

RESEARCH ARTICLE

Meta-Analysis of the Associations of p-Cresyl Sulfate (PCS) and Indoxyl Sulfate (IS) with Cardiovascular Events and All-Cause Mortality in Patients with Chronic Renal Failure

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Abstract

Background

Indoxyl sulfate (IS) and p-cresyl sulfate (PCS) are protein-bound uremic toxins that increase in the sera of patients with chronic kidney disease (CKD), and are not effectively removed by dialysis. The purpose of this meta-analysis was to investigate the relationships of PCS and IS with cardiovascular events and all-cause mortality in patients with CKD stage 3 and above.

Methodology/Principle Findings

Medline, Cochrane, and EMBASE databases were searched until January 1, 2014 with combinations of the following keywords: chronic renal failure, end-stage kidney disease, uremic toxin, uremic retention, indoxyl sulfate, p-cresyl sulfate. Inclusion criteria were: 1) Patients with stage 1 to 5 CKD; 2) Prospective study; 3) Randomized controlled trial; 4) English language publication. The associations between serum levels of PCS and IS and the risks of all-cause mortality and cardiovascular events were the primary outcome measures. Of 155 articles initially identified, 10 prospective and one cross-sectional study with a total 1,572 patients were included. Free PCS was significantly associated with all-cause mortality among patients with chronic renal failure (pooled OR = 1.16, 95% CI = 1.03 to 1.30, $P = 0.013$). An elevated free IS level was also significantly associated with increased risk of all-cause mortality (pooled OR = 1.10, 95% CI = 1.03 to 1.17, $P = 0.003$). An elevated free PCS level was significantly associated with an increased risk of cardiovascular events among patients with chronic renal failure (pooled OR = 1.28, 95% CI = 1.10 to 1.50,

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$P = 0.002$), while free IS was not significantly associated with risk of cardiovascular events (pooled OR = 1.05, 95% CI = 0.98 to 1.13, $P = 0.196$).

Conclusions/Significance

Elevated levels of PCS and IS are associated with increased mortality in patients with CKD, while PCS, but not IS, is associated with an increased risk of cardiovascular events.

Introduction

The prevalence of cardiovascular disease (CVD) is markedly higher in patients with chronic kidney disease (CKD) [1], and CVD disease is the primary cause of death in CKD patients, especially in those with end stage renal disease (ESRD) [2,3]. Traditional CVD risk factors such as hypertension and diabetes and non-traditional such as hyperhomocysteinemia, however, do not fully account for the increased CVD risk in patients with CKD [4,5].

Impairment of renal function results in the retention of a large number of compounds which are normally excreted in the urine [6,7]. While some compounds such as urea are removed by dialysis in patients with ESRD, many others are not [6,7]. These retained compounds are called uremic retention solutes or uremic toxins [6,7].

Indoxyl sulfate (IS) is a protein-bound uremic solute resulting from bacterial metabolism of dietary tryptophan to indole [6,8,9]. It is normally cleared by renal proximal tubular secretion; but in patients with CKD impaired renal function can lead to its accumulation [6,8,9]. Another uremic solute, p-cresyl sulfate (PCS) is synthesized by intestinal anaerobic bacteria from the amino acids tyrosine and phenylalanine, and like IS it is normally secreted in the urine [6,8,9]. Both PCS and IS cannot be removed efficiently by hemodialysis or peritoneal dialysis because protein binding limits their clearance [10]. Levels of IS and PCS increase with the severity of CKD, and both have been shown to have a strong negative correlation with renal function (estimated glomerular filtration rate [eGFR]) in patients with CKD [11–14]. Furthermore, studies have shown that IS and PCS are associated with CVD, mortality, and deterioration of renal function in patients with CKD [15–18].

The purpose of this systematic review of the literature and meta-analysis is to investigate the relationships of PCS and IS levels with cardiovascular events and all-cause mortality in patients with CKD.

Materials and Methods

Search strategy and selection criteria

Medline, Cochrane, and EMBASE databases were searched until January 1, 2014 with combinations of the following keywords: chronic renal failure, end-stage kidney disease, uremic toxin, uremic retention, indoxyl sulfate, p-cresyl sulfate. Reference lists of relevant published articles (as “other resources” in Fig 1) were also searched. Inclusion criteria were: 1) patients with stage 1 to 5 CKD, or undergoing hemodialysis or peritoneal dialysis; 2) prospective study; 3) randomized controlled trial; 4) English language publication. Case reports, comments, editorials, letters, and non-English publications were excluded.

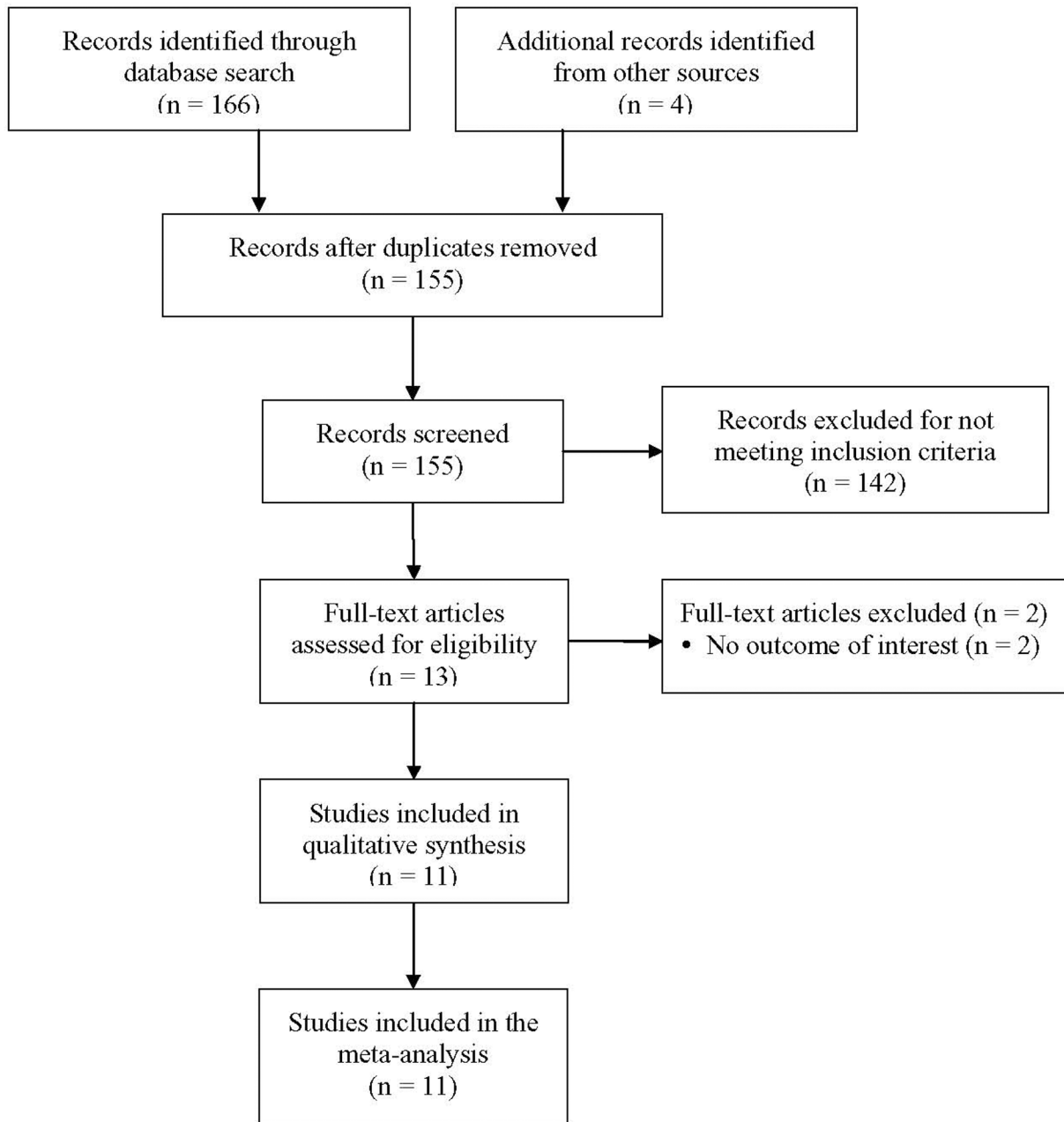


Fig 1. Flow chart of study selection.

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Study selection and data extraction

Studies were identified via the search strategy by two independent reviewers. When there was uncertainty regarding eligibility, a third reviewer was consulted. The following data were extracted from studies that met the inclusion criteria: the name of the first author, year of publication, study design, number of participants in each group, participants' age, gender, and CKD stage, and IS, PCS, albumin, creatinine, and parathyroid hormone (PTH) levels.

Quantitative data synthesis and outcome measures

The associations between serum levels of PCS and IS, and the risks of all-cause mortality and cardiovascular events were the primary outcome measures. The risks of all-cause mortality and cardiovascular events associated with albumin, creatinine, and PTH levels were also determined for comparison. The odds ratio (OR) and/or hazard ratio (HR) and corresponding 95% confidence interval (CI) from each study were used for evaluation. For prospective studies, the HR was considered as the OR in the statistical analysis since there was barely numeric difference between the two [19]. Heterogeneity among studies was assessed by calculating Cochran Q and the I^2 statistic. For the Q statistic, $P < 0.10$ was considered to indicate statistically significant heterogeneity. The I^2 statistic indicates the percentage of the observed between-study variability caused by heterogeneity. Heterogeneity determined using the I^2 statistic was defined as follows: 0 to 24% = no heterogeneity; 25 to 49% = moderate heterogeneity; 50 to 74% = large heterogeneity; and 75 to 100% = extreme heterogeneity. If heterogeneity existed between studies (a Q statistic with $P < 0.1$ [20] or an I^2 statistic $> 50\%$ [21]), a random-effects model (DerSimonian-Laird method) was performed [22]. Otherwise, a fixed-effect model was used (Mantel-Haenszel method). Combined ORs were calculated, and a two-sided P value < 0.05 was considered to indicate statistical significance.

The associations between serum levels of free PCS and IS and the risk of all-cause mortality, as well as cardiovascular events, were further examined by sensitivity analysis which was performed based on the leave-one-out approach. Publication bias was assessed by constructing funnel plots for the associations between the serum levels of free PCS and IS, and the risk of all-cause mortality and cardiovascular events. It was also quantitatively detected by Egger's test [23]. The absence of publication bias is indicated by the data points forming a symmetric funnel-shaped distribution and $P > 0.10$ in Egger's test. Moreover, the Duval and Tweedie's trim-and-fill method was used to adjust for potential publication bias. The trim-and-fill method formalizes the interpretation of any asymmetry in the funnel plot by imputing suspected missing studies and calculating an adjusted result. The adjusted result is neither intended to find the values of missing studies, nor to give a better effect size estimate in itself, but is used as a form of sensitivity analysis to help ascertain the probable effect of publication bias on the meta-analysis [24–26]. All statistic analyses were performed using the Comprehensive Meta-Analysis statistical software, version 2.0 (Biostat, Englewood, NJ, USA).

Results

Literature search

A summary of the literature search and study selection is shown in Fig 1. After initially identifying 155 articles after the removal of duplicates, 142 were excluded as they did not meet the stringent inclusion criteria. Thirteen articles were thus included in the full text review. Of the 13 articles, two were excluded as they did not provide data with respect to the outcome of interest [13,14]. Thus, eleven studies were included in the meta-analysis [11,12,15–18,27–31].

Study characteristics

The basic characteristics of the 11 studies included in the meta-analysis are summarized in Table 1. Among the eleven studies included, there were 10 prospective studies and one cross-sectional study with a total of 1,572 patients. The total number of patients in each of the studies ranged from 46 to 521, and the follow-up duration of the prospective studies ranged from 20 months to 5 years. A summary of the serum levels of the five biomarkers (free PCS, free IS, albumin, creatinine, and PTH) is also presented in Table 1.

Table 1. Characteristics of studies included in the meta-analysis.

Author (Year)	Study Type	Number of Patients	Type of Patients	Age (y)	Male (%)	Follow-up Time	Free IS(mg/L)	Free PCS(mg/L)	Albumin (g/dL)	Creatinine (mg/dL)	Parathyroid Hormone (pg/mL)
Lin et al. (2013) [27]	Prospective	46	ESRD	47.4 ± 12.8	45.60%	5 y	4.3 ± 2.6	1.1 ± 1.0	3.9 ± 0.3	13.3 ± 3.4	382.9 ± 279.4
Lin et al. (2013) [28]	Prospective cohort	50	Hemodialysis	70.50 ± 3.45	NR	38 mo	4.27 ± 2.90	1.59 ± 1.12	4.05 ± 0.34	10.36 ± 2.32	291.44 ± 235.09
Melamed et al. (2013) [15]	Prospective cohort	521	Dialysis	58.3 ± 14.7	46.00%	NR	NR	NR	3.6 ± 0.4	7.3 ± 2.4	NR
Lin et al. (2012) [29]	Prospective cohort	70	Stable CKD (stages 3–5)	60.6 ± 9.7	48.60%	36 mo	NR	NR	4.02 ± 0.4	3.76 ± 2.7	132 ± 176
Wu et al. (2011) [16]	Prospective cohort	112	Hemodialysis	72.6 ± 6.3	69.60%	3.5 y	3.7 (0.01, 19.7) †	2.2 (0.01, 25.6) †	3.6 ± 0.4	NR	197 (10.1, 3104) †
Chen et al. (2012) [30]	Cross-sectional	91	Hemodialysis	57.6 ± 1.2	57.10%	NR	2.9 ± 0.2	2.1 ± 0.2	3.9 ± 0.3	11.3 ± 0.2	NR
Wu et al. (2011) [31]	Prospective observational	268	Pre-dialysis CKD	66.9 ± 12	42.50%	24 mo	NR	NR	3.9 ± 0.4	1.9 ± 1.4	89.9 (1, 692) †
Lin et al. (2010) [17]	Prospective	100	Hemodialysis	62.36 ± 8.03	42.00%	20 mo	4.42 ± 2.41	1.57 ± 0.95	4.05 ± 0.27	10.89 ± 2.07	335.53 ± 192.98
Liabeuf et al. (2010) [11]	Prospective cohort	139	Stage 1–5 CKD	67 ± 12	60%	779 ± 185 d	NR	2.6 (5.1)	3.8 (6)	NR	137 (138)
Barreto et al. (2009) [12]	Prospective cohort	139	Stage 1–5 CKD	67 ± 12	60%	605 ± 217 d	NR	2.6 (5.1)	3.8 (6)	NR	137 (138)
Bammens et al. (2006) [18]	Prospective	175	Stage 5 CKD	64.7 ± 1.1 †	38.30%	30.1 mo	NR	2.59 ± 0.17	3.64 ± 0.03	8.6 ± 0.2	NR

Data are reported as mean ± standard deviation unless otherwise indicated.

ESRD, end stage renal disease; CKD, chronic kidney disease; IS, indoxyl sulfate; PCS, p-cresyl sulfate; NR, not reported.

† Mean ± SEM.

‡ Median (range).

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Associations between biomarkers and all-cause mortality

Free PCS was significantly associated with all-cause mortality among patients with chronic renal failure (pooled OR = 1.16, 95% CI = 1.03 to 1.30, $P = 0.013$, Fig 2A). An elevated free IS level was also significantly associated with increased risk of all-cause mortality (pooled OR = 1.10, 95% CI = 1.03 to 1.17, $P = 0.003$, Fig 2B). There was significant association between albumin level and all-cause mortality among patients with chronic renal failure (pooled OR = 0.83, 95% CI = 0.69 to 1.00, $P = 0.047$, Fig 2C), while there was no significant association between creatinine and PTH levels and all-cause mortality (creatinine: pooled OR = 0.94, 95% CI = 0.83 to 1.06, $P = 0.304$, Fig 2D; PTH: pooled OR = 0.999, 95% CI = 0.997 to 1.000, $P = 0.077$, Fig 2E).

Associations between biomarkers and cardiovascular events

An elevated free PCS level was significantly associated with an increased risk of cardiovascular events among patients with chronic renal failure (pooled OR = 1.28, 95% CI = 1.10 to 1.50, $P = 0.002$, Fig 3A), while free IS was not significantly associated with risk of cardiovascular events (pooled OR = 1.05, 95% CI = 0.98 to 1.13, $P = 0.196$, Fig 3B). Of albumin, creatinine, and PTH, only creatinine level had a significantly negative association with the risk of cardiovascular events (pooled OR = 0.86, 95% CI = 0.78 to 0.96, $P = 0.009$, Fig 3C and 3D).

Sensitivity analysis

Results of the sensitivity analysis for the association between free PCS and IS levels and the risks of all-cause mortality and cardiovascular events in which the studies were omitted one-by-one, are shown in Fig 4. As a whole, the direction and magnitude of the pooled ORs did not vary markedly with removal of any study, indicating good reliability in this meta-analysis.

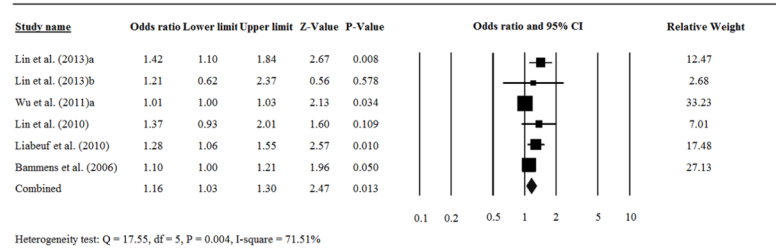
Publication Bias

The funnel plot for publication bias regarding the association between free PCS and mortality risk showed evidence of asymmetry and publication bias (Fig 5A). Egger's test also indicated there was significant evidence of publication bias ($t = 4.50$, $df = 4$, $P = 0.005$). When the Duval and Tweedie's trim-and-fill method was used to adjust for the effect of publication bias, and when possibly missed studies were imputed (Fig 5A), the adjusted point estimates of the OR decreased from 1.16 (95% CI = 1.03 to 1.30) to 1.03 (95% CI = 0.93 to 1.16). This suggests that publication bias may have exaggerated the observed effect size, and the significant association found between serum free PCS level and mortality risk should be interpreted with caution.

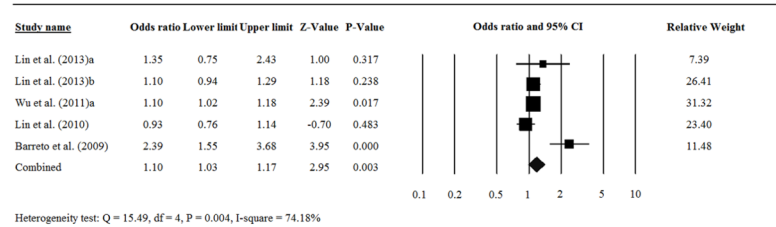
The funnel plot for publication bias regarding the association between free PCS and risk of cardiovascular events showed evidence of asymmetry and publication bias (Fig 5B). Egger's test also indicated there was significant evidence of publication bias ($t = 10.73$, $df = 4$, $P < 0.001$). When the Duval and Tweedie's trim-and-fill method was used to adjust for the effect of publication bias, and when possibly missed studies were imputed (Fig 5B), the adjusted point estimates of the OR decreased from 1.28 (95% CI = 1.10 to 1.50) to 1.10 (95% CI = 0.93 to 1.27). This suggests that publication bias may have exaggerated the observed effect size, and the significant association found between serum free PCS level and the risk of cardiovascular events should be interpreted with caution.

Due to the small number of studies (≤ 5), it was inappropriate to assess for publication bias using the funnel plot [25] with regard to the associations between free IS level and mortality risk or the risk of cardiovascular events.

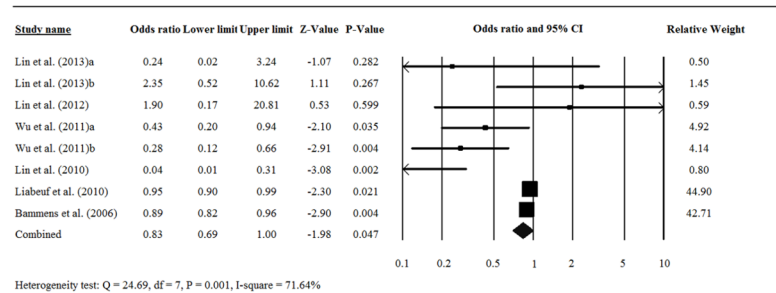
(A) Free PCS



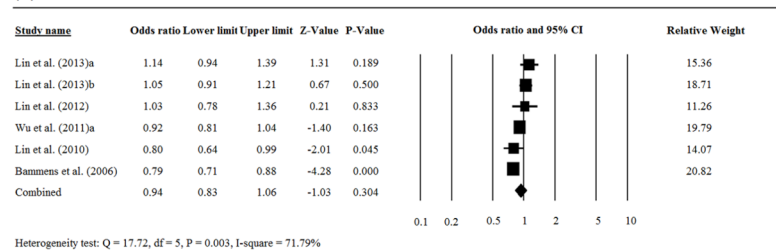
(B) Free IS



(C) Albumin



(D) Creatinine



(E) PTH

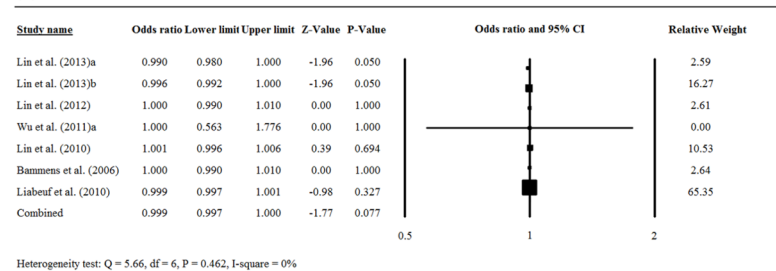
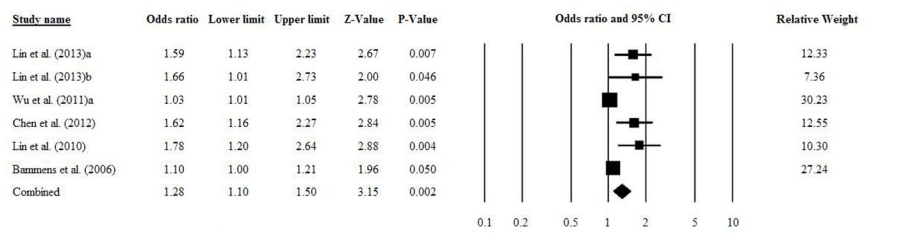


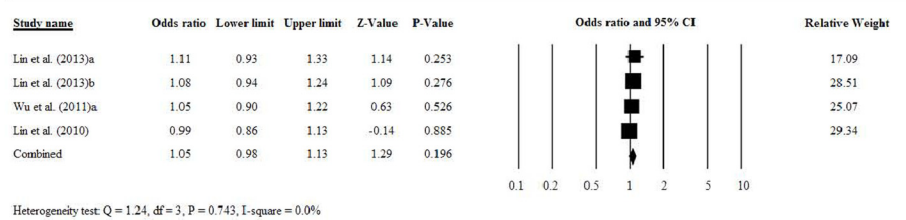
Fig 2. Forest plots of the associations between serum levels of renal biomarkers and the risk of all-cause mortality among patients with chronic renal failure: (A) free PCS, (B) free IS, (C) albumin, (D) creatinine, (E) PTH.

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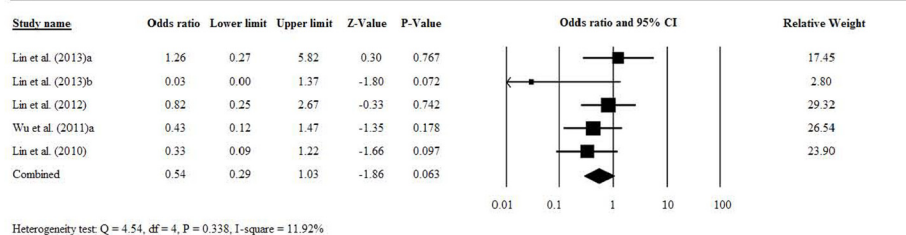
(A) Free PCS



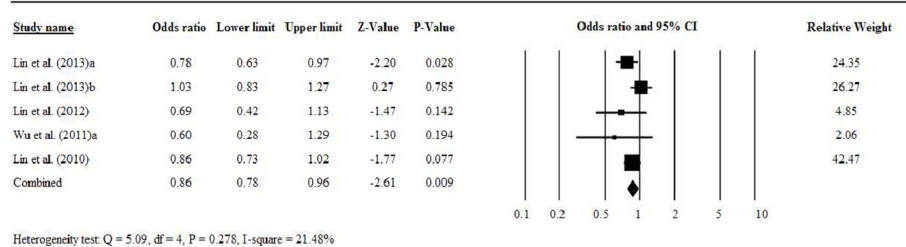
(B) Free IS



(C) Albumin



(D) Creatinine



(E) PTH

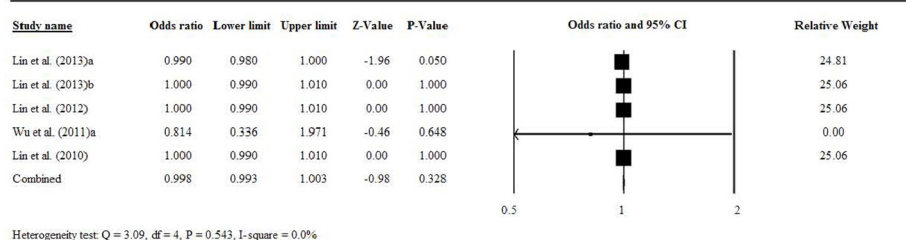
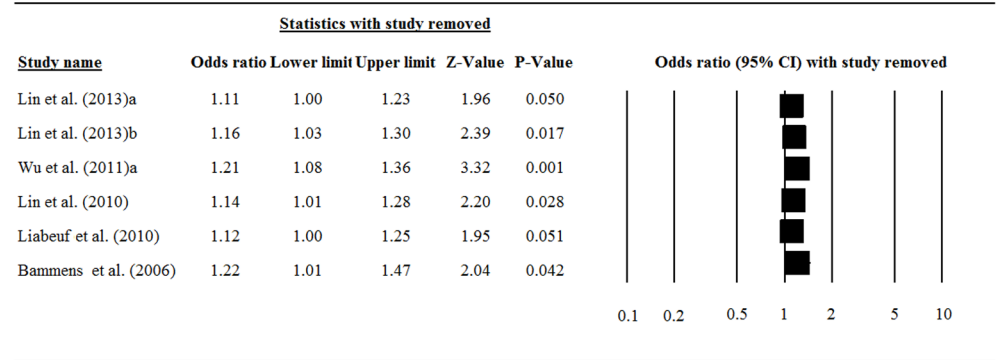


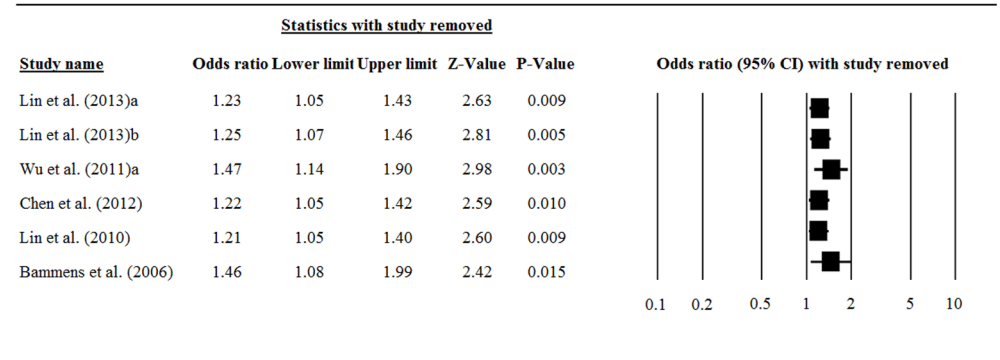
Fig 3. Forest plots of the associations between serum levels of renal biomarkers and the risk of cardiovascular events among patients with chronic renal failure: (A) free PCS, (B) free IS, (C) albumin, (D) creatinine, (E) PTH.

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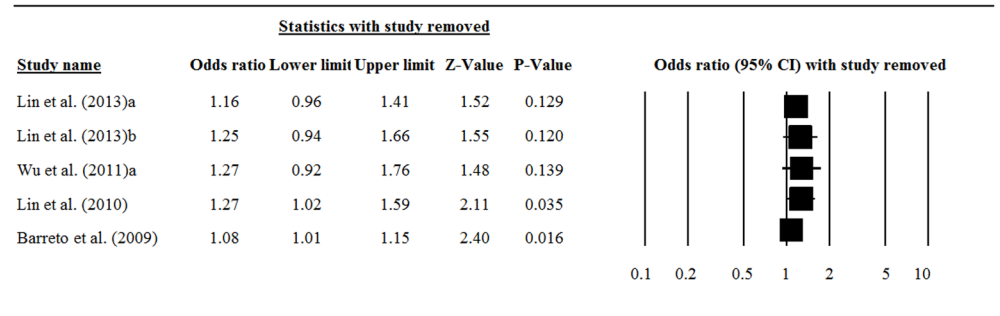
(A)



(B)



(C)



(D)

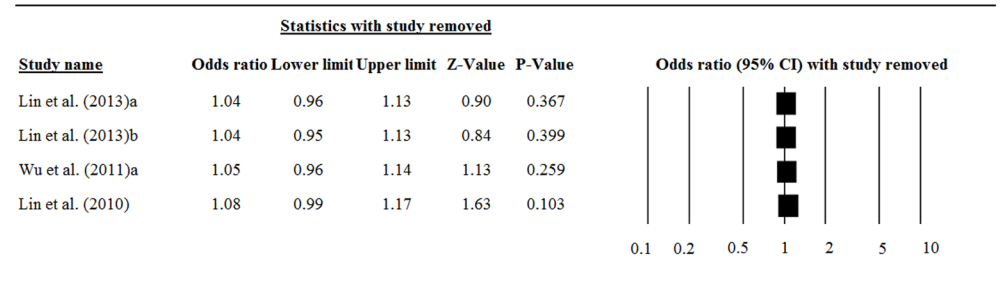


Fig 4. Sensitivity analysis performed by leave-one-out approach: (A) association between serum level of free PCS and the risk of mortality, (B) association between serum level of free PCS and the risk of

cardiovascular events, (C) association between serum level of free IS and the risk of mortality, (D) association between serum level of free IS and the risk of cardiovascular events.

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Discussion

PCS and IS are two of more than 80 uremic toxins that increase in the sera of patients with CKD and are not removed by dialysis [6]. Study has shown that the serum concentrations of IS and PCS in patients with CKD are 54 and 17 times higher, respectively, than in healthy individuals, and because they are bound to albumin only approximately 30% are eliminated by hemodialysis [7,10,32]. The results of this meta-analysis indicate that elevated levels of PCS and IS are associated with increased mortality in patients with CKD, while PCS, but not IS, is associated with an increased risk of cardiovascular events.

A growing number of publications have confirmed the negative effects of IS and PCS. Barretto et al. [12] studied 139 patients with stage 2 to 5D CKD and reported that IS level was inversely related to renal function, and high IS was significantly associated with cardiovascular and overall mortality after adjustment for age, gender, diabetes, albumin, hemoglobin, phosphate, and aortic calcification. In further analysis of the same 139 patients, Liabeuf et al. [11] found that higher free PCS levels were associated with mortality independent of factors such as age, vascular calcification, anemia, and inflammation. Meijer et al. [33] reported that PCS level was predictive of cardiovascular risk in 499 patients with mild to moderate CKD. A cross-section observational study by Rossi et al. [34] showed that more advanced stages of CKD were associated with progressive increases in total and free serum IS and PCS, and that their levels were independently associated with structural and functional markers of cardiovascular disease. In a prospective study of 200 patients with stage 1 to 5 CKD, Poesen et al. [35] found that urinary excretion of PCS was predictive of cardiovascular events independent of eGFR.

While most results of the studies included in this meta-analysis are generally consistent, there are some differences. Lin et al. [27] found that total PCS was associated with cardiovascular events, free PCS was associated with all-cause mortality, and total IS was associated with dialysis failure in stable peritoneal dialysis patients. In a separate study of elderly (> 65 years of age) patients receiving hemodialysis, Lin et al. [28] found that free and total PCS were significantly associated with cardiovascular events and the total PCS was associated with all-cause mortality. In a recent report (2014), Lin et al. [36] examined 72 pre-dialysis patients and found that a serum PCS level > 6 mg/L was associated with cardiovascular events and initiation of dialysis. Melamed et al. [15], however, found that elevated PCS levels (defined as greater than the population median) were not associated with either all-cause or cardiovascular mortality, and that elevated IS levels were associated with all-cause mortality, but not with cardiovascular mortality. Wu et al. [16] reported that free PCS was associated with all-cause and cardiovascular mortality after adjusting for traditional risk factors such as age, gender, and diabetes status in elderly patients receiving hemodialysis. Bammens et al. [18] also found that a higher level of free PCS was independently associated with mortality in hemodialysis patients.

While the number of publications supporting the predictive value of PCS and IS in patients with CKD is increasing, Vanholder et al. [37] have pointed out that the interpretation of the results may be affected by different cutoff values of PCS and IS, and low albumin concentrations in the study populations. To this end the authors performed a systematic review of the literature, and after excluding studies biased by albumin binding considerations they concluded that PCS and IS indeed play a role vascular and renal disease progression. The exact mechanisms by which elevated levels of IS and PCS contribute to CVD and mortality, however, have not been elucidated. Studies have suggested that IS and PCS may suppress the activity or

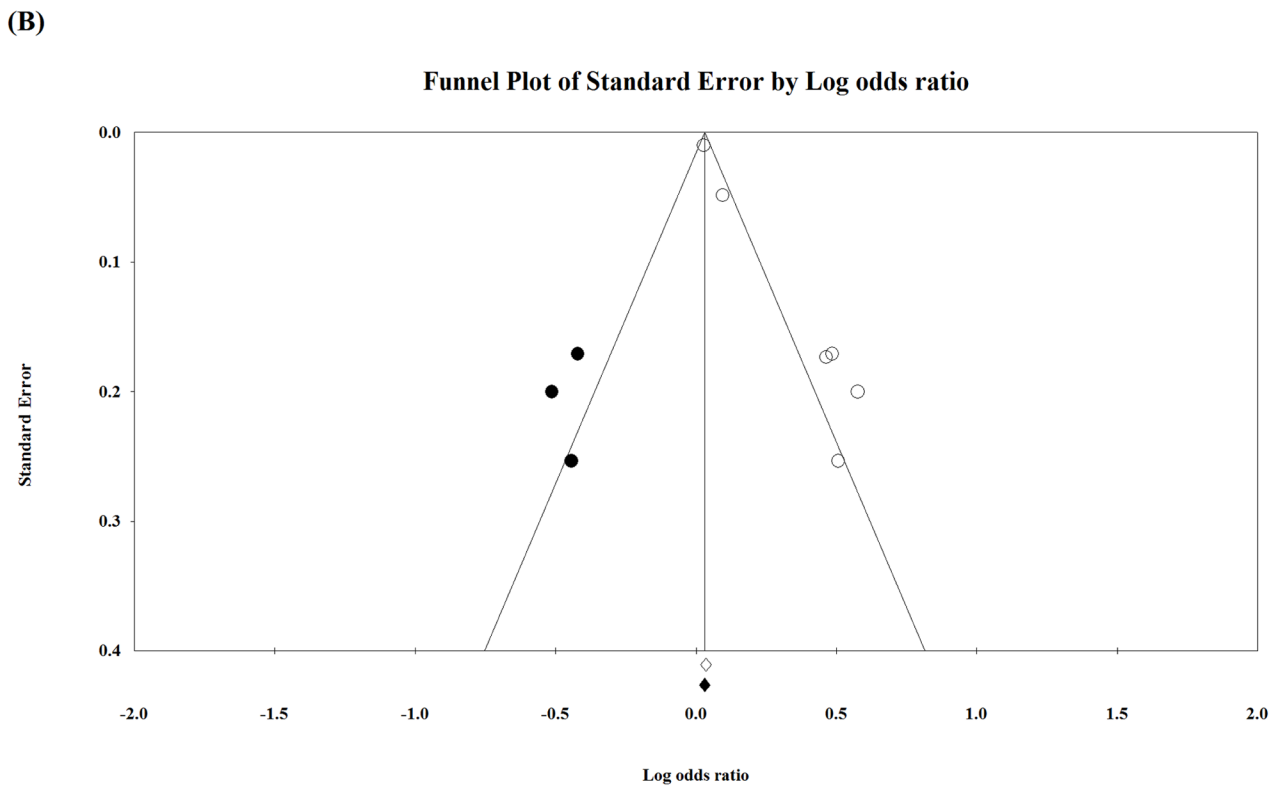
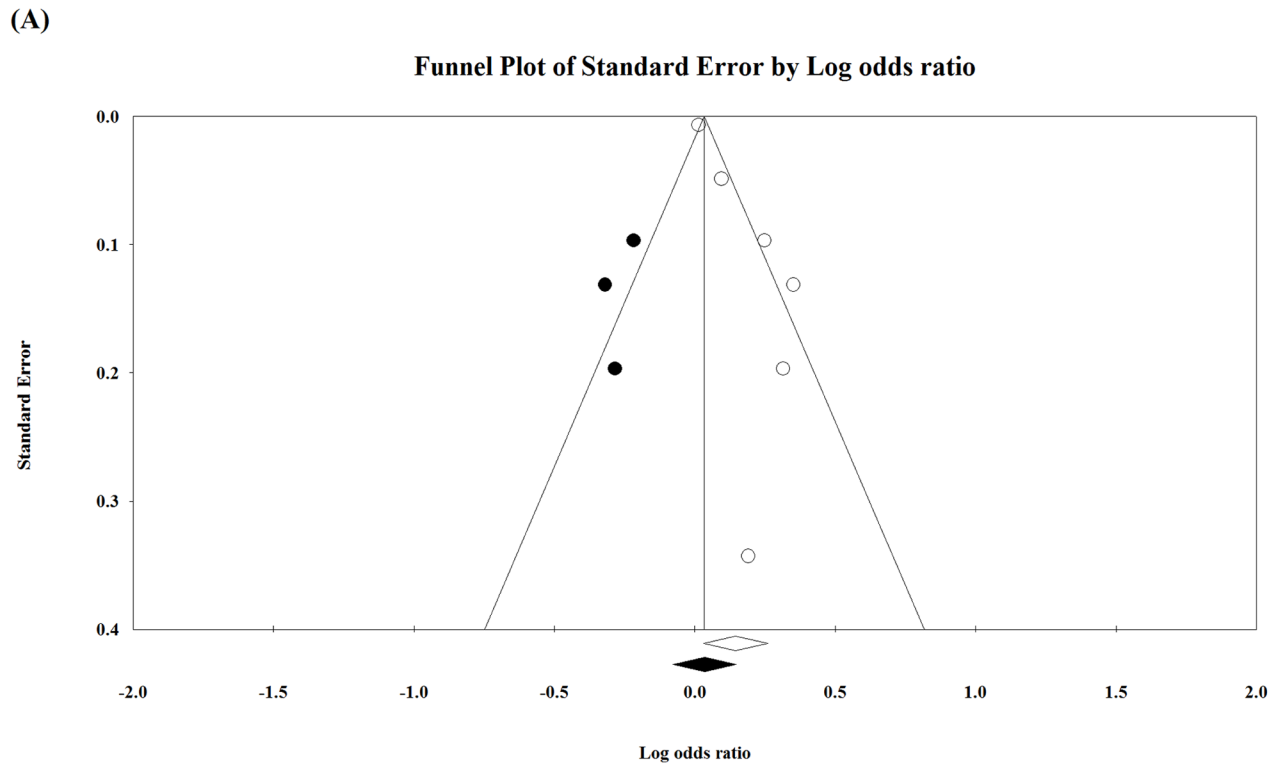


Fig 5. Funnel plot for evaluating publication bias regarding the association between free PCS and (A) mortality risk and (B) the risk of cardiovascular events. White circles represent observed studies, and black circles represent possibly missed studies imputed using Duval and Tweedie's trim-and-fill method. White and black rhombuses represent observed and theoretical combined effect size, respectively.

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activated leucocytes, inhibit the release of platelet-activating factor by macrophages, and contribute to endothelial dysfunction and oxidative stress [9,38,39].

There are limitations of this study that should be considered. Given the number of studies in the literature examining the roles of IS and PCS in outcomes of patients with CKD, the number of studies included in the meta-analysis is relatively small due to the relatively strict inclusion criteria. Publication bias may have exaggerated the significant association found between PCS level and the risk of cardiovascular events and all-cause mortality, but could not be assessed with respect to IS level and the risks of mortality and cardiovascular events due to the small number of studies. Furthermore, confounding factors (age, gender, diabetic or dialysis status etc.) of mortality for which multivariate analytic data were adjusted varied among studies, and additional multi-variable analyses for those factors with mortality association were not performed.

Conclusions

This meta-analysis indicated that elevated levels of PCS and IS are associated with increased mortality in patients with CKD, and PCS, but not IS, is associated with an increased risk of cardiovascular events. Although it is clear that PCS and IS are associated with negative outcomes in patients with CKD, further studies are necessary to determine their exact roles and mechanisms, and possible treatment options to reduce their negative impact in patients with CKD.

Supporting Information

S1 PRISMA Checklist.
(DOC)

Author Contributions

Conceived and designed the experiments: CJL CJW. Performed the experiments: CJL CJW. Analyzed the data: PCW. Contributed reagents/materials/analysis tools: VW. Wrote the paper: CJL. Corrected the manuscript: VW.

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