials (RCT) were identified from MEDLINE, EMBASE, www.clinicaltrials.gov, the Cochrane Controlled Clinical Trials Register Database and Nephrology Filters. Independent extraction of articles was performed using predefined data fields. The primary outcome was relative risk (RR) of CVD, CHD, stroke and all-cause mortality for the pooled trials. A stratified analysis was planned, assessing the RR for cardiovascular events between the patients on and not on dialysis. Overall, ten studies met the inclusion criteria. The estimated RR were not significantly different for any outcomes, including CHD (RR 1·00, 95 % CI 0·75, 1·31, $P = 0·97$), CVD (RR 0·94, 95 % CI 0·84, 1·05, $P = 0·30$), stroke (RR 0·83, 95 % CI 0·57, 1·19, $P = 0·31$) and all-cause mortality (RR 1·00, 95 % CI 0·92, 1·09, $P = 0·98$). In the stratified analysis, the estimated RR were not significantly different for cardiovascular events regardless of dialysis or in combination with vitamin B therapy or the degree of reduction in Hcy levels. Our meta-analysis of RCT supports the conclusion that Hcy-lowering therapy was not associated with a significant decrease in the risk for CVD events, stroke and all-cause mortality among patients with CKD.

Key words: CVD: Chronic kidney disease: Folic acid: Homocysteine-lowering therapy: Homocysteine: Meta-analyses

CVD is the leading cause of mortality both among the general population and among patients with chronic kidney disease (CKD). The rate of mortality from CVD among patients on haemodialysis (HD) is 20-fold higher than that among the general population[1], and impaired renal function *per se* is a very strong cardiovascular prognostic factor[2,3]. Therefore, much attention has been focused on managing risk factors to attempt to reduce the associated CVD risks, especially among patients with CKD. Elevated plasma total homocysteine (Hcy) has been proposed as a risk factor for cardiovascular morbidity and mortality in patients either with normal renal function or with CKD[4,5]. Among patients with CKD, plasma Hcy levels tend to increase with decreasing glomerular

filtration rate[6]; thus, hyperhomocysteinaemia may potentially further increase the risk of CVD in this special population. In the Cardiovascular Risk Extended Evaluation in Dialysis trial, investigators followed-up on 175 patients with kidney failure for 29 months and found that an increase in Hcy of 10 μmol/l was associated with a 20 % increased risk for CVD events[7]. In another study, Hcy was found to be a strong independent predictor of mortality among patients on HD with a 3 % increase in mortality for each 1 μmol/l increase in plasma Hcy concentration[8].

Paradoxically, it has not been confirmed that therapy to lower the concentration of Hcy decreases the risk for CVD events among the general population[9,10] or among patients

with CKD[11]. Several small randomised, controlled trials (RCT) of Hcy-lowering therapy for patients on dialysis have produced inconsistent results[12–14]. Recently, a meta-analysis from seven Hcy-lowering therapy trials in patients with CKD showed that folic acid therapy reduced the risk of CVD by 15 % (RR 0·85, 95 % CI 0·76, 0·96, $P=0.009$), and a greater beneficial effect was observed among those trials with a decrease in Hcy levels > 20 % (RR 0·83, 95 % CI 0·73, 0·95, $P=0.007$)[15]. However, this meta-analysis did not include three subsequently published RCT of Hcy-lowering and CVD, and so this result leads to a controversy. Considering the inconsistent results obtained with Hcy-lowering therapy in patients with CKD, we thought it important to attempt to resolve the question of whether Hcy-lowering therapy could reduce the risk of CVD morbidity and all-cause mortality in CKD patients who either are or are not on dialysis. Therefore, we conducted a meta-analysis of published RCT to pool the results and, thus, improve the statistical power of the analysis.

Methods

Search strategy

RCT were identified in MEDLINE (source PubMed, 1966–2010), EMBASE (1974–2010), www.clinicaltrials.gov, the Cochrane Controlled Clinical Trials Register Database and Nephrology Filters (http://hiru.mcmaster.ca/hiru/HIRU_Hedges_Nephrology_Filters.aspx). We used the following terms to search all trial registers and databases: 'homocysteine OR HCY' AND 'chronic kidney disease OR End Stage Renal Disease (ESRD)' AND 'cardiovascular events' AND 'Folic acid', presence of a randomised control group, duration of intervention ≥ 12 months, availability of primary CVD or secondary CVD outcome data for morbidity, stroke and all-cause mortality among patients with CKD, including those on dialysis and those not on dialysis. We obtained 134 papers. In the second step, we then kept all of the clinical trials and deleted review papers and those involving animal experiments; thus, 124 studies were excluded. The detailed steps are shown in Fig. 1. The bibliographies of all retrieved articles were also checked manually. Finally, ten studies met the inclusion criteria for our meta-analysis. All included trials were published in English-language medical journals.

Eligibility criteria

Studies were eligible for inclusion if the study was: a randomised, controlled trial; duration of intervention was more than 12 months; availability of primary CVD or second CVD outcomes, stroke and all-cause death among patients with CKD including those on dialysis and those not on dialysis; the intervention with folic acid and/or in combination with vitamins available for change of Hcy concentrations during therapy.

Data collection

Data were collected by all authors and extracted independently by two authors (H. M. Jin and Y. Pan) for participants' characteristics (study design, participant's age, sex, duration of
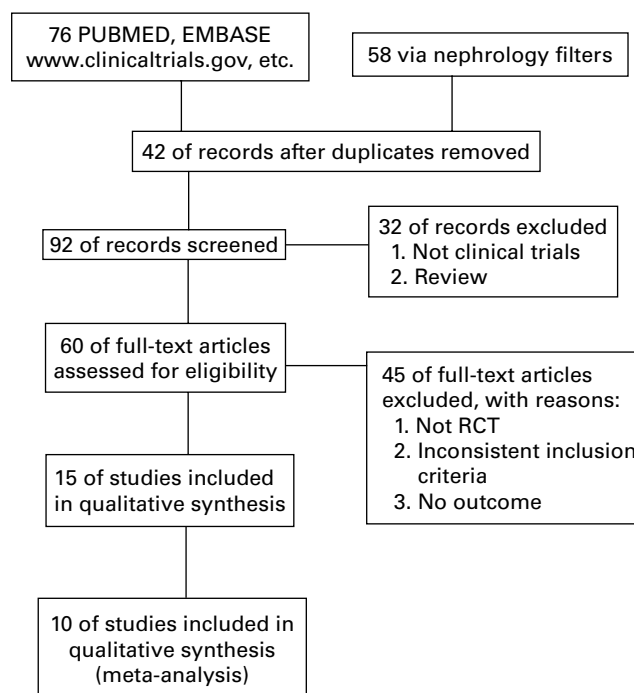

Fig. 1. Flow diagram of selection process for inclusion of studies in the meta-analysis. RCT, randomised, controlled clinical trials.

follow-up, co-interventions (folic acid and/or vitamin (Vit) B_6 and $VitB_{12}$), baseline concentrations of Hcy and net change in Hcy from baseline). Any disagreement in data extraction was resolved by discussion between them and in consultation with the other authors (Table 1).

Summary measures and synthesis of results

The primary outcome was relative risk (RR) for cardiovascular events, CHD, stroke and all-cause mortality from the pooled trials. We also used subgroup analysis to assess the confounding factors to affect the outcomes such as whether on dialysis or not on dialysis, prescription of $VitB_6$ or $VitB_{12}$ and the rate of Hcy-lowering. To assess the potential for publication bias, we constructed funnel plots for each outcome in which the log RR was plotted against their standard errors. We also conducted a sensitivity analysis in which each study was extracted in turn to evaluate the influence of the study on the estimate.

Statistical analyses

All different units of Hcy were converted to μmol/l, using the conversion factor 1 mg/l $= 7.397$ μmol/l[10]. RR were used as a measure of the association between the treatment group $v.$ the control group and risk of CVD, CHD, stroke and all-cause mortality. RR for each trial were based on the number of events in each group. RR and 95 % CI for each side effect were calculated. We also carried out subgroup analyses based on whether the patients were on dialysis or not on dialysis, prescription of $VitB_6$ or $VitB_{12}$, and the extent of decrease in Hcy levels to further analyse the potential confounding factors in exploration of the different CVD

Table 1. Baseline characteristics of participants in randomised, controlled trials

(Mean values and standard deviations)

Study	Study design	Participants (n)	Follow-up (years)	Age (years) Mean	Age (years) SD	Men (%)	Diabetes mellitus (%)	Pre-existing condition	Outcomes
Righetti et al.[16]	Randomised Placebo-controlled	81	1	64	14	55·7	12·9	Haemodialysis	CVD
Wrone et al.[13]	Randomised Double-blind Placebo-controlled	510	2	60·2	15·1	50	45·5	92 % Haemodialysis; 8 % peritoneal dialysis	CHD, CVD, stroke, all-cause death
Zoungas et al.[12]	Randomised Placebo-controlled Multicentre	315	3·6	56	13·5	32·3	23·2	60 % Haemodialysis; 25 % peritoneal dialysis	CHD, CVD, stroke, all-cause death
Righetti et al.[14]	Randomised Placebo-controlled	88	2·4	64·3	11·7	56·0	16·2	Haemodialysis	CHD, CVD, stroke, all-cause death
Vianna et al.[17]	Randomised Double-blind Placebo-controlled	186	2	49·3	13·5	66	22	Haemodialysis	CVD
Jamison et al.[20]	Randomised Double-blind Placebo-controlled	2056	3·2	66·2	11·5	98	55	Pre-dialysis	CVD, stroke, all-cause death
Nanayakkara et al.[18]	Randomised Double-blind Placebo-controlled	93	2	52	13	63	NR	Pre-dialysis	CVD
Mann et al.[19]	Randomised Double-blind Placebo-controlled	619	5	72·2	6·6	66	40	Pre-dialysis	CVD, all-cause death
House et al.[21]	Randomised Double-blind Placebo-controlled Multicentre	238	3	60·7	11·6	90	100	Pre-dialysis	CHD, CVD, stroke, all-cause death
Heinz et al.[22]	Randomised Double-blind Placebo-controlled Multicentre	650	2·1	61	13	58	43	Haemodialysis	CHD, CVD, stroke, all-cause death

NR, not reported.

outcomes. STATA version 10.0 (STATA Corporation) was used to pool the data and calculate RR. Statistical significance was set at $P < 0.05$ for all analyses. All analyses were conducted in parallel by two investigators (Y. Pan and H. M. Jin).

Results

Trial flow and study characteristics

The decision process that was used to differentiate among studies considered for inclusion is shown in Fig. 1. Overall, ten eligible studies with a total of 4836 participants were included in the review (2511 participants in the treatment group and 2325 participants in the control group)[12–14,16–22]. Of these, five of the selected trials focused on patients on dialysis, three focused on patients not on dialysis, and the remaining trial included patients among whom 60 % were on HD, 25 % were on peritoneal dialysis and 15 % were not on dialysis[12]. Effectively, nine of these trials provided data on the number of target events (including CVD, CHD, stroke and all-cause mortality) in each group, and one trial reported myocardial infarction as the cardiovascular event[20]. The net changes in blood Hcy are shown in Table 2. All trials showed a reduction in Hcy levels, in the range -2.4 to $-26.0\,\mu$mol/l (-8.9 % to -53.8 %). However, the characteristics of baseline Hcy existed in a hung range from 14·7 (SE 4·9) to 50·3 (SE 6·0) among included studies.

However, there was no significant association between the baseline of Hcy concentrations and the extent of Hcy-lowering.

Relative risk for CHD, CVD, stroke and all-cause mortality

The results from random-effect models pooling the RR for CHD, CVD, stroke and all-cause mortality are shown in Figs. 2–5. As noted, Jamison et al.[20] reported myocardial infarction as the cardiovascular event. The RR estimated were not statistically significantly different for any outcomes. The total number of CHD events was ninety-five among 981 in the treatment group and ninety among 820 in the control group, respectively (RR 1·00, 95 % CI, 0·75, 1·31, $P = 0.97$; Fig. 2); the total number of cardiovascular events was 501 among 2511 in the treatment group and 522 among 2325 in the control group (RR 0·94, 95 % CI 0·84, 1·05, $P = 0.30$; Fig. 3); the total number of strokes was eighty-five among 2013 in the treatment group and ninety-three among 1844 in the control group (RR 0·83, 95 % CI 0·57, 1·19, $P = 0.31$; Fig. 3); and the total all-cause mortality was 810 among 2320 in the treatment group and 757 among 2156 in the control group (RR 1·00, 95 % CI 0·92, 1·09, $P = 0.98$; Fig. 4).

Subgroup analysis of relative risk for CVD

Whether on dialysis or not on dialysis, prescriptions of $VitB_6$ or $VitB_{12}$, or the degree of Hcy-lowering were potential

Table 2. Reported changes in homocysteine (Hcy) levels

(Mean values and standard deviations; mean values and 95 % confidence intervals)

Study	Intervention groups	Baseline Hcy (μmol/l)*		Change from baseline	
		Mean	SD	μmol/l	%
Righetti et al.[16]	Folic acid: 5 mg/d or 15 mg/d	50·3	6·0	−26·0	−51·7
Wrone et al.[13]	Folic acid: 5 mg/d or 15 mg/d	32·9	20	−3·6	−10·9
Zoungas et al.[12]	Folic acid: 5 mg/d or 5 mg, every other day	35·0	13·1	−15·1	−49·3
Righetti et al.[14]	Folic acid: 15 mg/d	27	13·0	−2·4	−8·9
Vianna et al.[17]	Folic acid: 10 mg, three times a week	23·5	15	−10·5	−44
Jamison et al.[20]	Folic acid: 40 mg/d VitB$_6$: 100 mg/d VitB$_{12}$: 2 mg/d	22·5	10	−6·3	−25·8
Nanayakkara et al.[18]	Folic acid: 5 mg/d VitB$_6$: 100 mg/d VitB$_{12}$: 1 mg/d	20·2	6·8	−12·1	−53·8
Mann et al.[19]	Folic acid: 2·5 mg/d VitB$_6$: 50 mg/d VitB$_{12}$: 1 mg/d	15·9	7·3	−4·0	−25·2
House et al.[21]	Folic acid: 2·5 mg/d VitB$_6$: 25 mg/d VitB$_{12}$: 1 mg/d	14·7	4·9	−2·2	−15·0
Heinz et al.[22]	Folic acid: 5 mg VitB$_6$: 20 mg VitB$_{12}$:50 μg			−10·4	−35·9
Mean		29·0			
95 % CI		14·1, 63·3			

Vit, vitamin.

confounders related to both the outcome and the primary variable of interest. As Fig. 6 shows, there were six trials focused on patients on dialysis and four trials focused on pre-dialysis. The estimated RR indicated that there was no statistical association between Hcy-lowering and incidence of CVD events in both pre-dialysis and dialysis conditions (RR 0·91, 95 % CI 0·74, 1·12, $P=0·39$; RR 0·94, 95 % CI 0·74, 1·20, $P=0·62$, respectively). Furthermore, there was also no significant relevance between incidence of CVD events and whether the degree of Hcy decreased by $< 20\%$ or $\geq 20\%$ (RR 1·20, 95 % CI 0·78, 1·86, $P=0·41$; RR 0·87, 95 % CI 0·76, 1·01, $P=0·06$, respectively), although there was a declining tendency of CVD incidence in Hcy-lower $\geq 20\%$ subgroup ($P=0·06$). Similarly, there was no significant difference in CVD incidence whether folic acid was alone or was in combination with VitB$_6$ and VitB$_{12}$ (RR 0·88, 95 % CI 0·75, 1·03, $P=0·12$; RR 0·98, 95 % CI 0·76, 1·26, $P=0·88$, respectively).

Sensitivity analyses and publication bias

In sensitivity analyses, exclusion of any one study from the analysis did not alter the overall findings of the non-significant association between Hcy-lowering therapy and the risk of cardiovascular outcome. Within the sensitivity analysis, we considered variance in results as a possible source of small heterogeneity. Publication bias was assessed by visually examining a funnel plot of precision against hazard ratio (not shown), with asymmetry being formally assessed with the Egger test; and no significant bias was found (bias $= -0·07$, $P=0·94$).

Discussion

In this meta-analysis, ten RCT were combined to assess the effects of folic acid-based therapy with or without other vitamins on the risk for CVD events and all-cause mortality among patients with CKD who were either on or not on dialysis. The pooled results confirmed no significant benefit of Hcy-lowering therapy for the risk of CVD, stroke and all-cause mortality among this population of patients with CKD.

Numerous conflicts and inconsistencies have obscured the interpretation of the literature on this topic. For example, the plausibility of a link between the levels of Hcy and the risk of CVD was supported by a number of studies.
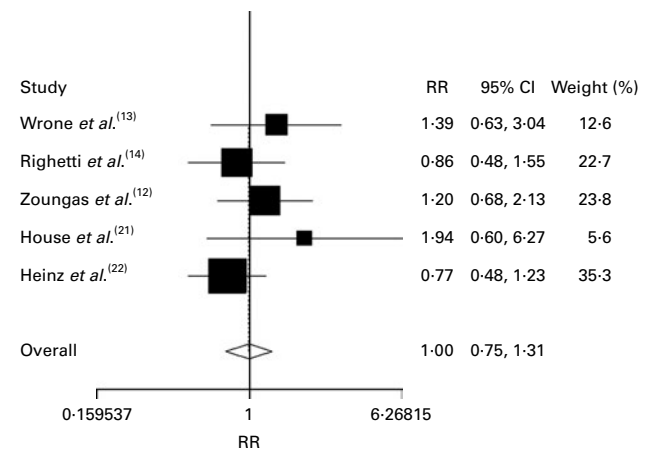

Fig. 2. Relative risk (RR) for CHD in pooled trials. The RR estimated were not significantly different for CHD (RR 1·00, 95 % CI 0·75, 1·31, $P=0·974$).
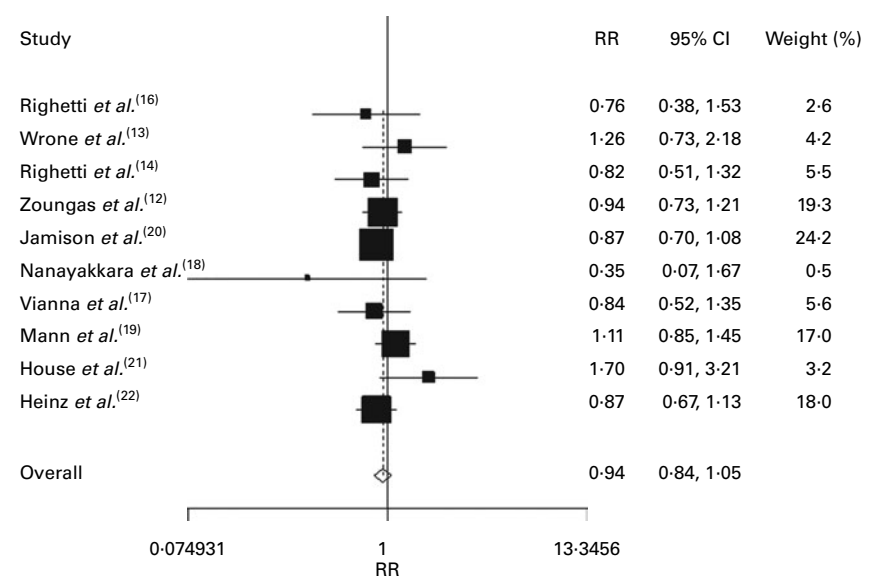
Fig. 3. Relative risk (RR) for CVD in pooled trials. The RR estimated were not significantly different for CVD (RR 0·94, 95% CI 0·84, 1·05, *P*=0·30).

One report demonstrated that endothelial cells can develop abnormal vasoreactivity and may adopt a prothrombotic phenotype and secrete leucocyte chemotactic factors, adhesion molecules and inflammatory cytokines when exposed to elevated Hcy[23]. Endothelial dysfunction is a key process in atherosclerosis, which independently predicts CVD events[24]; and it becomes progressively more common as renal function declines[25]. However, studies of supplementation with either 5 mg/d folic acid or 5 mg/d folic acid combined with 4 g/d betaine for 12 weeks, followed by either 1 or 5 mg/d folic acid for 40 weeks showed no improvement of endothelial function in patients on peritoneal dialysis or HD[26,27].

Another problem that complicates the interpretation of the literature on Hcy-lowering therapy is the lack of data supporting either an optimal dose of folic acid, an optimal target level

of Hcy, or the required duration of intervention in order to see an effect. Arnadottir *et al.*[28] observed that an average dose of 2·14 mg/d folic acid was as effective as either 5 or 10 mg/d over a 6-week period among HD patients for lowering Hcy; so 2 mg of folic acid has been recommended as the optimum dose for CKD patients. However, in clinical trials, attempts to normalise Hcy with either 1 or 5 mg/d folic acid were unsuccessful over a 40-week period among HD patients[27]. In fact, reduction to a normal Hcy level occurs in only about 20% of treated patients. Also, to the best of our knowledge, there are no studies that have determined a threshold value for Hcy level for effective prevention of cardiovascular events in the general population or in patients with CKD. Observational data in a meta-analysis show that a 25% reduction in Hcy was associated with a 32% lower risk of CVD among
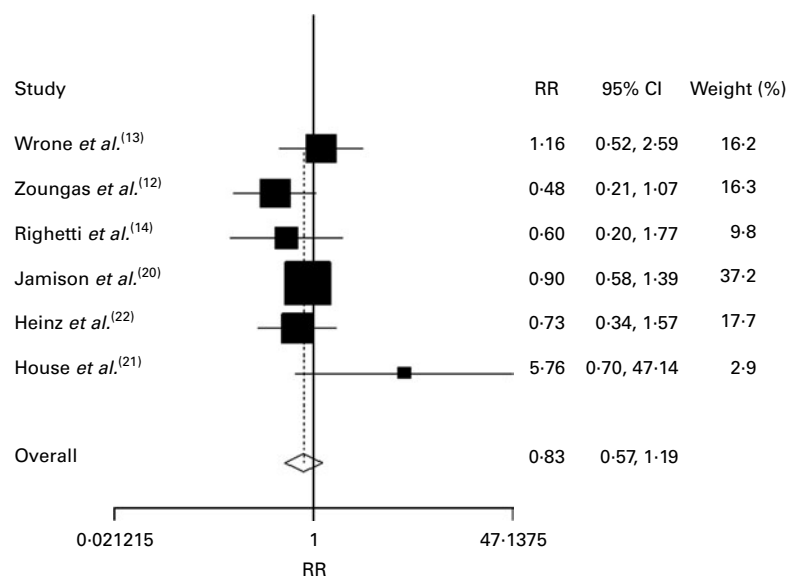

Fig. 4. Relative risk (RR) for stroke in pooled trials. The RR estimated were not significantly different for stroke (RR 0·83, 95% CI 0·57, 1·19, *P*=0·31).
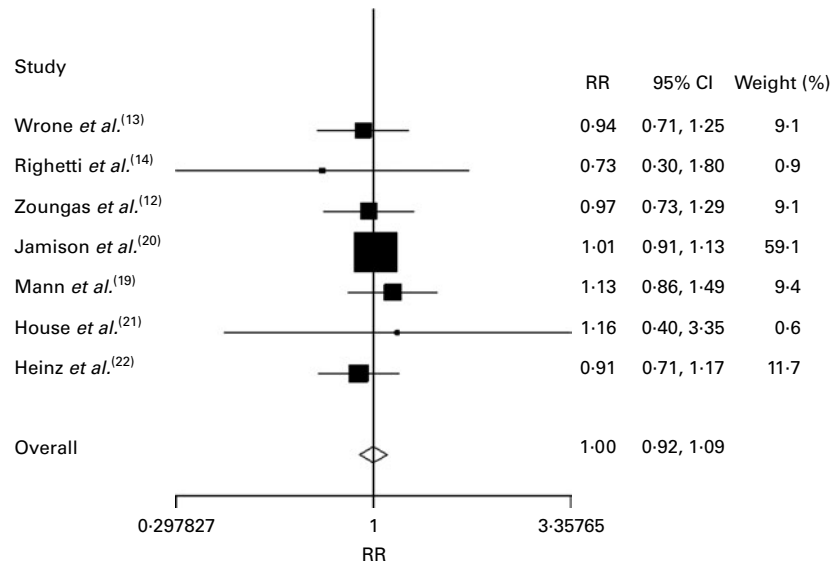
Fig. 5. Relative risk (RR) for all-cause mortality in pooled trials. The RR estimated were not significantly different for all-cause mortality (RR 1·00, 95 % CI 0·92, 1·09, $P = 0.98$).

women and a 15 % lower risk among men[29]. However, the reported decreases in Hcy levels vary substantially; and the ten papers in our meta-analysis reported a wide range of Hcy-lowering from 8·9 % to 53·8 %, which is consistent with the observed range of decrease in patients without CKD. In this meta-analysis, we could not observe the benefits when Hcy levels decreased by 20 %, which was in contrast to a previous meta-analysis[15]. It is unclear whether there are benefits if the reduction of Hcy levels is greater. In fact, the degree of Hcy-lowering in each patient in response to folic acid therapy is different. It has been proposed that the mutant TT genotype at nucleotide 677 of the gene regulating methylenetetrahydrofolate reductase activity is associated with increased Hcy levels and increased mortality in general and CKD populations. In the methylenetetrahydrofolate reductase TT group, the greatest reductions of Hcy concentration after folate
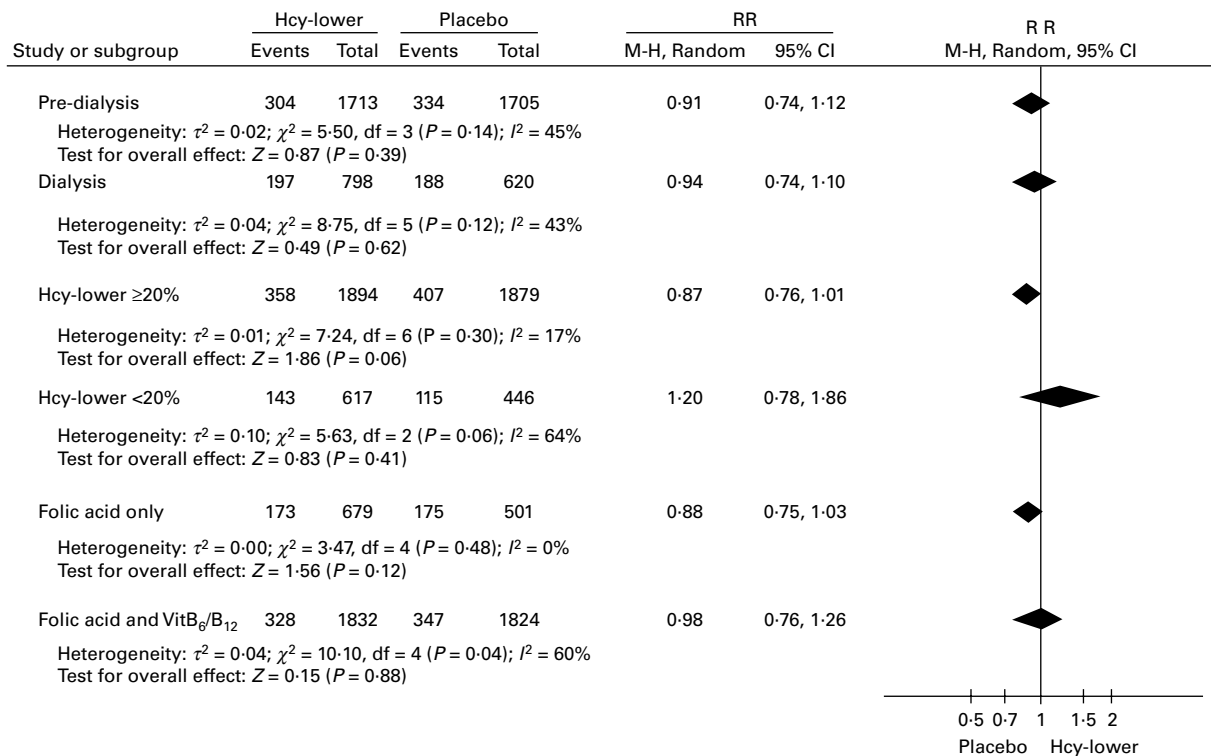

Fig. 6. Relative risk (RR) for CVD associated with dialysis condition, homocysteine (Hcy)-lowering degree, and in combination with vitamin (Vit) $B_{6/12}$.

therapy were observed[30]. However, it could not be observed in other studies[31,32], since Hcy concentration decreased in all genotypes.

Another confounding factor that could affect the outcomes of Hcy-lowering therapy among patients on dialysis might be the dialysis procedure and membrane pore size. However, dialysis kinetics does not affect the levels of Hcy reduction[33,34]. Furthermore, studies comparing non-albumin-leaking with albumin-leaking high-flux membranes also failed to find a significant difference in plasma Hcy levels before dialysis despite greater clearance per dialysis session[35,36]. Finally, a recent study found no difference in Hcy levels between patients on HD compared to those on peritoneal dialysis[37].

A third unresolved issue is whether elevated plasma Hcy is paralleled by increased Hcy concentration in the coronary arteries of CKD patients, and few studies have addressed this question. In patients with coronary artery disease (CAD)[38,39], it was observed that although the CAD group presented with high plasma Hcy levels (27·7 (SE 12·8) μmol/l), the media and intimal layers containing the endothelium exhibited the lowest enrichment of Hcy (media: 20·8 (SE 4·4)%; intima: 6·1 (SE 2·3)%). In contrast, the control group (from heart donors) revealed an extensive Hcy enrichment that co-localised with vascular smooth cells by immunohistochemical staining. Similar investigations in patients with CKD have not been performed.

Now that there are no benefits for the prevention of CVD events and mortality in individuals during treatment with folic acid and/or in combination with vitamins, another concern has also been raised about the safety of folic acid, particularly in relation to CVD and mortality risk. Small trials were designed to deal with this negative effect. In a double-blind, placebo-controlled trial with a total of 6837 patients with IHD, after a median 39 months of follow-up, it had been observed that treatment with folic acid plus VitB$_{12}$ was associated with increased all-cause mortality, with a hazard ratio of 1·18 (95% CI 1·04, 1·33, $P=0·01$)[40]. In the House *et al.*[21] study, a multicentre, randomised, double-blind, placebo-controlled trial, it was also found that high doses of B vitamins resulted in a greater decrease in glomerular filtration rate and an increase in vascular events as myocardial infarction, stroke, revascularisation (peripheral, cardiac angioplasty or cardiac bypass). Considering this potential side effect, more RCT are needed to confirm the results.

There are several limitations in this meta-analysis to improve the statistical power to assess the effect of Hcy-lowering therapy on CVD events and mortality in patients with CKD. One is that there are still relatively few trials. Only ten RCT compared folic acid-based active therapy with or without other vitamins with placebo, and were included in the meta-analysis. Second, there was considerable variation among patients (CKD without dialysis and on dialysis) and the degree of reduction in plasma Hcy concentration. Third, the study by Nanayakkara *et al.*[18] was included in the meta-analysis although it was not mainly designed to examine the clinical end points.

Despite these limitations, the present meta-analysis supports the conclusion that Hcy-lowering therapy was not associated with a significant decrease in the risk for CVD events, stroke and all-cause mortality among patients with CKD. Although these results cannot rule out the possibility that normalisation of Hcy in CKD would benefit patients with CKD or under dialysis, it seems to be very difficult to normalise the Hcy concentration in this special population. In conclusion, more large-scale clinical trials evaluating the effect of Hcy-lowering therapy on cardiovascular risk are expected to clarify whether Hcy-lowering therapy has the potential to clearly decrease cardiovascular risk among this special population.

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