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Original Article

Clinic value of compound kushen injection combined with chemotherapy in breast cancer: a systematic review and meta-analysis

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Abstract: Purpose: To evaluate the efficacy and safety of compound kushen injection (CKI) combined with chemotherapy in the treatment of breast cancer. Methods: Electronic databases, including PubMed (n=2), EMBASE (n=2), The Cochrane Library (n=1), Web of Science (n=14), CNKI (n=56), VIP (n=34), CBM (n=41) and Wan Fang (n=45) were searched for relevant original articles. Random or fixed effect models were adopted to estimate the summary odds ratio (OR) and 95% confidence interval (CI). Results: Thirty-one trials including 2234 cases were identified for meta-analysis with RevMan5.3. The meta-analysis showed that there were significant differences in the tumor response (OR=2.07, 95% CI [1.61, 2.67], *P*<0.00001), KPS score (OR=2.63, 95% CI [1.97, 3.52]; *P*<0.00001), $CD4^+$ cells (MD=10.21, 95% CI [8.70, 11.71]; *P*<0.00001), $CD8^+$ cells (MD=12.72, 95% CI [10.45, 14.99]; *P*<0.00001), the ratio of $CD4^+/CD8^+$ (MD=0.47, 95% CI [0.25, 0.69]; *P*<0.00001), IL-4 (MD=27.56, 95% CI [25.64, 29.48]; P<0.00001), IL-10 (MD=17.96, 95% CI [17.03, 18.90]; *P*<0.00001), leucopenia (OR=0.33, 95% CI [0.24, 0.45]; *P*<0.00001), gastrointestinal adverse reactions (OR=0.41, 95% CI [0.31, 0.53]; *P*<0.00001), hepatic insufficiency (OR=0.33, 95% CI [0.21, 0.52]; *P*<0.00001), TBIL (OR=0.29, 95% CI [0.12, 0.66]; *P*=0.004), renal insufficiency (OR=0.47, 95% CI [0.28, 0.77]; *P*<0.003), alopecia (OR=0.36, 95% CI [0.22, 0.58]; *P*<0.00001) and PLT (MD=48.51, 95% CI [44.19, 52.84]; *P*<0.00001) between CKI combination group and control group, While there were no differences between two groups in RBC (MD=0.06, 95% CI [-0.50, 0.63]; *P*=0.82), the change of ECG (OR=0.70, 95% CI [0.40, 1.26]; *P*=0.24) and bone marrow depressions (OR=0.27, 95% CI [0.07, 1.03]; *P*=0.06). Conclusion: CKI is effective to improve the efficacy of chemotherapy and reduce side effects in the treatment of breast cancer. The quantity and quality of RCTs are lower so that we still have to enhance research levels through scientific design and normative report.

Keywords: Kushen injection, chemotherapy, breast cancer, meta-analysis

Introduction

Breast cancer is one of the most frequent female malignant tumors in the world, and it's the leading cause of death in female cancer patients [1]. Breast cancer is the breast epithelial cells in a variety of carcinogenic factor, the occurrence of a genetic mutation, resulting in uncontrolled cell proliferation [2]. The American Cancer Society estimates that there will be 235,030 new cases (232,670 female, 2,360 male) of breast cancer and 404,302 die from the disease in the United States by 2014 [3]. Based on the statistics, more than 169,000 women suffer annually from breast cancer, being the second most common form of tumor among women, and about 45,000 will die of breast cancer this year [4]. Despite the advance in diagnosis and treatment, which had led to reduce the mortality rate in recent decades, breast cancer remains a major public health problem, and needs for strong prevention and treatment programs.

In recent years, chemotherapy has become the preferred treatment for breast cancer. It includes preoperative neoadjuvant chemotherapy and adjuvant chemotherapy after surgery

[5]. Clinical trial data showed that chemotherapy can reduce breast cancer recurrence rate 30% and 50% [6]. Chemotherapy uses powerful drugs to control any cancer cell growth, invasion, metastasis, and eventually kill cancer cells. However, it lacks of target specificity, and kills healthy cells as well as cancerous ones, especially vigorously growing cells in blood and lymphoid tissues. The toxicity and side effects of the chemotherapy can damage the patient's quality of life. For example, marrow suppression, immune suppression, digestive disorder, etc. So many people couldn't stand the pain of chemotherapy and give up treatment. As a result, the focus of breast cancer clinic research is to explore the ideal ways and methods to enhance clinic efficacy and decrease these side effects [7].

From traditional Chinese medicine (TCM) aspect, chemotherapy is actually eliminating pathogenic factors. The induced toxicity and side effects are the manifestations of consumptive disease, which is resulted from damaging primordial qi and blood [8]. The treatment of malignant tumor with integrated traditional Chinese and Western medicine therapy has its unique advantage. In the recent years, TCM therapy, especially compound preparations extracted from Chinese natural herbs should be a positive response of reducing toxicity and side effects including marrow depression, heart or peripheral nerve toxicity as well as side effects of digestive, urinary and respiratory systems, and decreasing the probability of recurrence and metastasis of advanced cancer. At present, TCM therapy is more and more popular for its good result. According to the National Center for Complementary and Alternative Medicine (NCCAM) investigation and study, 55% of cancer patients choose to complementary and alternative medicine, including traditional Chinese medicine [9].

The compound kushen injection (known as the YanShu injection) is extracted from two Chinese herbs (kushen [Radix Sophoraeflavescentis] and baituling [Rhizoma Smilacis Glabrae]) [10]. It is a Chinese patent medicine approved by the China Food and Drug Administration (CFDA) for the treatment of various types of solid tumors [11]. Currently, this being China, CKI has been widely used in clinic in the treatment of breast cancer. Few domestic and foreign researchers have been published in English written journals to report the effectiveness and safety of many commonly used TCM therapies. Hence, the authors performed a systematic review and meta-analysis of published randomized, controlled trials to assess the clinical efficacy and safety of CKI plus chemotherapy in the treatment of breast cancer in order to clarify whether the combination can really enhance immune function and reduce adverse effects.

Methods

Literature search strategy

Two researchers conducted a systematic literature search through databases (PubMed, EMBASE, The Cochrane Library, Web of science, Chinese National Knowledge Infrastructure Database (CNKI), *VIP Database for Chinese Technical Periodicals (VIP),* wanfang Database and *Chinese* BioMedical Literature *Database* (CBM)), all from time of inception up to January 2016. The keywords used in this search were shown as follows: compound kushen injection, compound matrine injection, yanshu injection, breast cancer. The search results were downloaded in a reference database and screened further.

Inclusion and exclusion criteria

Inclusion criteria: The inclusion criteria were as follows: (1) Patients were confirmed cytologically or pathologically, or diagnosed by imaging studies with breast cancer; (2) Trials were described as randomized clinical trials (RCTs), No blinding restriction was used; (3) The experimental group received CKI plus chemotherapy while control group received chemotherapy only; (4) The published data of primary interest were the clinical efficacy, immune function and safety evaluation; (5) There were not heavily damage for liver and kidney function before the subjects included in the study; (6) Expected lifetime is more than 3 months; (7) All the publication languages were restricted to Chinese and English.

Exclusion criteria: Trials were excluded if they did not meet the criteria above and included the following: (1) Reviews, nonclinical studies, and case observations; (2) Animal studies or in vitro studies; (3) The research couldn't find the outcome measurements; (4) Duplicate publications of other studies previously identified in our systematic evaluation.

Documents screening

The literature searches were performed using Endnote software. Duplicate records were deleted. Two independent investigators read related studies by the title and summary to exclude the references which did not met the inclusion criteria. Then, reading full-text in the remaining studies as mentioned above. Finally, determines whether these references included were final studies or not, according to the inclusion and exclusion criteria.

Other two independent investigators performed the data extraction according to use a standardized data collection form. Disagreements between the two investigators were resolved by consensus and discussion of two coauthors, the following information was collected from each study: (1) The information about patients: the number of patients allocated, age, clinical stage, and KPS score; (2) The characteristics of methods: the randomization procedure, concealment of allocation, blinding procedure, withdrawal and reasons, and selective reporting; (3) The characteristics of interventions: Chemotherapy regimens, dosage and duration of CKI combined with chemotherapy; (4) The outcomes: the tumor response, quality of life, immune function expression, and adverse events.

Outcome measurement

The main outcome measurements were as follows: (1) Tumor response was evaluated according to the WHO standard for evaluating therapeutic efficacy on solid tumors [12, 13]. Based on the degree of tumor regression, efficacy was evaluated as following: CR (complete response, CT and/or MRI revealed complete clearance of the lesion); PR (partial response, lesion decreased more than 50%); SD (lesion decreased less than 50% or increased less than 25%); PD (size of lesion increased more than 25% after treatment). Tumor responses were defined as CR+PR. (2) Quality of life was evaluated according to the Karnofsky performance score (KPS) [14]. Which was classified as: Improvement (KPS improved ≥10 points after treatment); Stabilization (KPS improved <10 points or decreased <10 points); Deterioration (KPS decreased ≥10 points after treatment). (3) The change of immune function indexes ($CD3^+$ cells, $CD4^+$ cells, $CD8^+$ cells, $CD4^+/CD8^+$, IL-4, IL-10). (4) Adverse events were assessed by the grading of acute and subacute toxicity (WHO criteria) [15]. Hematologic toxicity: leucopenia, erythropenia thrombocytopenia. The non-hematological adverse events: gastrointestinal adverse reactions, hepatic insufficiency, TBIL, renal insufficiency, ECG, bone marrow depressions, alopecia.

Study quality assessment

Two independent reviewers judged the methodological quality using the Cochrane Handbook for Systematic Reviews of Interventions [16]. The evaluation was performed as follows: (1) Selection bias (random sequence generation and allocation concealment); (2) Performance bias (blinding of participants and personnel); (3) Detection bias (blinding of outcome assessment); (4) Attrition bias (incomplete outcome data); (5) Reporting bias (selective reporting); (6) Other bias (other sources of bias). The quality judgment of each term was assessed using three levels: 'Low risk' of bias (adequate and correct description of methods or procedures), 'High risk' of bias (incorrect description of methods or procedures) or 'Unclear risk' of bias (no description of methods and procedures) and ('Yes' for 'low risk', 'No' for 'high risk', and Unclear for 'unclear risk').

Statistical analysis

The meta-analysis was conducted using Review Manager 5.3 (Cochrane Collaboration, Oxford, UK). Heterogeneity among studies was estimated using the Cochran's Q statistic and I^2 tests. $P<0.10$ or $I^2>50\%$ were defined to have heterogeneity [17]. The random effects model was used when there was significant statistical heterogeneity; otherwise the fixed effects model was used. Dichotomous data were treated as Odds ratio (OR) with 95% confidence intervals (CI), and for continuous data, the mean differences (MD) were calculated with 95% CI. It was necessary to make a subgroup analysis to seek the source of the heterogeneity. Funnel-plot was used to identify the publication bias.

Results

Search results

A total of 195 potentially related articles were identified through database searching, and

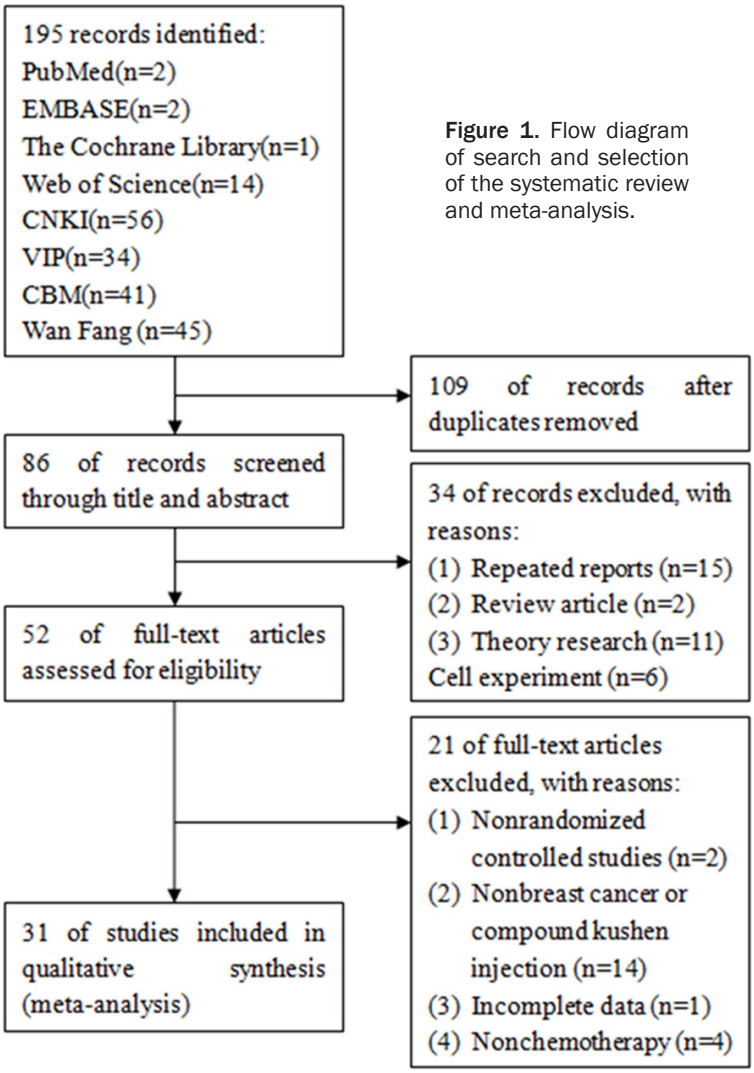

Figure 1. Flow diagram of search and selection of the systematic review and meta-analysis.

109 articles were excluded after duplicate review. By reading title and abstract, 34 articles were excluded for the following reasons: 15 studies were repeated reports, 2 studies were review articles, 11 studies were theory research, 6 studies were cell experiment. A total of 52 full-text articles were read. 21 studies were excluded for the following reasons: 2 articles were not RCTs, 1 article did not address the complete data, 14 articles were associated with other Chinese medicine therapies in experimental group or in control group or other cancer, 4 articles were not chemotherapy. 31 relevant studies were finally included in the systematic review and meta-analysis (**Figure 1**).

Study characteristics and quality assessment

There were 31 clinical trials [18-48] with 2234 breast cancer patients, the case of CKI plus chemotherapy and chemotherapy alone were 1141 and 1090, respectively. All of these trials were reported in Chinese journals and published between 2007 and 2016. All of the patients were Chinese women, and mainly middle-aged and old women. 26 studies [18-33, 36, 37, 47, 39, 40, 43-48] were reported the TNM-staging. The dose of administered CKI ranged from 12 to 30 mL/day, and there were many chemotherapy regimens. However, the combination of PTX, CTX, ADM, EPI, 5-FU, THP or Docetaxelin the chemotherapy treatment was the most common regimen. The studies lasted 2 to 6 cycles. Detailed characteristics of included studies are listed in **Table 1**.

All of the included studies mentioned RCTs, but only 8 trials reported the method of random sequences generation. The results indicated that there was a possibility of high selectivity bias in our study. No trials described information on allocation concealment. One [47] reported blind method. It suggested that a possibility of high performance bias have existed in our article. 6 trials [21, 27, 31, 33, 37, 45] reported the withdrawals and dropouts. 31 studies described baseline information in detail about research object, such as gender and age. But study was not multi-center RCT. The quality assessment of included randomized controlled trials was shown in **Figure 2**.

The clinical efficacy assessment

Tumor response: In the 31 included trials, 15 trials [18, 20, 23-29, 32, 35, 36, 38, 43, 44] with 1075 cases were identified with the CR+PR outcome measurement of tumor response. The heterogeneity test results (Chi^2=11.39, df=14 (P=0.66); I^2=0%) indicated that there was no statistical heterogeneity between studies. Based on the results of the heterogeneity, a fixed-effects model was applied to calculate the

Table 1. Intervention characteristics of the included trials

First author Year	Country	Sample size (E/C)	Age Year (E/C)	TMN	Intervention (E/C)	Dosage (ml/d)	Course (C/D)	KPS
CLJ 2010	China	35/32	55.9/56.3	II-III	CKI+TAC	20	6 C	Unclear
CW 2012	China	52/52	55/53	II-III	CKI+CAF	20	6 C	≥60
CXY 2014	China	32/32	54.9/56.3	II-III	CKI+TAC	20	6 C	Unclear
DHD 2012	China	40/39	49±7.74/47±8.52	I-IV	CKI+T	12	5 D	≥80
GQF 2015	China	26/26	50.2	II-III	CKI+FEC	30	6 C	Unclear
GXD 2015	China	45/44	47.22±2.4/47.8±3.1	III	CKI+FEC	15	3 C	Unclear
HYH 2012	China	20/20	55	III-IV	CKI+TA	20	2 C	>70
HZF 2010	China	30/30	36.7±11.3/38.1±11.9	III-IV	CKI+CTF	20	2 C	≥60
JJS 2008	China	35/33	46/43	III-IV	CKI+TA	20	2-4 C	>70
LJB 2015	China	43/37	67.82±6.90/68.09±6.58	I-IV	CKI+TA	30	6 C	≥60
LLY 2016	China	34/34	45.6±4.2/46.5±3.1	III-IV	CKI+TA	20	2 C	>70
LYX 2012	China	30/30	49.3±0.9	II-IV	CKI+CTF	12	2 C	≥60
MHY 2014	China	38/38	44.8±10.2/44.1±10.9	II-III	CKI+ACD	30	4 C	>70
MYQ 2011	China	32/31	44/46	III-IV	CKI+TEC	20	3 C	≥60
QJH 2012	China	57/52	35-75	IV	CKI+DP	20	2 C	≥60
RJO 2010	China	62/60	39-65	II-III	CKI+CAF	30	6 C	≥60
RW 2015	China	20/20	47	Unclear	CKI+CAF	15	3 C	Unclear
SRF 2009	China	64/54	22-70	Unclear	CKI+GN	20	2 C	≥70
SX 2008	China	38/32	51	IV	CKI+TA	20	2 C	Unclear
SXM 2008	China	30/29	49.6	II-III	CKI+CAF	30	6 C	Unclear
WL 2007	China	30/30	42.5	Unclear	CKI+CTF	20	2-3 C	Unclear
WXH 2010	China	60/60	32-65/28-67	I-III	CKI+CAF	20	3 C	≥60
WXH 2011	China	34/34	50	II-III	CKI+TA	30	6 C	>70
WYH 2010	China	12/12	52	Unclear	CKI+CTF	20	2 C	≥70
XY 2015	China	33/26	46	Unclear	CKI+TAC	Unclear	6 C	Unclear
YX 2012	China	30/30	41.50±10.29	III-IV	CKI+TA	16	3 C	≥70
ZH 2014	China	35/28	53.5/54.3	II-III	CKI+TE	20	12 D	≥60
ZJQ 2010	China	48/44	Unclear	II-III	CKI+HFE	15	2 C	Unclear
ZXJ 2014	China	61/62	42.7±10.5/43.5±11.2	I-III	CKI+CAF	20	6 C	>70
ZXM 2011	China	21/17	46.2±7.6	II-III	CKI+ACT	20	4 C	≥60
ZZJ 2015	China	65/65	43.2±17.9	I-II	CKI+AC	12	6 C	≥60

Note: E/C: experimental group/control group; C: cycle; D: day; KPS: Karnofsky; CKI: compound kushen injection; TAC: Docetaxel, ADM (Adriamycin) and CTX (cyclophosphamide); CAF: CTX, ADM and 5-fu (5-fluorouracil); FEC: 5-fu, EPI (epirubicin) and CTX; T: PTX (paclitaxel); CTF: CTX, THP (Therarubicin) and 5-fu; TA: PTX and ADM; ACD: dox (doxorubicin), CTX and Docetaxel; TEC: Docetaxel, EPI and CTX; DP: Docetaxel and DDP (Cisplatin); GN: GEM (emcitabine) and NVB (Vinorelbine), TE: Docetaxel and EPI; HFE: HCPT (hydroxycamptothecine), 5-FU and ADM; ACT: ADM, CTX and PTX.

combined OR and 95% CI (OR=2.07, 95% CI [1.61, 2.67]; *P*<0.00001), which demonstrated that CKI combined with chemotherapy in the treatment of breast cancer could significantly improve the tumor response compared with chemotherapy alone (**Figure 3A**).

Quality of life: There were 11 trials [24-26, 28, 29, 31-33, 35, 36, 48] with 908 cases contained a KPS improvement of >10 points. No significant heterogeneity was found among these trials (Chi^2=4.08, df=10 (P=0.94); I^2=0%). The fixed effect model was used for statistical analysis (OR=2.63, 95% CI [1.97, 3.52]; P<0.00001). Which meant that CKI combined with chemotherapy might improve the KPS increase rate, to further improve the quality of life compared with chemotherapy alone (**Figure 3B**).

Immune function

T lymphocytes subsets expression level: The most commonly detection indexs for tumor-infil-

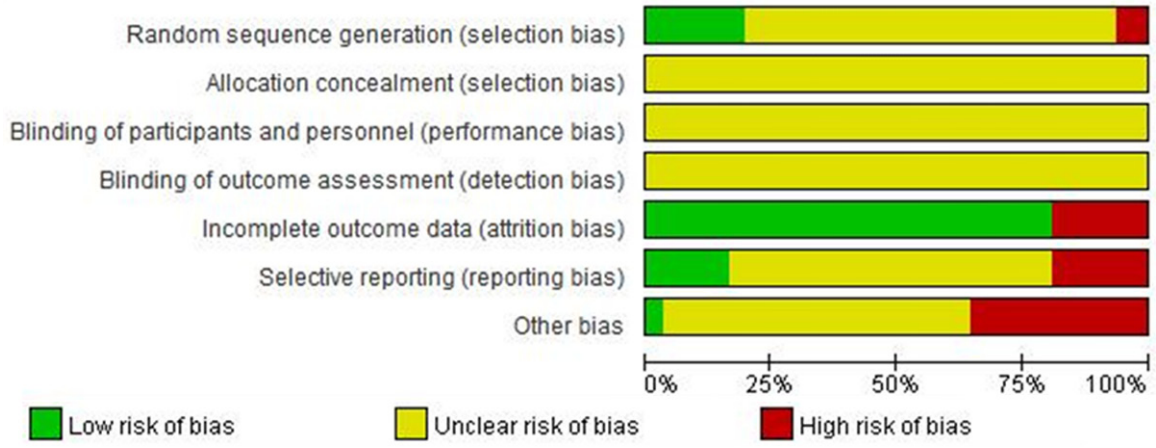

Figure 2. Risk of bias graph Quality assessment was conducted by Review Manager 5.3.

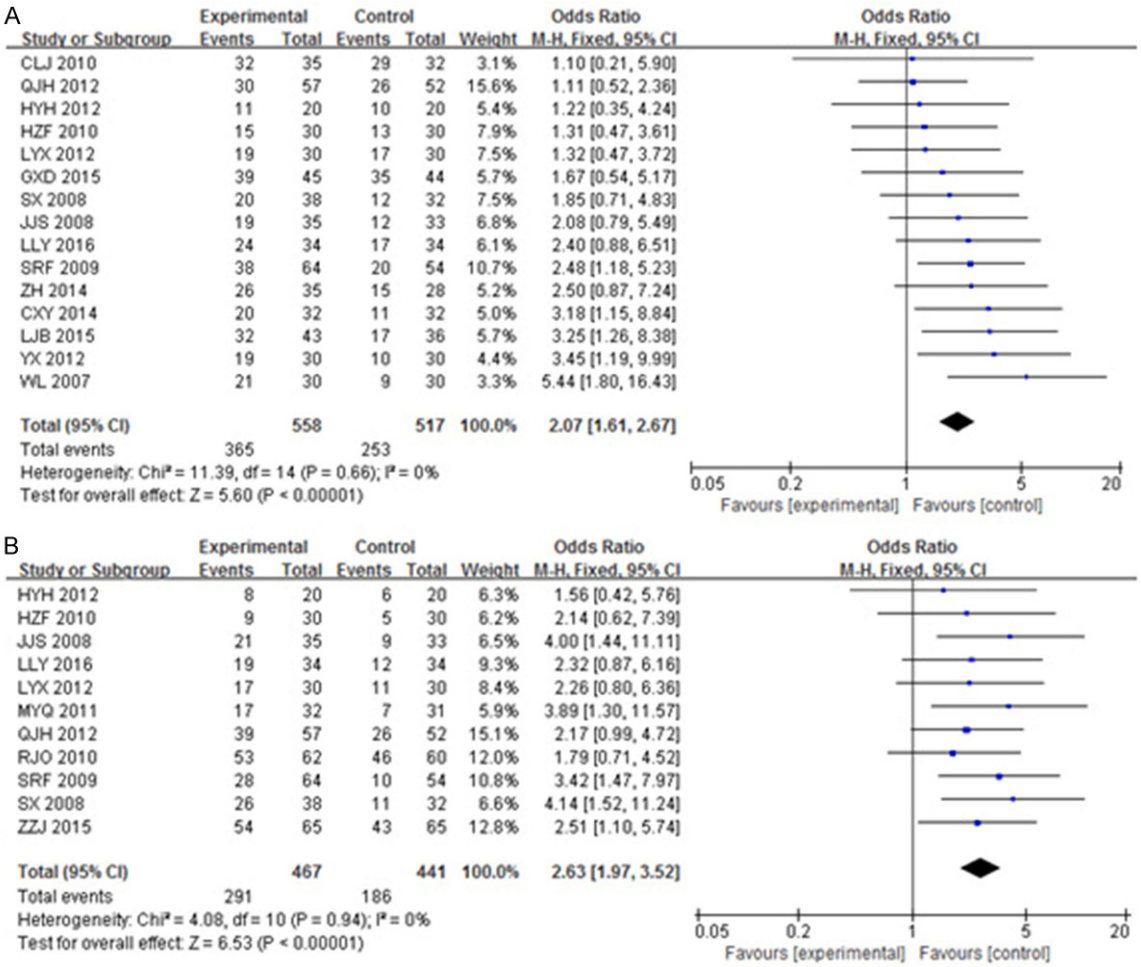

Figure 3. Forest plot of improved the tumor response and quality of life CKI plus chemotherapy versus chemotherapy alone.

trating lymphocytes subset in breast cancer are $CD4^+$ cells, $CD8^+$ cells and the ratio of $CD4^+/CD8^+$. Of the 31 trials, 8 trials [25, 27, 30, 36, 40, 41, 43, 46] reported T lymphocytes sub-

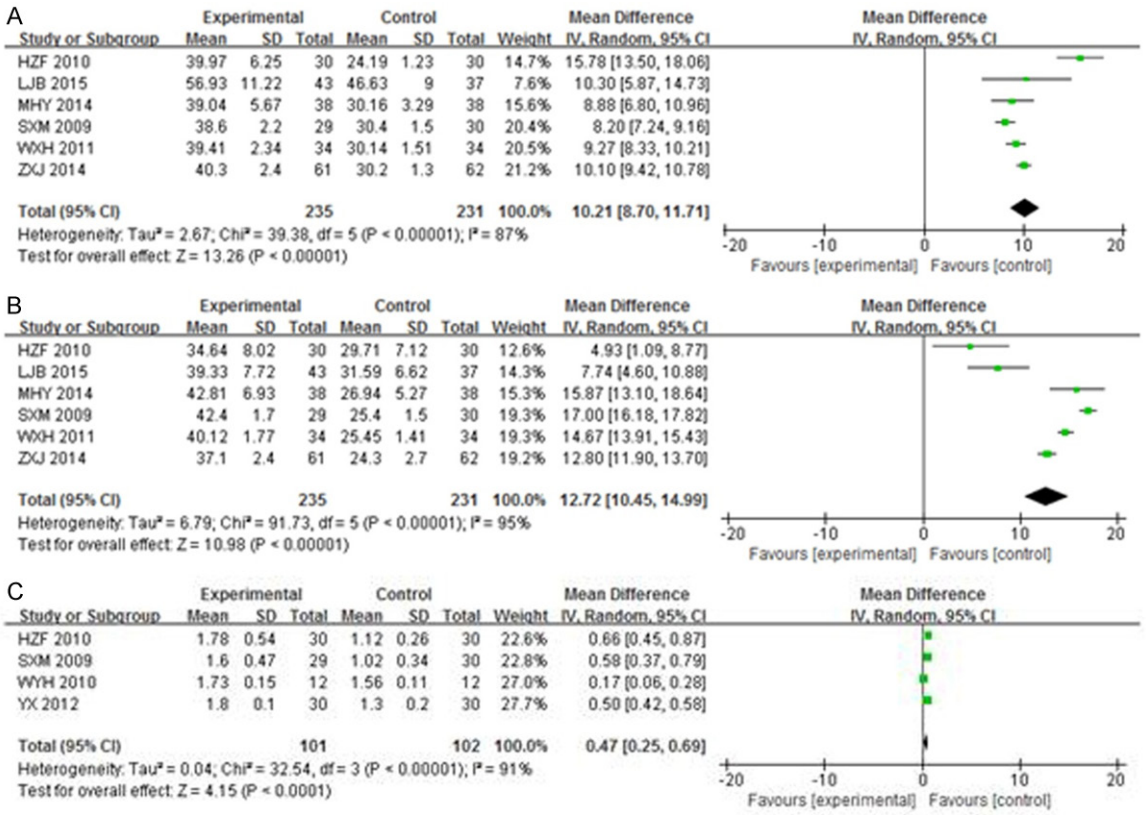

Figure 4. Forest plot of T lymphocytes subsets expression level CKI plus chemotherapy versus chemotherapy alone. A: $CD4^+$ cells; B: $CD8^+$ cells; C: ratio of $CD4^+CD8^+$.

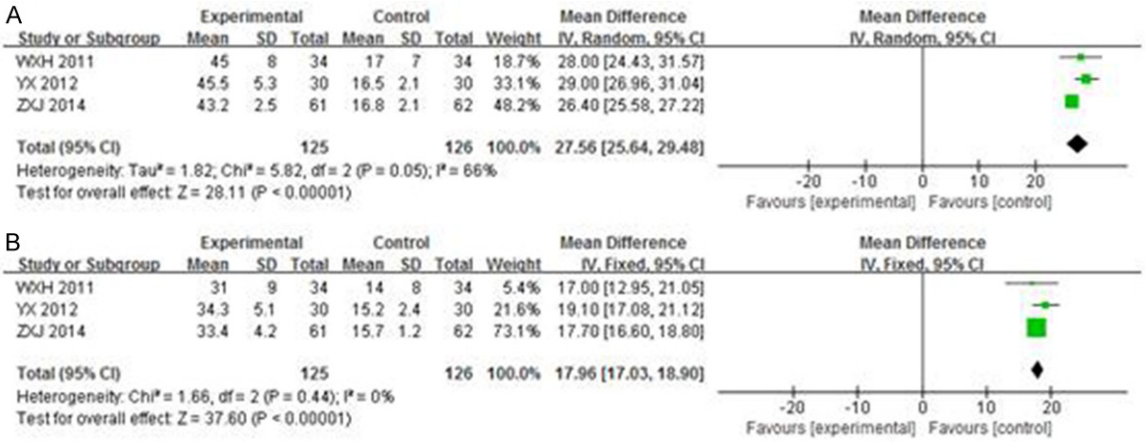

Figure 5. Forest plot of interleukin level CKI plus chemotherapy versus chemotherapy alone. A: IL-4; B: IL-10.

sets. The heterogeneity test showed that there was large statistical heterogeneity between studies (**Figure 4**). The random-effects model was used to calculate the combined MD and 95% CI, $CD4^+$ cells (*n*=466, MD=10.21, 95% CI [8.70, 11.71]; *P*<0.00001), $CD8^+$ cells (n=466, MD=12.72, 95% CI [10.45, 14.99]; *P*<0.00001), and the ratio of $CD4^+/CD8^+$ (n=203, MD=0.47, 95% CI [0.25, 0.69]; *P*<0.00001). Meta-analysis indicated that CKI plus chemotherapy can mount a more effective immune response in the treatment of breast cancer.

Interleukin level: 3 trials [40, 43, 46] with 251 patients were reported using the IL-4 outcome. The random effect model was used (Chi^2=5.82,

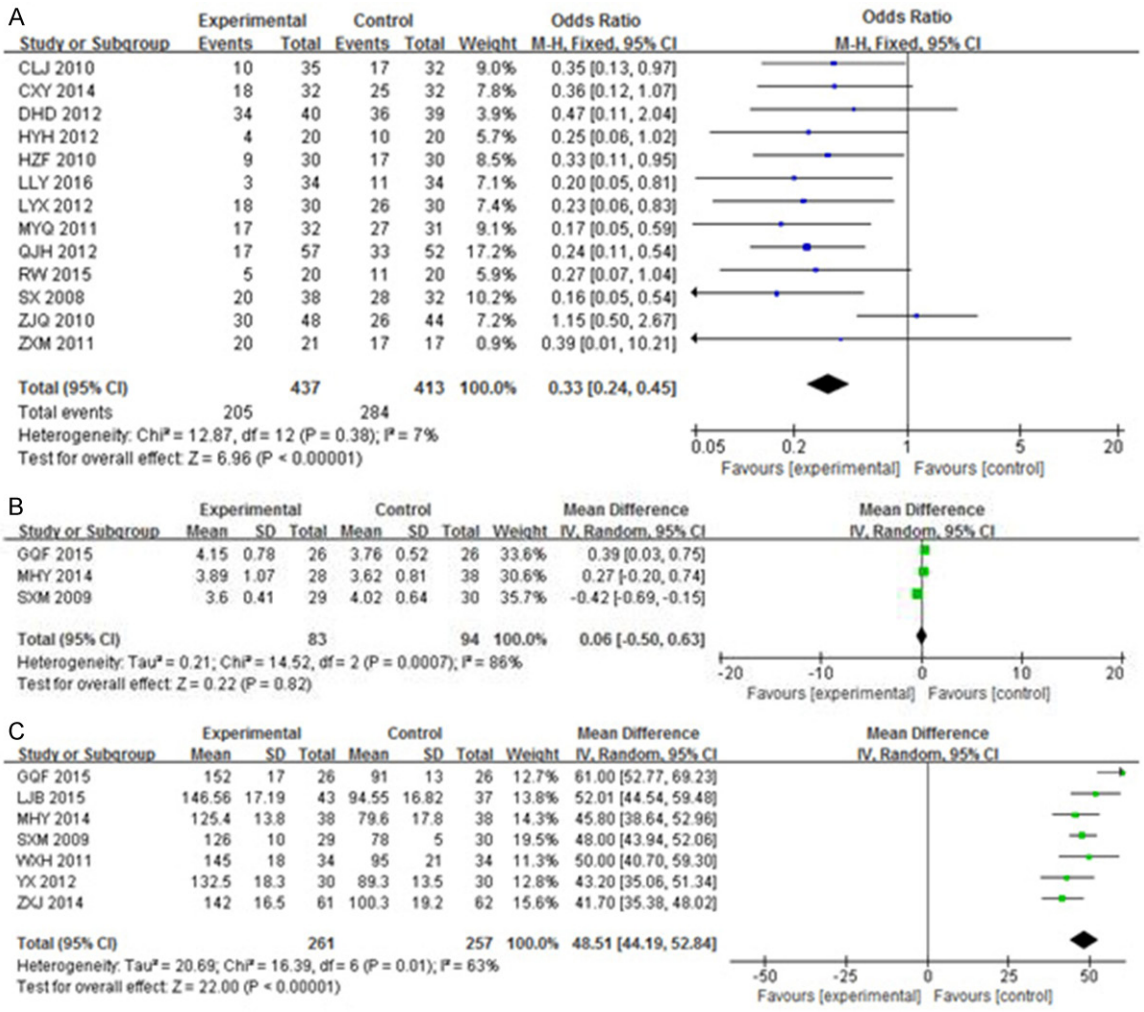

Figure 6. Forest plot of the blood system CKI plus chemotherapy versus chemotherapy alone. A: WBC; B: RBC; C: PLT.

df=2 (P=0.05); I^2=66%). CKI combined with chemotherapy improved the IL-4 level in patients with breast cancer compared to chemotherapy alone (MD=27.56, 95% CI [25.64, 29.48]; P<0.00001) (**Figure 5A**). Meanwhile, 3 studies were reported using the IL-10 outcome. No heterogeneity (Chi²=1.66, df=2 (P=0.44); I^2=0%) was noted among these studies. The fixed effect model was used. CKI combined with chemotherapy improve the IL-10 level in the treatment of breast cancer (MD=17.96, 95% CI [17.03, 18.90]; P<0.00001) (**Figure 5B**).

Adverse events

Adverse events associated with hematology: The incidence of leucopenia was recorded in 13 studies (850 cases) [18, 20, 21, 24, 25, 28, 29, 31, 32, 34, 36, 45, 47], which showed that there was seldom statistical heterogeneity between 13 trials (Chi²=12.87, df=12 (P=0.38); I^2=7%). The incidence of leukopenia in CKI combined with chemotherapy group was significantly lower than those in control group in the treatment of breast cancer (OR=0.33, 95% CI [0.24, 0.45]; P<0.00001) (**Figure 6A**).

The counts of red blood cells (RBC) data extracted from 3 studies (177 cases) [22, 30, 37], which showed that there were large statistical heterogeneity among trials (Chi²=14.52, df=2 (P=0.0007); I^2=86%). The random effect model was used for statistical analysis. The results indicated that there was no statistical difference between two groups, CKI combined with chemotherapy did not improve the RBC count in patients (MD=0.06, 95% CI [-0.50, 0.63]; P=0.82) (**Figure 6B**).

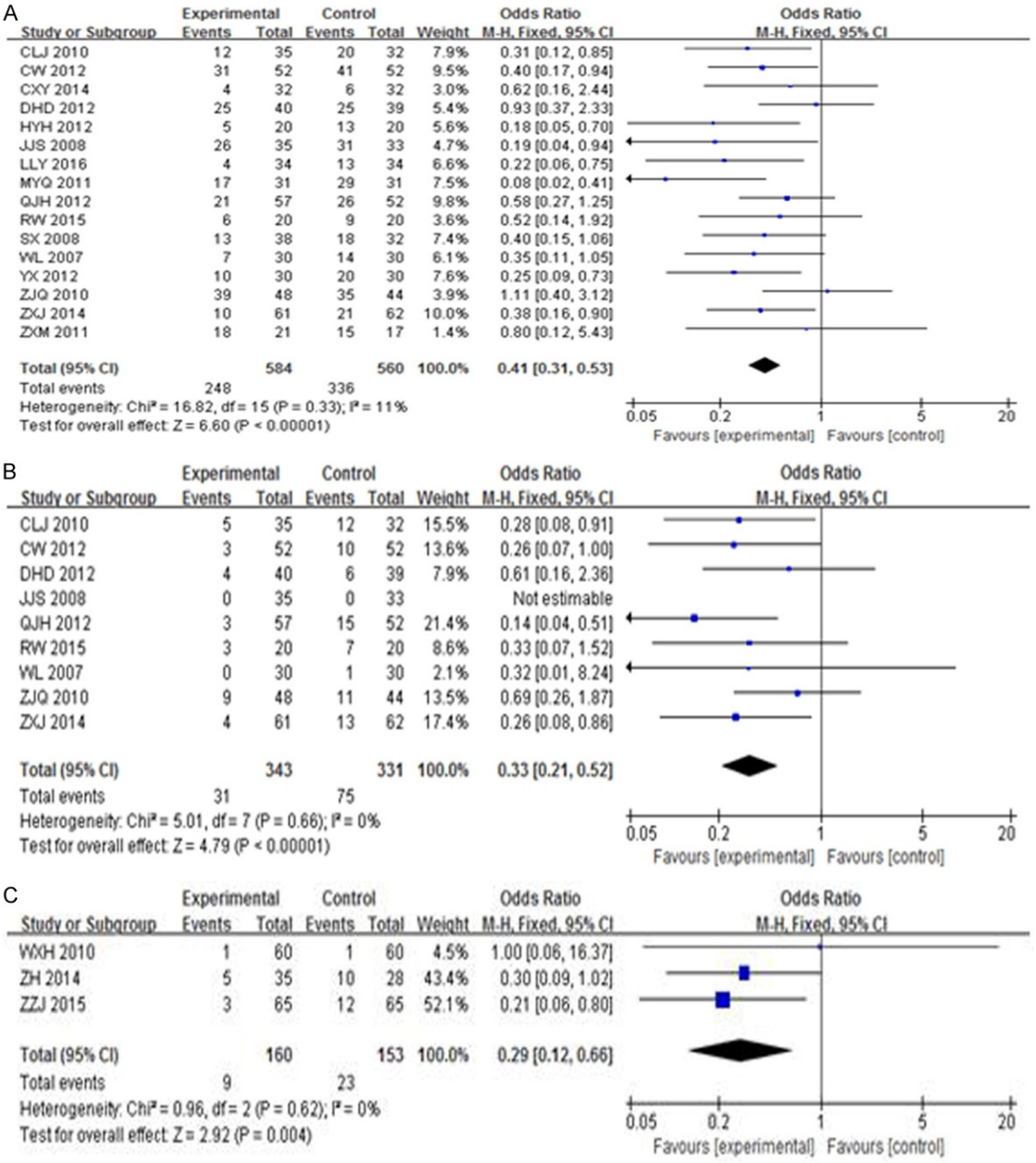

Figure 7. Forest plot of the non-hematological adverse events. A: Gastrointestinal adverse reactions; B: Hepatic insufficiency; C: TBIL.

7 studies [22, 27, 30, 36, 40, 43, 46] with 518 cases reported the platelets (PLT) count, which showed heterogeneity among trials (Chi^2=16.39, df=6 (P=0.01); I^2=63%). A meta-analysis of these studies using a random effect model demonstrated that CKI combined with chemotherapy could remarkably increase the PLT count in the treatment of breast cancer (MD=48.51, 95% CI [44.19, 52.84]; P<0.00001) (**Figure 6C**).

The non-hematological adverse events: 16 trials [18-21, 24, 26, 28, 31, 32, 34, 36, 38, 43, 45-47] including 1144 patients reported the gastrointestinal adverse reactions occurrence rate. Meta-analysis showed the heterogeneity test (Chi^2=16.82, df=15 (P=0.33); I^2=11%). A fixed effects model was used to calculate the combined OR and 95% CI (OR=0.41, 95% CI [0.31, 0.53]; P<0.00001) (**Figure 7A**). CKI combined with chemotherapy resulted in a lower

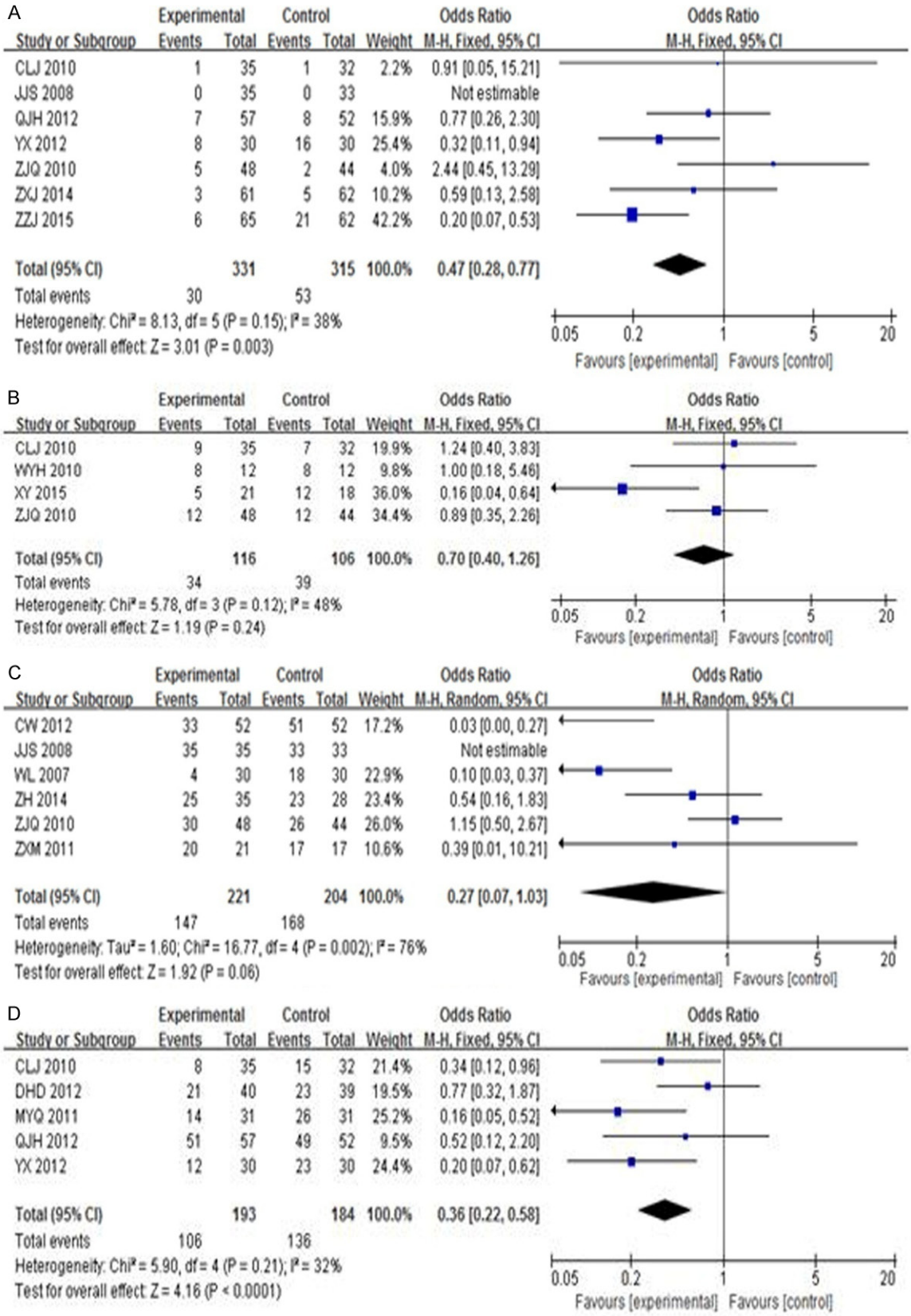

Figure 8. Forest plot of the non-hematological adverse events. A: Renal insufficiency; B: Electrocardiogram; C: Bone marrow depressions; D: Alopecia.

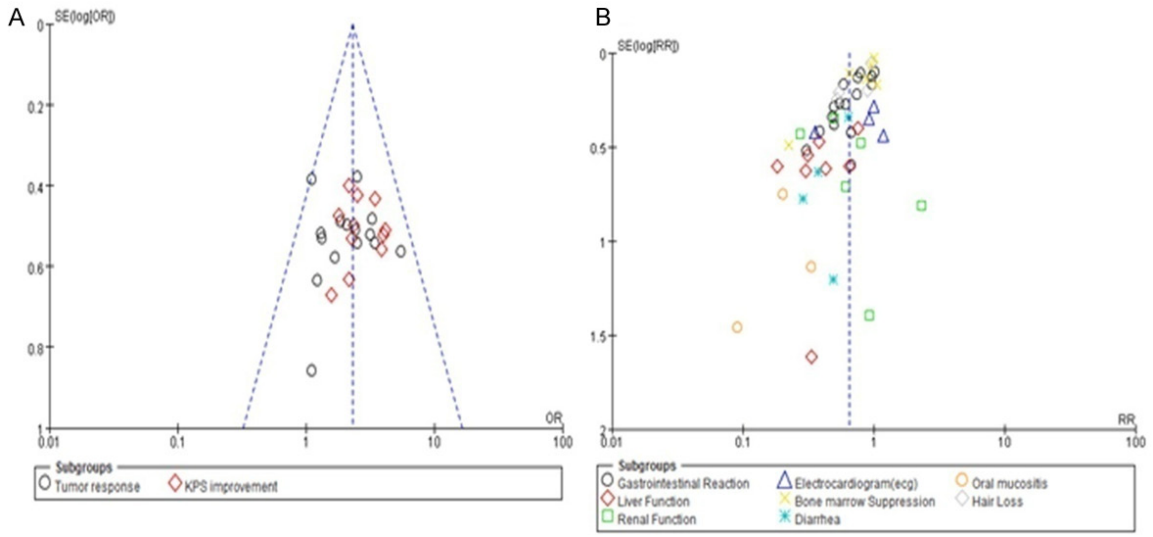

Figure 9. Funnel plot for publication bias. A: Funnel plot of clinical efficacy; B: Funnel plot of safety.

incidence of gastrointestinal adverse reactions in the treatment of breast cancer when compared with chemotherapy alone.

The hepatic insufficiency was provided by 9 trials [18, 19, 21, 26, 32, 34, 38, 45, 46] with 742 patients. No statistical heterogeneity was observed among studies (Chi^2=5.01, df=7 (P=0.66); I^2=0%), so the fixed effects model was used. The results indicated that CKI combined with chemotherapy could reduce the rate of hepatic insufficiency in the treatment of breast cancer (OR=0.33, 95% CI [0.21, 0.52]; P<0.00001) (**Figure 7B**).

The increased bilirubin (TBIL) rate data extracted from 3 studies [39, 44, 48] with 313 cases, the results indicated that there was no statistical heterogeneity between studies (Chi^2=0.96, df=2 (P=0.62); I^2=0%). The fixed effect model was applied to calculate the combined OR and 95% CI (OR=0.29, 95% CI [0.12, 0.66]; P=0.004). Meta-analysis explained that CKI combined with chemotherapy demonstrated a lower rate of TBIL when compared with chemotherapy alone (**Figure 7C**).

Of 31 trials, 7 studies (646 cases) [18, 26, 32, 43, 45, 46, 48] reported the renal insufficiency. The heterogeneity test indicated that there was seldom heterogeneity between trials (Chi^2=8.13, df=5 (P=0.15); I^2=38%). The fixed effect model was used for meta-analysis. CKI plus chemotherapy could reduce the incidence rate of renal insufficiency in patients when compared with chemotherapy (OR=0.47, 95% CI [0.28, 0.77]; P<0.003) (**Figure 8A**).

The change of Electrocardiogram (ECG) was reported by 4 studies [18, 41, 42, 45] with 222 patients. The fixed effect model was used because heterogeneity was moderate (Chi^2=5.78, df=3 (P=0.12); I^2=48%). The meta-analysis indicated that no better improvements were observed in CKI combined with chemotherapy group for cardiac function (OR=0.70, 95% CI [0.40, 1.26]; P=0.24) (**Figure 8B**).

6 studies (425 patients) [19, 26, 38, 44, 45, 47] provided the bone marrow depressions. The random effect model was used because the results of heterogeneity test (Chi^2=16.77, df=4 (P=0.002); I^2=76%). The pooled OR revealed that CKI combined with chemotherapy could not reduce the incidence of bone marrow depressions in the treatment of breast cancer (OR=0.27, 95% CI [0.07, 1.03]; P=0.06) (**Figure 8C**).

Alopecia was provided in 5 studies [18, 31, 32, 43]. No statistically significant heterogeneity was found among trials (Chi^2=5.90, df=4 (P=0.21); I^2=32%). The fixed effect model was applied for meta-analysis. CKI combined with chemotherapy could reduce the incidence of alopecia in the treatment of breast cancer (OR=0.36, 95% CI [0.22, 0.58]; P<0.00001) (**Figure 8D**).

Publication bias analysis

The funnel plot was used to assess the publication bias on clinical efficacy and safety. The funnel plot of the tumor response and KPS improvement was symmetrical in general, (**Figure 9A**) and it prompted that publication bias for the literatures was controlled passably. But the funnel plot showed evident asymmetry of safety evaluation, and publication bias may have existed in our study, it might influence the results of our analysis (**Figure 9B**). For other research items, there were only very little studies, so we did not make the funnel plot.

Discussion

The peak of incidence rate of breast cancer is at age group 40 to 60 (paralleling the menopausal age). And the morbidity of young patients (≤35 years) increases gradually [49]. At present, chemotherapy already was used extensively at treating breast cancer for many years. The side-effects or toxicity of tumor patients after chemotherapy treating are main limiting factors in the clinical treatment. A large number of clinical studies have proven that TCM could improve clinical effective and immunity in the cancer patients, and reduce the incidence of poor reactions [50].

Alkaloids are the main constituents of CKI, including matrine, oxymatrine and sophoridin. The CKI has diverse activities, such as anti-inflammatory, anti-allergic, anti-viral, anti-fibrotic and cardiovascular protective effects, especially anti-tumor and raising tumor patient immunity [51]. The anti-tumor mechanisms of CKI involved in: (1) Reduces cancer cell proliferation, and induces differentiation and apoptosis; (2) Inhibits invasion and metastasis; (3) Enhances the antitumor immunity ability; (4) Restrains angiogenesis; (5) Protects against the development of chronic inflammation for the tumor; (6) Reverses the multi-drug resistance and adverse events; (7) Enhances the anti-cancer potential combination chemotherapy regiments with other chemotherapeutic drugs [52-55]. During the survey period, there was only one case of adverse reaction in the 1141 identified patients who received CKI [37], which disappeared after withdrawal. As a result, CKI had good safety.

The meta-analysis showed that CKI plus chemotherapy indeed improves the tumor response, quality of Life, T lymphocytes subsets ($CD4^+$, $CD8^+$, $CD4^+/CD8^+$) and Interleukin (IL-4, IL-10), PLT. Meanwhile there were statistically significant reductions in the incidences of leucopenia, gastrointestinal adverse reactions, hepatic insufficiency, TBIL, renal insufficiency, and alopecia. However, the current evidence does not support the efficacy of CKI for RBC, the change of ECG and bone marrow depressions. It may be closely related with the small sample size included.

Cycle and dosage were the important objective index in evaluating clinical efficiency of CKI. Therefore, we performed the subgroup analysis. First, according to the dosage of CKI, the studies were divided into two groups: the high-dose groups (≥20 ml/d) and low-dose groups (<20 ml/d). As a result, the high-dose groups of CKI weresuperior to low-dose groups on tumor response, and the difference was significant between them ($P<0.05$). According to the cycle course of treatment, the studies were divided into two groups, (1) The course of the consecutive treatment of CKI exceeded 3 period (≥3C); (2) The course of the other trials were less than 3 period (<3C). The different cycle did not result in differences in tumor response. However, there were still some limitations and shortcomings in the trial design, such as different the tumor grade of breast cancer, different chemotherapy regimens, and these regimens had different adverse drug reactions. Although, Traditional Chinese Medicine has now been widely recognized and used in worldwide, such as a recent study, for analyze the anti-cancer molecular mechanism of CKI and identified potential primary target pathway, CKI could disrupt multiple pathways to induce apoptosis of MCF-7 cells [56]. But, the clinical use of CKI is currently limited to China that all of the articles were from China. It was necessary to examine the results using a more varied population sample. We did not carry out the subgroup analysis based on different TMN stage and age and chemotherapy regimens.

In spite of the poor quality of included trials, the results of meta-analysis provided scientific evidence of the effectiveness and safety of CKI combined with chemotherapy in the treatment of breast cancer. In the future the larger, longer-term, rigorously designed, multi-center, randomized, double-blind, controlled trials were required to fully assess whether the combination is more outstanding.

Conclusion

In summary, our findings suggest that CKI combined with chemotherapy may significantly improve tumor response and KPS, enhance the immune function of patients, and reduce in the incidence of adverse events. However, the interpretation results must be careful, because of the small sample size and limitations, and the mechanism of CKI was a complex process and still not completely understood.

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Disclosure of conflict of interest

None.

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