

ORIGINAL ARTICLE

Kallikrein expression as a prognostic factor in ovarian cancer: a systematic review and meta-analysis

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Summary

Purpose: Kallikrein is considered as a mediator of tumorigenesis. Various studies examining the relationship between high kallikrein expressions with the clinical outcome in patients with ovarian cancer have yielded controversial conclusions.

Methods: We conducted a meta-analysis of 10 studies (N=1478) that evaluated the relationship between positive kallikrein expression and overall survival and progression-free survival (PFS). Data were analyzed with random effect and combined hazard ratios (HR) by STATA software.

Results: Positive kallikrein expression was significantly associated with worse OS (HR for OS was 2.01, 95%CI: 1.68-2.34, $p<0.05$). Subgroup analysis showed that kallikrein detected by RT-PCR was related with OS (HR=2.51, 95%CI: 2.16-2.86, $p<0.05$), as well as by non-PCR methods (HR=1.6, 95%CI: 1.08-2.12, $p<0.05$). The heterogeneity among studies

was significant ($I^2=91\%$, $p=0.000$). Begg's and Egger's test showed $p=0.813$ and $p=0.938$, respectively. The estimated HR for PFS was 1.83, 95%CI: 1.51-2.14, $p<0.05$). The heterogeneity among studies was significant ($I^2=88.9\%$, $p=0.000$). Begg's and Egger's test showed $p=0.93$ and $p=0.88$, respectively. Furthermore, confunnel plot (contour-enhanced funnel plot) was undertaken which also showed absence of publication bias for both OS and PFS.

Conclusion: Although the presence of some modest bias cannot be avoided, positive kallikrein expression seems to be associated with worse OS and PFS in patients with ovarian cancer.

Key words: meta-analysis, kallikrein, ovarian cancer, overall survival

Introduction

Ovarian cancer is the leading cause of death among gynecological tumors and the seventh most common cancer in women worldwide [1]. Most of the ovarian cancers are diagnosed at an advanced stage, and the prognosis is extremely poor with an expected 5-year overall survival (OS) rate less than 30% [2]. Five-year OS following ovarian cancer diagnosis is dependent on a spectrum of biological and clinical factors including histological classification, preoperative serum CA125 level, ascites, FIGO (International Federation of Gynecology and Obstetrics) stage, age and the extent of postoperative residual disease [3]. Despite these clinical determinants of ovarian cancer survival,

physicians still lack the appropriate tools to confidently determine individual prognosis of ovarian cancer patients at diagnosis. In the case of ovarian cancer, especially in tumor marker-negative disease, these existing prognostic factors do not sufficiently differentiate the patients who will be cured by adjuvant chemotherapy from those having higher risk of metastases. The identification and validation of additional ovarian cancer prognostic factors have the potential to improve the quality of individualized care for ovarian cancer patients.

Considerable efforts have been made to explore and identify novel markers for predicting

ovarian cancer prognosis. Supplemental prognostic factors may be derived from the expression of candidate proteins (MMP-9, E-cadherin, HER-2, VEGF and protease inhibitors) shown to regulate ovarian cancer vascularization, invasion and metastasis [4-7]. Recent microarray analyses have revealed molecular markers as well as gene expressions that may bear prognostic significance. One candidate marker for ovarian cancer are human kallikreins, a group of 15 trypsin and chymotrypsin-like secreted serine proteases that are found in diverse tissues and biological fluids [8]. Clinical evidence suggests that subtypes of the kallikreins are differentially produced in hormone-dependent cancers such as prostate, ovarian, and breast cancers [9,10]. The kallikrein-related peptidases may be able to activate each other or combine with other molecules like cytokines and vascular growth factors in a cascade of events leading to tumorigenesis [11]. On the other hand, extracellular matrix is degraded by kallikrein, enhancing the ability of tumor invasion and metastasis [12].

This systematic review and meta-analysis tried to evaluate the evidence considering expression of kallikrein as a prognostic indicator for OS and PFS among women with ovarian cancer. A statistically significant risk differentiation according to kallikrein levels may encourage the clinical validation of this protein as an independent prognostic factor in ovarian cancer.

Methods

Search strategy

The objective of this review was to examine OS and PFS in ovarian cancer as a function of kallikrein expression in the tumor. A search in PubMed, Medline, EMBASE and Sciencedirect was carried out to identify all related articles focused on kallikrein and ovarian cancer. Publication time was limited between 1995 and May 1st, 2014. Searched key words were 'kallikrein or KLK', 'ovarian cancer', 'ovarian neoplasm'. Furthermore, references from eligible articles as well as reviews and editorials were reviewed manually to draw further information for our search. We tried to avoid duplication of data by selecting the larger dataset. Our literature search was language-restricted (English) and yielded 206 potentially relevant papers.

Selection of studies

Studies measuring kallikrein in patients with ovarian cancer were accepted. We didn't weight each study by a quality score because no such score has received general agreement for meta-analyses of prog-

nostic studies [13]. Overlapping patients from the same clinical center was blocked by retaining the largest study to avoid duplicate information. Inclusion criteria were as follows: (1) Kallikrein measurements were performed by RT-PCR, IHC or ELISA; (2) The main outcome of the study was OS and/or PFS; (3) Sufficient data for determining an estimate of HR and its 95% CI; (4) All observed patients should have pathological diagnosis of ovarian cancer and more than 50 patients should be enrolled in each study; (5) The study population was divided into high kallikrein (or positive) and low kallikrein group (or negative) for survival analysis; (6) Only articles written in English were included; (7) Studies should have at least 2 years of follow-up.

Data extraction and analysis

For every single study, we marked the results as 'positive' when kallikrein positive expression predicted poorer survival. For obtaining OS and DFS, we measured kallikrein's impact on survival by combining HR and its 95% CI. The following information from eligible studies was collected: first author/year of publication, number of patients, FIGO stage, types of survival analyses, methods, HR and 95% CI. Data extraction was conducted independently by two researchers (Wu and Lu). Disagreement was resolved by a third researcher (Zhou) through discussion. Heterogeneity between studies was evaluated by Q test and expressed by I^2 index. As $I^2 > 35\%$ indicated heterogeneity, we chose random effect (I-V heterogeneity) models which allowed that results may differ genuinely between studies. We considered a worse survival when observing combined $HR > 1$ for kallikrein-positive populations (using STATA 12.0). This impact of kallikrein on OS and PFS was considered as having statistical significance if the combined HR and its 95% CI didn't overlap.

Publication bias was evaluated by Begg's and Egger's tests and Contour-enhanced funnel plot (carried out by STATA 12.0). Publication bias was considered when $p < 0.05$. Furthermore, contour-enhanced funnel plot was helpful to indicate regions of statistical significance, to interpret funnel plot and to identify whether the cause of asymmetry was due to factors such as variable study quality.

Results

The primary search retrieved a total of 206 references and 132 full text reports were evaluated. However, 102 original articles and 17 reviews were excluded after detailed reading for irrelevance to kallikrein and prognosis. Thirteen reports with kallikrein measurement in patients with ovarian cancer were identified. For all the enrolled patients, measurement had been done in the primary tumor without adjuvant chemotherapy or targeted therapy. Of the published studies, 3 were excluded for overlapping with another

Table 1. Main characteristics of 10 included studies

| First author/year | Patients N | FIGO stage (N) | Follow-up (months, median) | Outcome | HR (95% CI) | Subtypes | Methods |
|-----------------------|------------|-------------------------|----------------------------|-----------|---------------------------------------|----------|---------|
| Kim H/2001 | 142 | I-II(38) III-IV(104) | 48 | OS PFS | 1.83 (1.09-3.06) 1.68 (1.09-2.59) | KLK5 | RT-PCR |
| Shan JS /2006 | 280 | I-II(85) III-IV(195) | 52 | OS PFS | 1.16 (0.81-1.67) 1.54 (1.11-2.14) | KLK7 | ELISA |
| Kyriakopoulou LG/2003 | 102 | I-II(89) III-IV(13) | 64 | OS DFS | 3.74 (1.15-12.13) 1.57 (0.96-2.57) | KLK7 | RT-PCR |
| Shigemasa K/2004 | 51 | I-II(25) III-IV(26) | 45 | OS | 3.9 (1.2-12.6) | KLK11 | RT-PCR |
| Borgono CA/2006 | 136 | I-II(33) III-IV(103) | 42 | OS PFS | 0.47 (0.21-1.08) 0.48 (0.24-0.95) | KLK8 | ELISA |
| Hoffman BR/2002 | 180 | I-II(57) III-IV(123) | 62 | OS PFS | 1.88 (1.09-3.21) 1.71 (1.11-2.64) | KLK6 | IHC |
| Youself GM/2003 | 168 | I-II(42) III-IV(126) | 62 | OS PFS | 1.96 (1.16-3.31) 2.33 (1.52-3.55) | KLK15 | RT-PCR |
| Obiezu CV/2001 | 147 | I-II(38) III-IV(109) | 48 | OS PFS | 2.45 (1.43-4.22) 1.95 (1.26-3.02) | KLK4 | RT-PCR |
| Kountourakis P/2009 | 126 | I-II(7) III-IV(119) | 34 | PFS | 1.013 (0.34-1.03) | KLK8 | IHC |
| Diamandis EP/2003 | 146 | I-II(43) III-IV(103) | 25 | OS PFS | 3.15 (1.36-7.29) 4.1 (2.28-7.36) | KLK6 | ELISA |

OS: overall survival, PFS: progression free survival

study [14,15]. Finally, 10 studies fulfilled the inclusion criteria, encompassing 1478 ovarian cancer patients [16-25]. The main study characteristics are shown in Table 1.

Analysis of kallikrein impact on survival

HRs for OS were available in 9 studies with 1352 patients. Kallikrein positive cases were associated with a worse prognosis regarding the risk of death during follow-up. The estimated HR of kallikrein positive cases was 2.01 (95% CI: 1.68-2.34; Figure 1). Since the heterogeneity among studies was significant ($I^2=91\%$, $p=0.000$), the random effect method was selected. We performed Begg's and Egger's tests which showed $p=0.813$ and $p=0.938$, respectively. Furthermore, confunnel plot (contour-enhanced funnel plot) was undertaken which also indicated absence of publication bias (Figure 2). The prognostic value of kallikrein for OS was significant in the 'RT-PCR' subgroup (HR=2.51, 95%CI: 2.16-2.88) and in "non RT-PCR" subgroup (HR=1.6, 95% CI: 1.08-2.12).

HRs for PFS were available in 9 studies with 1427 patients. Kallikrein positive cases were associated with a worse prognosis regarding the risk of progression during follow-up. The estimated HR of the kallikrein positive group was 1.83 (95% CI: 1.51-2.14; Figure 3). Since the heterogeneity among studies was significant ($I^2=88.9\%$, $p=0.000$), the random effect model was select-

ed. Begg's and Egger's tests showed $p=0.93$ and $p=0.88$, respectively. Furthermore, confunnel plot (contour-enhanced funnel plot) was undertaken which also indicated absence of publication bias (Figure 4).

Discussion

Owing to the absence of specific clinical symptoms in ovarian cancer, population screening is a milestone for improving ovarian cancer prognosis. CA125 is widely used but its levels are elevated in <30% of ovarian cancer patients [26]. The development of new biomarkers for patients with ovarian cancer is necessary as these markers could play an important role in the decision-making regarding therapy, outcomes as well as to improve the prognostic power of CA125 [27]. It is beneficial to screen available biomarkers to gain as much information as possible. In this paper, we examined the correlation of kallikrein-positive expression with OS and PFS. In our systematic review and meta-analysis we evaluated 10 studies comparing survival data in patients with kallikrein positive and negative expression. Summary estimates showed that kallikrein positive expression is associated with worse OS and PFS in patients with ovarian cancer. All studies but 2 came to the same conclusion. Although prognostic value on mortality was seen in the two largest studies, results

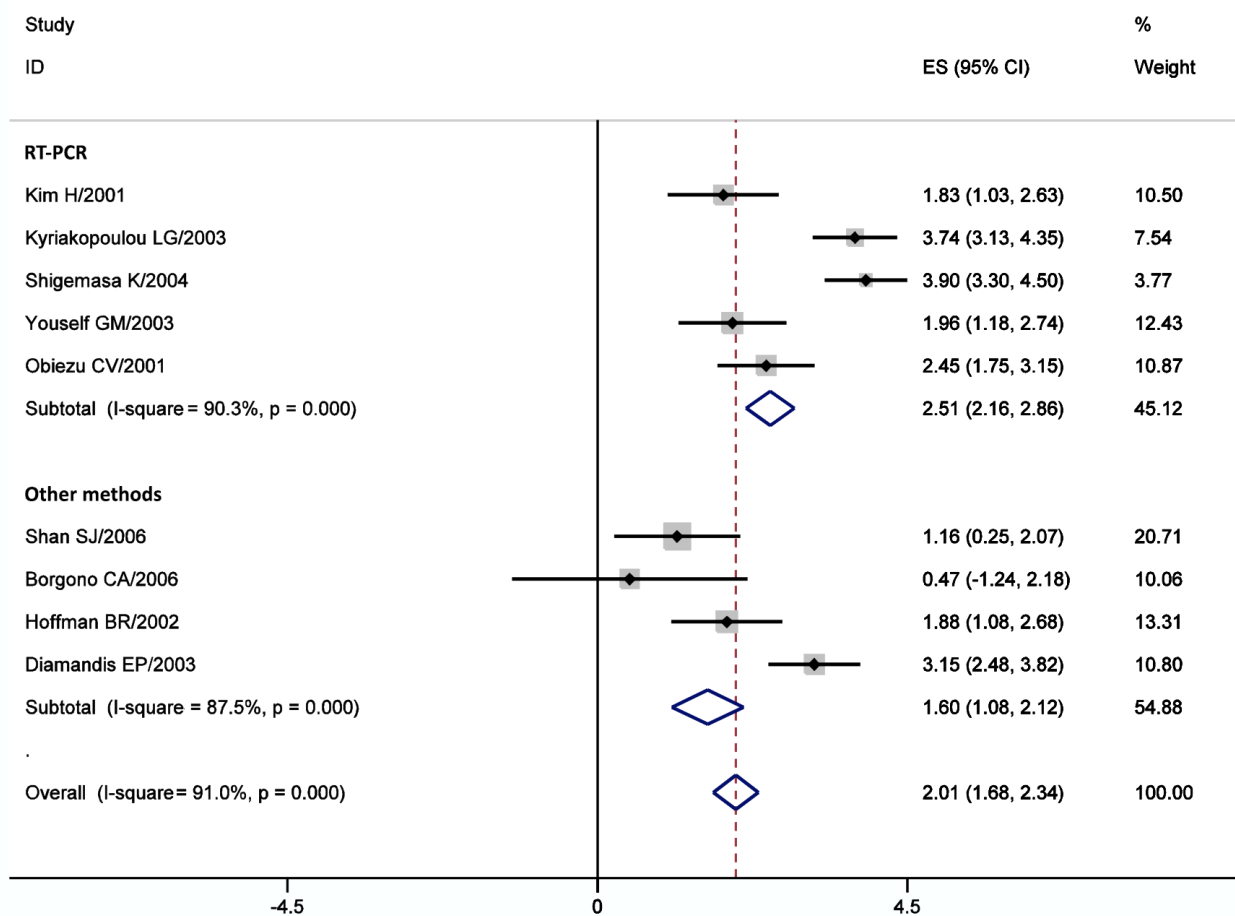


Figure 1. Metaanalysis of 9 eligible studies. Combined HR with a random-effects model for overall survival in kallikrein positive group in ovarian cancer. Subgroup analysis for detecting methods, HR= 2.51 (2.16-2.86) for RT-PCR, HR and 95% CI=1.6 (1.08-2.12) for other methods.

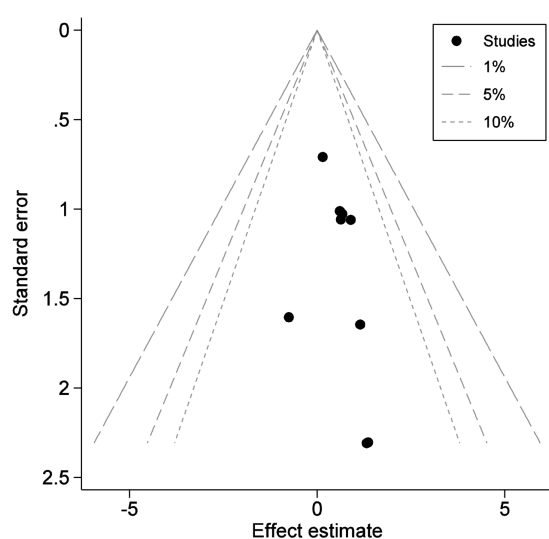


Figure 2. Contour-enhanced funnel plot of 9 eligible studies evaluating overall survival in patients with ovarian cancer showing that all studies were in the non-significant area, meaning absence of publication bias.

should be interpreted with caution. Kallikrein might be a potential prognostic marker in ovarian cancer, but the associations with clinical prognostic factors such as FIGO stage or histology may contribute to its prognostic effect. Five studies have examined kallikrein in ovarian cancer using methods other than RT-PCR (ELISA or IHC). Our subgroup analysis for OS showed that different methods are not interchangeable, and that findings are consistent with the “RT-PCR” subgroup.

Human tissue kallikreins (hKs) is a subfamily of serine proteases, and constitute a group of 15 trypsin and chymotrypsin-like secreted serine proteases encoded by kallikrein (KLK) genes on chromosome 19q13.3-q13.4 [28]. It has been confirmed that kallikreins play a crucial role in activating the growth of angiogenic factors, and degrading of extracellular matrix components [29,30]. Prostate-specific antigen (KLK3) is a well-known biomarker for the early detection of prostate cancer. Recent studies suggest that, in addition to KLK3,

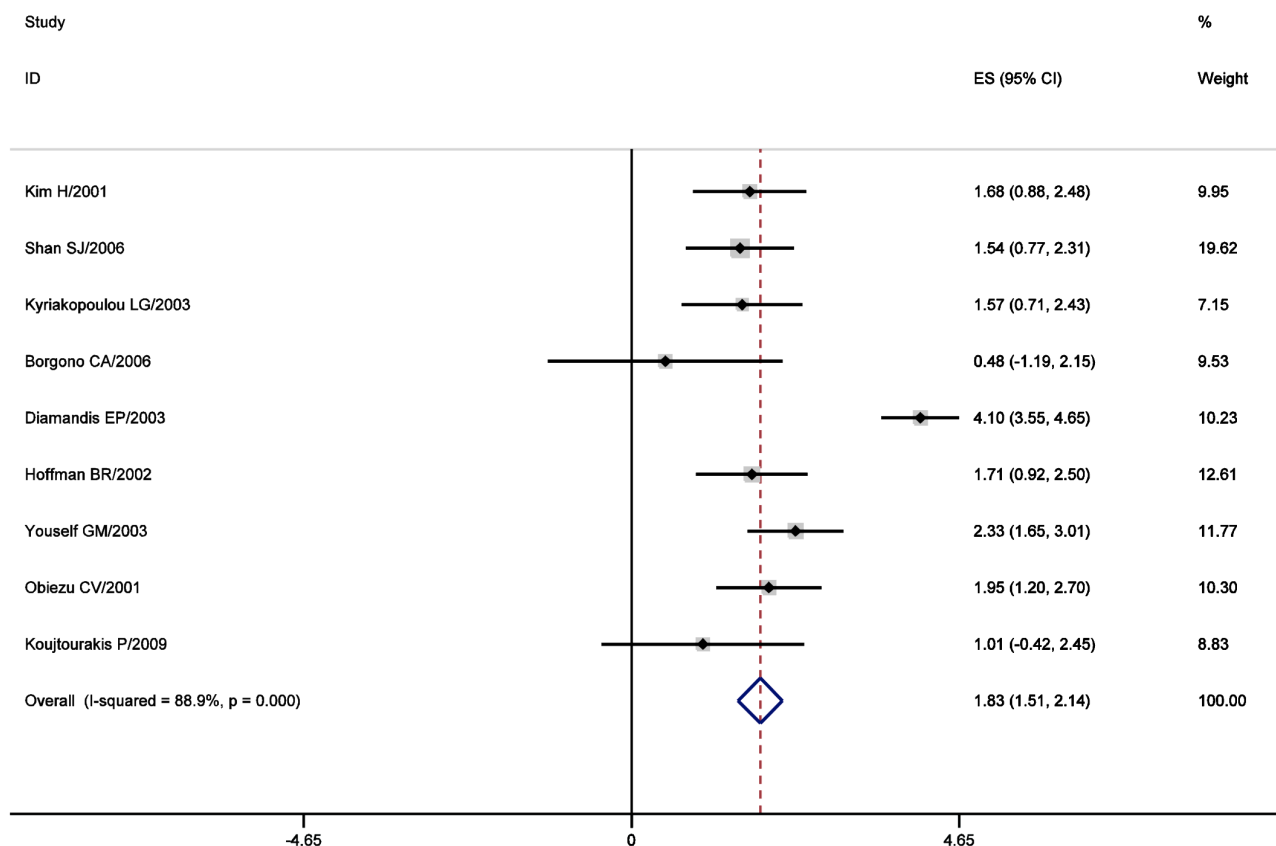


Figure 3. Metaanalysis of 9 eligible studies evaluating kallikrein positive group in progression-free-survival. HR and its 95% CI: 1.83 (1.51-2.14).

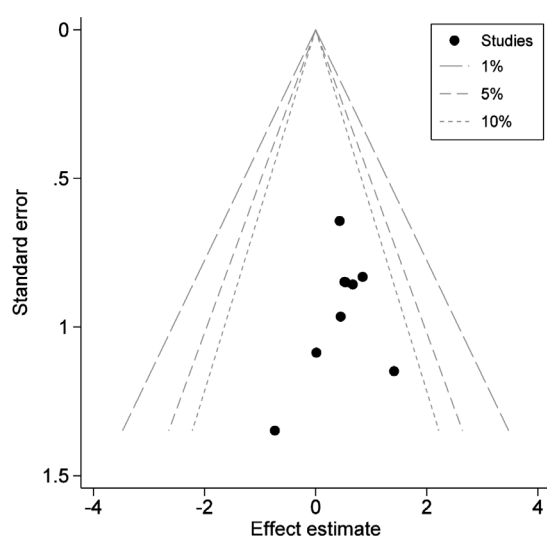


Figure 4. Contour-enhanced funnel plot of 9 eligible studies evaluating progression-free survival in patients with ovarian cancer. Figure shows that all studies were in the non-significant area, indicating absence of publication bias.

kallikreins are promising biomarkers for several cancer types [31]. For ovarian cancer, at least 6 of the 15 hKs members (hKs 5, 6, 7, 8, 11 and 15) are overexpressed and associated with prognosis.

KLK8 is a brain-related trypsin-like serine protease implicated in neurologic processes in epilepsy [31-33]. In addition to its implied role in the brain, Borgono et al. [24] found that hK8 is an independent marker of favorable prognosis in ovarian cancer. They found that women with hK8-positive tumors most often had lower-grade tumors, no residual tumor after surgery, and successful debulking surgery. On the other hand, KLK4 was reported to have a strong positive association with FIGO stage indicating that patients with ovarian tumors positive for KLK4 expression had an increased risk for relapse and death. Kim et al. [16] found KLK5 was highly expressed in ovarian cancer samples while quite low in normal ovarian tissue. These results indicated strong correlation between KLK5 expression and tumor grade and

disease stage which was consistent with the KLK8. Hoffman et al. found that KLK6 positive group had worse PFS and DFS [18]. In addition, Diamandis et al. [17] evaluated the association between serum hK6 concentration and prognosis in ovarian cancer. They found that serum hK6 concentration correlated moderately with CA-125 and was higher in patients with late-stage, higher-grade disease. KLK7, also named human stratum corneum chymotryptic enzyme, is produced in the keratinizing squamous epithelium as well as in various serous cavity fluids including malignant ascites in ovarian cancer patients, where its presence suggests potential evidence of late-stage ovarian cancer. Shan et al. [20] showed that hK7 positivity was associated with significantly shorter PFS but not OS, and it was correlated with the size of residual tumor after staging surgery. Studies focusing on KLK11 and KLK15 presented the same results of poor survival in ovarian cancer patients [19,23]. These results suggested that KLKs were powerful predictors in ovarian cancer. The current meta-analysis summarizes the results of 10 studies on the prognostic value of human kallikrein family in ovarian cancer with 1478 patients. The results indicated that high kallikrein expression is

connected with poor patient prognosis.

Some limitations of the present study should be discussed. Firstly, only published studies were included in our systematic review in order to eliminate potential impact of publication bias. While our search excluded studies that were not published in English, we probably missed sources satisfying our inclusion criteria. Secondly, the variability in the definition of “positive”, outcomes, measurements may result in between-study heterogeneity while conducting a prognostic meta-analysis [34]. In our review, despite the fact that we tried to optimize the baseline, variability was unavoidable. Thirdly, the combined HR was not adjusted for tumor size, FIGO stage, and our available data didn’t allow examining whether kallikrein may affect the response to chemotherapy.

On the basis of our results, we believe kallikrein is a promising biomarker for ovarian cancer. Future studies could also emphasize the standardization of measuring kallikrein expression. Meanwhile, this meta-analysis appears to initially support the hypothesis that kallikrein positive expression is associated with poor OS and PFS.

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