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Review Article

Prognostic value of Wilms' tumor 1 in epithelial ovarian cancer: a systematic review and meta-analysis

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Abstract: Purpose: Although ovarian cancer has become a major cause of morbidity and mortality, the important question of specific prognostic factor has not been explicated thoroughly. Therefore, we conducted a systematic review and meta-analysis to investigate the prognostic value of Wilms' tumor 1 (WT1) expression level in patients with ovarian cancer. Methods: A comprehensive search was made for studies that evaluated the prognostic value of WT1 in ovarian cancer patients. PubMed, Embase, the Cochrane Library, the clinical trial registration website (ClinicalTrials.gov) and Ovid databases from the earliest available date to July 10, 2018 were used to identify the studies. References of selected studies were checked manually for further potential trials. Random-effects model was used to estimate hazard ratio (HR) statistics. All statistical analyses were performed by STATA (version 13.0; College Station, Texas, USA). Results: Nine studies were involved in this meta-analysis. According to higher versus lower WT-1 expression, the pooled HR of overall survival is 1.05 (95% confidence interval, CI = 0.57-1.52, P = 0.125). The pooled HR is 1.33 (95% CI = 0.77-1.85, P = 0.191) in disease-specific survival (DSS), and 1.17 (95% CI = 0.66-1.69, P = 0.177) in disease-free survival (DFS)/progression-free survival (PFS). Moreover, subgroup analysis of OS revealed that higher WT1 expression in ovarian cancer patients with III-IV stage might be a poorer prognostic biomarker for OS (HR = 1.98, 95% CI = 1.15-3.41). Conclusion: WT1 expression level is not associated with the OS, DSS, DFS or PFS in ovarian cancer patients. However, higher WT-1 may cause poorer survival in cases with advanced cancer stage.

Keywords: Ovarian cancer, Wilms' tumor 1, prognosis, biomarker

Introduction

According to the latest global cancer statistics [1], there were an estimated 238,700 new ovarian cancer cases and 151,900 deaths in 2012. In developed countries, it is ranked fifth and sixth most common cancer in incidence and mortality, respectively. World Health Organization (WHO) pathology classification divides ovarian cancer into several subtypes: epithelial tumor, sex cord-stromal tumor, germ cell tumor, germ cell sex cord-stromal tumor, tumor of the rete ovary and miscellaneous tumors. Among these subtypes, epithelial ovarian cancer is the most common disease.

Due to the nonspecific initial symptoms of ovarian cancer, more than 70% patients were diagnosed with advanced stage disease (International Federation of Gynecologists and Obstetricians [FIGO]-stages III-IV). Moreover, in epithelial ovarian cancer, the 5-year overall survival (OS) rate in early stage of the disease was 80.2%, whereas 5-year OS rate was 25.6% in the late stage [2]. Some studies illuminated several prognostic indexes or models to predict the outcome of ovarian cancer. The most well-known prognostic factors for ovarian cancer patients are tumor grade, tumor type, FIGO stage, amount of residual tumor after first surgery and the cancer antigen 125 (CA-125) in serum. However, these markers are still limited for evaluating the prognosis of individual patients, so more potential prognostic factors for the ovarian cancer patients should be discovered.

The Wilms' tumor 1 (WT1) gene, located on chromosome 11p13, was firstly identified as a tumor suppressor gene in Wilms tumor, and was involved in the cell-cycle regulation, cell apoptosis and mRNA metabolism [3-6]. WT1

acted as a tumor suppressor in clear cell renal cell carcinoma [7], but recent studies have found that the overexpression of the wild-type WT1 was found in several other types of neoplasms which acted as an oncogenic stimulator, such as in leukemias, breast, digestive tract and brain cancers [8-11].

Recent studies have demonstrated that WT1 can act as a prognostic marker in ovarian cancer patients. In addition, higher WT1 expression in tumors is associated with worse outcome in patients, but there is no consensus agreement. Therefore, we conducted a systematic review and meta-analysis evaluating the prognostic value of WT1 expression level in epithelial ovarian cancer.

Material and methods

Selection criteria

Published studies which were written in English were considered eligible if they reached the following criteria: (1) patients were pathologically diagnosed with ovarian cancer; (2) outcomes in studies should include the relationship between WT1 expression and prognosis, for instance, OS, disease-specific survival (DSS), disease-free survival (DFS) and progression-free survival (PFS); (3) presenting the exact hazard ratio (HR) and 95% confidence interval (CI) or Kaplan-Meier survival curves.

Search strategy

This meta-analysis was conducted according to the guideline of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [12]. We searched the PubMed, Embase, the Cochrane Library, the clinical trial registration website (ClinicalTrials.gov) and Ovid databases from the earliest available date to July 10, 2018 to identify the studies that reached the above criteria. The search strategy was performed using all following medical subject headings (MeSH) or non-MeSH terms including ovarian cancer, ovarian neoplasm, epithelial ovarian cancer, epithelial ovarian neoplasm, Wilms' tumor 1, WT1, outcome, overall survival, OS, disease-specific survival, DSS, disease-free survival, DFS, progression-free survival, PFS, recurrence-free survival and RFS. Each search strategy was conducted in each database. And we manually checked the references of selected studies for further potential trials.

Data extraction

Two authors independently (Lei Zhang and Xu Liu) performed the selection of potential eligible studies. Any discrepancies were resolved by consensus with a third reviewer (Yuan Zhao). And the data from the selected studies were collected on a standardized form by two authors, independently. Data should include author name, country, study type, study duration, population size, age, FIGO stage, diagnosis method, tumor histology, percentage of WT1 positive expression, cut-off value, follow-up time, HR, 95% CI and *p* value of OS, DSS, DFS, PFS or RFS and other covariates.

Outcome measures

The primary outcome measure in this meta-analysis was OS, which was defined as the length of time from the definitive surgery to death from any cause. The secondary outcome measure was DSS, defined as the time from definitive surgery to death due to ovarian cancer. Additionally, DFS and PFS were also determined as outcome measures, which were defined as the time from primary surgery to the first noting recurrence and the time from primary surgery to progression or relapse disease, respectively.

Quality assessment

Two independent authors (Lei Zhang and Xu Liu) assessed the quality of included studies. The Newcastle-Ottawa Scale (NOS) was used to evaluate the methodological quality of each cohort study, which was recommended by the Cochrane Nonrandomized Studies Methods Working Group [13]. There were three major aspects for assessing the quality score: method of selection of the study groups (0-4 stars), comparability of cohorts (0-2 stars) and ascertainment of the outcome (0-3 stars), with total score of 9 stars. The studies were divided into three groups: low (0-3 stars), moderate (4-6 stars) and high (7-9 stars). Any discrepancies were resolved by consensus with a third author (Yuan Zhao).

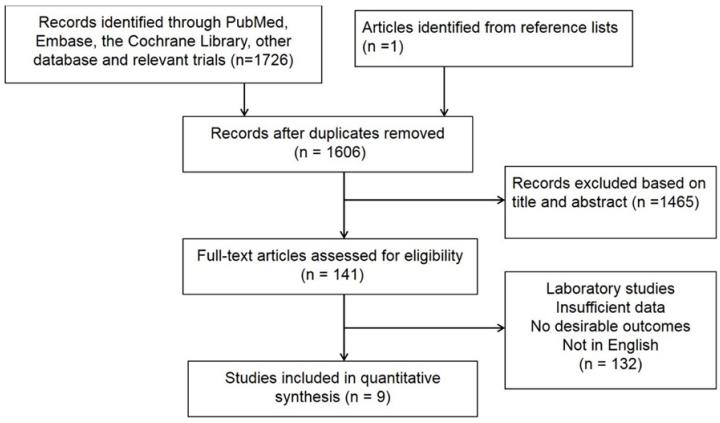

Figure 1. Selecting the flowchart for the inclusion of studies in the meta-analysis.

Statistical analysis

This analysis used HRs with 95% CIs to evaluate OS, DSS, DFS and PFS. Multivariate HRs and 95% CI were commonly used to calculate pooled HR, and univariate HRs and their 95% CI were used if multivariate HRs were not available. An HR>1 suggested higher WT1 expression which was associated with poorer prognosis. Random-effect model was used for pooled HRs regardless of high or low levels of heterogeneity. Patient characteristics, cut-off value and other confounding factors were not consistent between trials, so there was a prior advantage of a random-effect model, compared with a fixed-effect model in accounting for heterogeneity [14]. Heterogeneity was assessed by using the I^2 statistic and the Chi-square (Q statistic) test. An I^2 value <25% indicates a low level of heterogeneity, while values of 25%-50% and ≥50% represent moderate and high levels of heterogeneity, respectively [15]. Subgroup analysis of higher versus lower WT-1 expression and survival (OS, DSS, DFS and PFS) of epithelial ovarian cancer patients included histology, cancer stage, number of cases, and adjustment for covariates. We used Begg funnel plot and Egger regression asymmetry test to evaluate publication bias. Sensitivity analysis was carried out to examine the impact of single study. Every time one study was excluded, the rest was analyzed to evaluate whether single study affected results significantly. A two-sided test was used in analyses, and *P* value <0.05 was considered as statistically significant. All statistical analyses were performed using STATA version 13.0 (Stat Corporation, College Station, Texas, USA) program.

Results

1726 articles were identified through PubMed, Embase, the Cochrane Library and other database, and one article was searched from reference lists. 121 duplicates were removed. And 141 articles left after searching titles and abstracts. 132 additional studies were excluded, because they were laboratory studies having insufficient data, no desirable outcomes, or the articles were not in English. The final set of studies eligible for the quantitative synthesis included 9 studies [16-24]. The selection strategy is shown (**Figure 1**). The characteristic of relevant studies are outlined (**Table 1**). A total of 1,920 patients were included in this meta-analysis. All the studies were retrospective studies. Eight studies [16-22, 24] used immunohistochemistry (IHC) to diagnose WT1 expression, and only one study [23] used reverse transcription-polymerase chain reaction (RT-PCR). The median age of patients in these studies was about 60. Three studies only included serous ovarian cancer patients, and the other six studies included all the subtypes of ovarian cancer patients. Four studies used other covariates to calculate the multivariate Cox proportional hazards regression analyses of WT1 expression. The methodological NOS is listed in **Table 1**. The quality of cohort studies was mostly high. However, due to the nature of the cohort study designs, the level of evidence was still low.

Effect of WT1 expression on the primary outcome measure

OS was the primary outcome measure in this meta-analysis. For using the random-effects model, the pooled analysis of five studies' data showed that the higher WT1 expression was not associated with significantly poorer OS outcome (HR = 1.05, 95% CI = 0.57-1.52, P = 0.125, **Figure 2**), and the level of heterogeneity was high (I^2 = 45.1%, P = 0.121).

The high level of heterogeneity could be explained by the number of cases, cancer stage or adjusted for covariates. Subgroup analyses of OS outcome were shown in **Table 2**. The studies that included more than 100 cases showed

Table 1. Characteristic of relevant studies on WT1 and ovarian cancer patients included in the meta-analysis

First Author	Country	Study Type	Duration	Size	Age	Stage	Method	Histology	Positive Expression (%)	Cut-off Value	Follow-up Time	HR (95% CI) of OS	HR (95% CI) of DSS	HR (95% CI) of DFS/PFS	Covariates	Quality Score
Hylander, 2006	USA	Retrospective study	1995-2002	100	Median 63 (22-88)	I-IV	IHC	Epithelial	78/100 (78%)	>5%	1-126 m	1.05 (0.52, 2.12) UV	NA	NA	NA	7
Netinatsunthorn, 2006	Thailand	Retrospective study	1987-2004	99	≤60 (69) >60 (30)	III-IV	IHC	Serous	50/90 (56%)	≥median	1-168 m	1.98 (1.15, 3.41) MV	NA	3.36 (1.60, 7.04) MV	FIGO stage, histologic grade, chemotherapy and primary residual tumor	8
Hogdall, 2007	Denmark	Retrospective study	NA	560	Median 54 (35-79)	I-IV	IHC	Epithelial	89/560 (16%)	>10%	1-121 m	NA	1.22 (0.94, 1.59) MV	NA	Age at diagnosis, histological type and histological grade	8
Yamamoto, 2007	Japan	Retrospective study	1987-2004	119	Median 57	I-IV	IHC	Serous	99/119 (83%)	>10%	2-227 m	NA	1.92 (0.99, 3.73) MV	NA	Residual tumor size (≥2 vs <2 cm), histologic grade (3/2/1), and the intensity of WT1 immunoreactivity (high-level immunoreactivity vs others) as parameters	8
Kobel, 2008	Canada	Retrospective study	1984-2000	493	Mean 60.9 ± 0.8	I-III	IHC	Epithelial	174/493 (35%)	≥5%	Median 61 m	NA	0.73 (0.45, 1.18) MV	0.52 (0.32, 0.85) UV	Age, stage, and histological subtype	8
Vermeij, 2011	Netherlands	Retrospective study	1985-2006	229	Median 56.9 (16-89)	I-IV	IHC	Epithelial	129/229 (56%)	NA	1-60 m	NA	2.10 (1.41, 3.12) UV	2.30 (1.58, 3.35) UV	NA	7
Andersson, 2014	Sweden	Retrospective study	1993-2003	50	Median 56 (20-81)	I-IV	IHC	Epithelial	36/50 (72%)	>10%	1-229 m	2.12 (1.01, 4.44) UV	NA	3.25 (1.40, 7.53) UV	NA	7
Liu, 2014	Japan	Retrospective study	2008-2013	63	Median 57 (29-81)	I-IV	RT-PCR	Epithelial	18/63 (29%)	≥53.94	1-66 m	0.61 (0.16, 2.29) UV	NA	2.95 (1.06, 8.21) UV	NA	8
Taube, 2016	Germany	Retrospective study	NA	207	≤60 (105) >60 (102)	I-IV	IHC	Serous	109/207 (52.7%)	≥0.001	1-238 m	0.76 (0.64, 0.9) UV	NA	0.82 (0.71, 0.96) UV	NA	7

IHC = immunohistochemistry, RT-PCR = reverse transcription- polymerase chain reaction, UV = univariate, MV = multivariate, HR = hazard ratio, NA = not available.

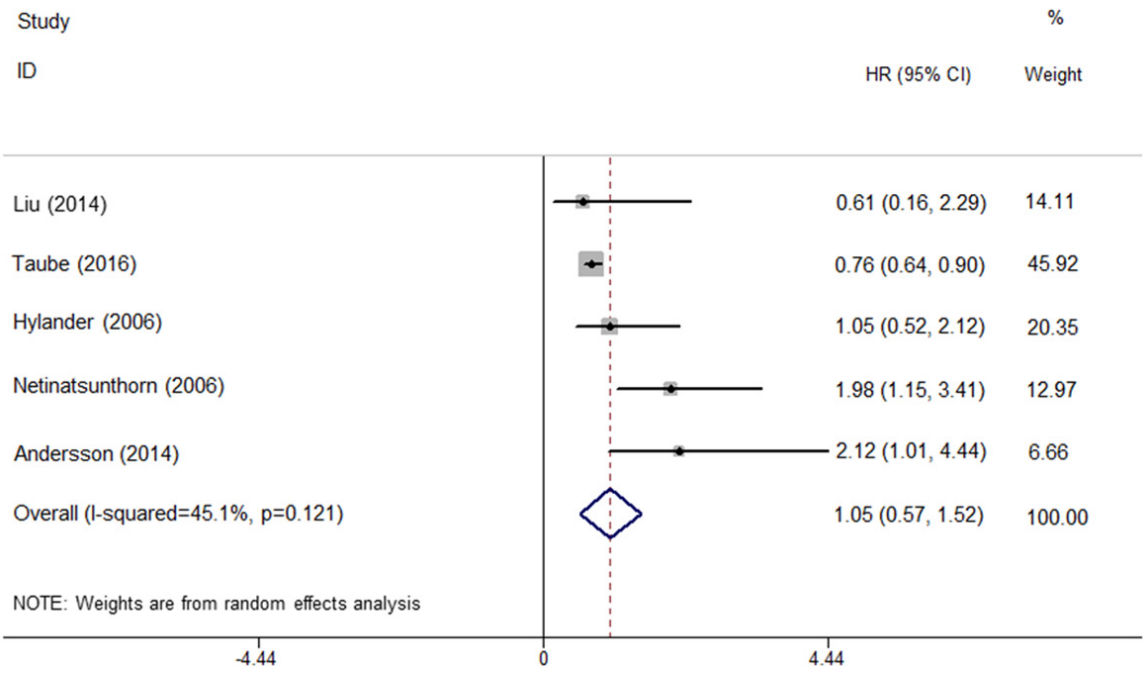

Figure 2. Forest plot showed hazard ratios (HRs) and 95% CIs for overweight and OS of ovarian cancer. HRs are for higher WT1.

Table 2. Random-effect summary estimates of the hazard ratios (HRs) of the association of OS of ovarian cancer with higher versus lower WT1 comparison

	Study	HR (95% CI)	*P*	*I*-squared (%)	$P_{heterogeneity}$
Histology					
Serous	2	1.24 (0.07, 2.40)	0.576	77.4%	0.036
Epithelial	3	1.05 (0.42, 1.68)	0.025	7.0%	0.341
Number of cases					
<100	3	1.47 (0.47, 2.48)	0.374	47.4%	0.149
≥100	2	0.77 (0.64, 0.90)	<0.001	0%	0.483
Cancer stage					
I-IV	4	0.77 (0.65, 0.90)	<0.001	0%	0.398
III-IV	1	1.98 (1.15, 3.41)	NA	NA	NA
Adjusted for covariates					
Yes	1	1.98 (1.15, 3.41)	NA	NA	NA
No	4	0.77 (0.65, 0.90)	<0.001	0%	0.398

HR = hazard ratio, NA = not available.

that the patients benefited from higher WT1 expression level (HR = 0.77, 95% CI = 0.64-0.90, P<0.001, heterogeneity P = 0.483, I^2 = 0%). Then, studies which involved all stages of ovarian cancer showed that HR was 0.77 (95% CI = 0.66-0.90, P<0.001, heterogeneity P = 0.398, I^2 = 0%), and only one study was adjusted for covariates (HR = 1.98, 95% CI = 1.15-3.41, P<0.001). No statistical differences were found in histology (**Table 2**). These data suggested that higher WT1 in patients with III-IV stage might act as a poorer prognostic marker.

Effect of WT1 expression on the secondary outcome measure

DSS, DFS and PFS were the secondary outcome measure in this meta-analysis. Pooled analysis showed that no significant differences in DSS, DFS or PFS were detected in patients

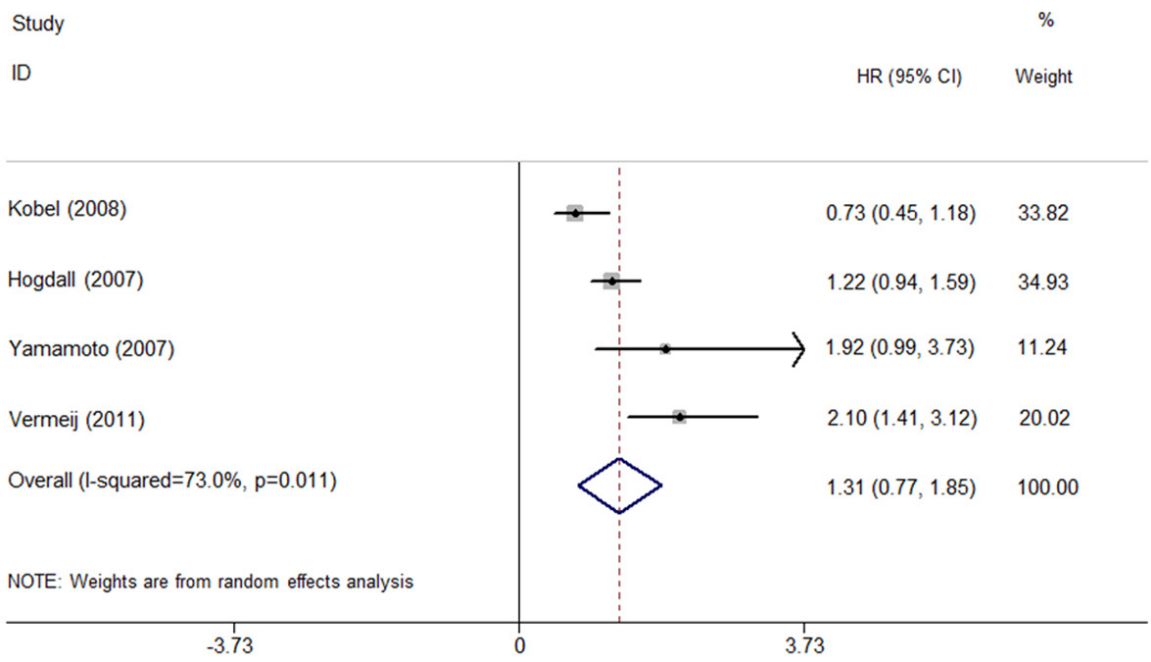

Figure 3. Forest plot showed hazard ratios (HRs) and 95% CIs for overweight and DSS of ovarian cancer. HRs are for higher WT1.

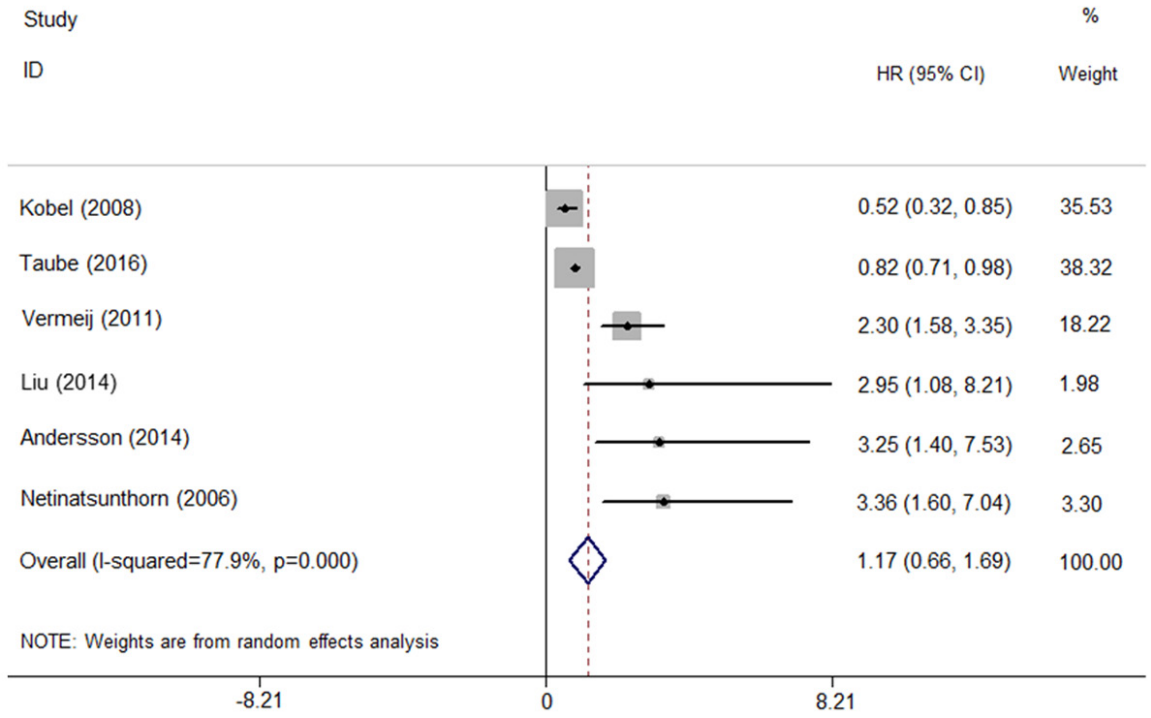

Figure 4. Forest plot showed hazard ratios (HRs) and 95% CIs for overweight and DFS/PFS of ovarian cancer. HRs are for higher WT1.

with higher WT1 expression level, with a pooled HR of 1.31 (95% CI = 0.77-1.85, P = 0.191) in DSS (**Figure 3**), and 1.17 (95% CI = 0.66-1.69, P = 0.177) in DFS/PFS (**Figure 4**). Both of them had high level of heterogeneity (DSS, I^2 = 73.0%, P = 0.011; DFS/PFS, I^2 = 77.9%, P<0.001).

Table 3. Random-effect summary estimates of the hazard ratios (HRs) of the association of DSS of ovarian cancer with higher versus lower WT1 comparison

	Study	HR (95% CI)	*P*	*I*-squared (%)	$P_{heterogeneity}$
Histology					
Serous	1	1.92 (0.99, 3.73)	NA	NA	NA
Epithelial	3	1.23 (0.65, 1.81)	0.195	79.4%	0.008
Number of cases					
<400	2	2.05 (1.32, 2.78)	<0.001	0%	0.827
≥400	2	0.98 (0.50, 1.46)	0.089	74.1%	0.049
Cancer stage					
I-III	1	0.73 (0.45, 1.18)	NA	NA	NA
I-IV	3	1.60 (0.95, 2.25)	0.176	52.7%	0.121
Adjusted for covariates					
Yes	3	1.08 (0.60, 1.55)	0.100	63.8%	0.063
No	1	2.10 (1.41, 3.12)	NA	NA	NA

Table 4. Random-effect summary estimates of the hazard ratios (HRs) of the association of DFS/PFS of ovarian cancer with higher versus lower WT1 comparison

	Study	HR (95% CI)	*P*	*I*-squared (%)	$P_{heterogeneity}$
Histology					
Serous	2	1.71 (0.01, 4.09)	0.461	70.1%	0.067
Epithelial	4	1.83 (0.33, 3.34)	0.217	83.8%	<0.001
Number of cases					
<100	3	3.22 (1.46, 4.99)	<0.001	0%	0.984
≥100	3	0.97 (0.48, 1.45)	0.137	87.0%	<0.001
Cancer stage					
I-IV	4	1.86 (0.57, 3.15)	0.418	78.9%	0.003
Others	2	1.60 (0.01, 4.31)	0.483	75.9%	0.042
Adjusted for covariates					
Yes	2	1.60 (0.01, 4.31)	0.483	75.9%	0.042
No	4	1.86 (0.57, 3.15)	0.408	78.9%	0.003

Subgroup analyses of DSS outcome were shown in **Table 3**. One potential explanation for DSS heterogeneity was number of cases in studies. The pooled HR in studies with less than 400 patients was 2.05 (95% CI = 2.05-2.78, P<0.001, heterogeneity P = 0.827, I^2 = 0%). No statistically significant outcome in DSS was detected in different histology or cancer stage (**Table 3**).

Subgroup analyses of DFS/PFS outcome were shown in **Table 4**. No benefit DFS/PFS outcome was seen in different histology, cancer stage and adjusted for covariates (**Table 4**). These data suggested that WT1 expression level was not associated with the DSS, DFS and PFS outcome, but subgroup analyses showed that WT1 expression level had a reverse effect on DSS, DFS and PFS outcome.

Publication bias and sensitivity analysis

Funnel plots of OS, DSS showed no publication bias (**Figures 5** and **6**), and Egger test showed P = 0.241 and 0.857, respectively. Funnel plot showed that there was publication bias existing in DFS/PFS (**Figure 7**), but Egger test showed P = 0.145, so this may not be caused by publication bias. Bias could be explained by tumor stage, and the number of case, et al. In sensitivity analysis, we excluded one study every turn and analyzed the rest articles. No significant change of pooled HR and 95% CI occurred when every single study was ignored.

Discussion

This study presents a meta-analysis of nine studies to evaluate the prognostic value of WT1 expression level in patients with ovarian cancer. We identified that WT1 expression level did not have a positive value in predicting prognosis of OS, DSS, DFS or PFS in patients with ovarian cancer. However, in subgroup analyses, we found that higher WT1 expression level in patients with high cancer stage showed poorer OS outcome.

WT1 is highly expressed in varies epithelial tumors and some hematopoitetic disorders,

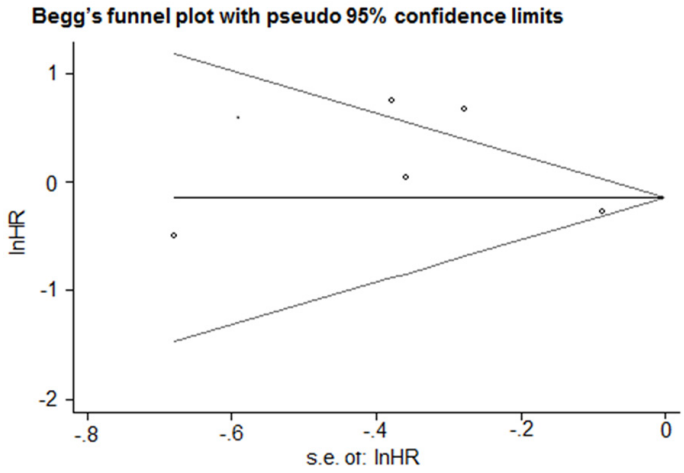

Figure 5. Begg funnel plot test for higher WT1 and OS of ovarian cancer.

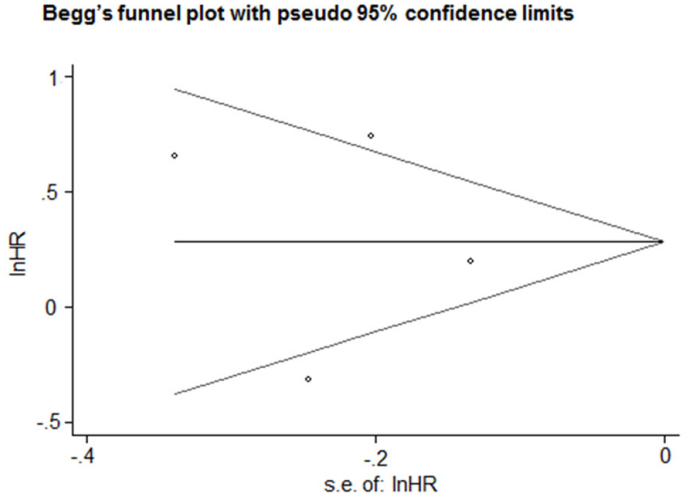

Figure 6. Begg funnel plot test for higher WT1 and DSS of ovarian cancer.

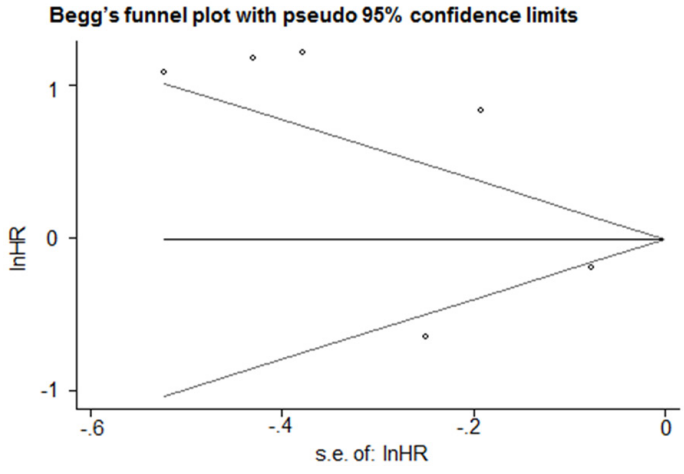

Figure 7. Begg funnel plot test for higher WT1 and DFS/PFS of ovarian cancer.

such as leukaemias and myeloproliferative neoplasms [25, 26], Moreover, high WT1 expression causes much endeavor in the pursuit of targeted therapies of WT1 epitopes [27]. In WT1 positive urinary cells, it can be used to detect patients with crescent formation [28]. Nevertheless, it is still unclear whether WT1 functions as a tumor suppressor or an oncogene.

Shimizu et al [29] reported that no significantly different survival rate was found between lower and higher WT1 expression level, and similar outcome was described by Hylander et al [16]. Liu et al [23] used a real-time qPCR method to quantify WT1 expression level, and showed that WT1 expression level had no significant impact on OS. Moreover, Taube et al [24] demonstrated that high WT1 expression could predict good prognosis. One potential reason was that histological type of all patients involved in this article was primary high-grade serous ovarian carcinoma. Thus, WT1 was originally defined as one tumor suppressor in Wilms tumor, but it is somewhat surprising that WT1 was regarded as a tumor activator in several adult cancers.

In OS outcome measure, no significant difference was found. However, high WT1 expression in studies with more than 100 cases showed its benefit for OS. In DSS outcome measure, high WT1 expression could only predict worse outcomes in univariate model, but not in multivariate model. Taking together, we could infer with caution that WT1 may be of limited prognostic value for DSS, DFS or PFS. However, WT1 expression level in patients with III-IV cancer stage could be a potential prognostic biomarker.

There were several limitations to this study. First, only English publications were included because of lacking of a translator. Second, individual patient data was difficult to be obtained, which is the gold standard for meta-analyses. Third, most involved res-

earches were studied in developed countries and researches in developing countries might be restricted by techniques, devices, and other factors. This could cause bias. Fourth, all involved researches are retrospective studies, and clinical evidence levels are lower than prospective ones. These limitations may make the results unstable, so further studies are still needed to explore the prognostic value in patients with ovarian cancer.

In conclusion, higher WT1 level in patients with III-IV cancer stage might be a poorer prognostic biomarker. And WT1 expression level is not associated with the DSS, DFS or PFS. More random controlled trials are needed to assess the prognostic value of WT1 expression level in patients with ovarian cancer.

Disclosure of conflict of interest

None.

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