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The prognostic value of phosphatase and tensin homolog negativity in breast cancer: A systematic review and meta-analysis of 32 studies with 4393 patients

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ABSTRACT

The prognostic value of phosphatase and tensin homolog (PTEN) negativity in breast cancer has been evaluated by many studies but remains controversial. We conducted a meta-analysis to assess the association of PTEN negativity with overall survival and disease-free survival. Thirty-two studies with 4393 patients were identified. PTEN negativity was significantly associated with unfavorable overall survival in breast cancer (hazard ratio = 1.89, 95% confidence interval 1.58–2.26), with low heterogeneity among the studies (I^2 = 25%, P = 0.160) and no evidence for publication bias. Meta-analysis of multivariate hazard ratios and sensitivity analyses did not materially change the results. The data on disease-free survival was heterogeneous (I^2 = 61.9%, P<0.001), with a summary hazard ratio of 1.57 (95% confidence interval

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meta-analysis

1.31–1.89). The exact source of heterogeneity remains unclear. We thus concluded that PTEN negativity was significantly associated with unfavorable prognosis in terms of overall survival in breast cancer.

1. Introduction

Breast cancer has long been the most frequent cancer among women (Siegel et al., 2013). Apart from effective treatments, clinical, pathological and biological factors that have prognostic and/or predictive value are also important for optimizing clinical management of the disease, as they can be used to inform risk stratification, treatment selection and development of new therapeutic strategy (Patani et al., 2013). Examples of such factors include tumor size, lymph node status, estrogen receptor (ER) status and human epidermal growth factor receptor 2 (HER2) status, which have been well integrated into clinical practice and contributed much to the improvement of outcome of breast cancer. Along with the emphasis on personalized medicine especially in oncology in recent years, increasing attention has been drawn to other biomarkers that may help explain residual risk not accounted for by traditional factors (Patani et al., 2013).

Phosphatase and tensin homolog (PTEN) is a protein encoded by the tumor suppressor gene *PTEN*. It antagonizes the phosphatidylinositol 3-kinase (PI3 K)/Akt signaling pathway that plays a key role in cell growth, differentiation and survival (Vazquez and Sellers, 2000). By inhibition of the pathway, PTEN may regulate the normal cell cycle and suppress the growth of cancer (Weng et al., 1999). Loss or impairment of PTEN expression (collectively referred to as PTEN negativity hereafter) could lead to constitute activation of PI3K/Akt pathway, which reduces apoptosis and promotes proliferation (Fresno Vara et al., 2004). PTEN negativity may result from such alterations of *PTEN* gene as mutation, deletion and promoter methylation (Nagata et al., 2004).

The prognostic value of PTEN negativity in human cancers has been heavily investigated. Previous meta-analyses showed that it was associated with poor overall survival in gastric, colorectal, hepatocellular, prostate, and endometrial cancers (Chen et al., 2014; Ocana et al., 2014). There are also some studies suggesting that PTEN negativity was associated with poor prognosis in breast cancer. For example, the study of Razis et al. with 182 patients found that PTEN negativity was significantly associated with poor overall survival (hazard ratio [HR] = 1.85, 95% confidence interval [CI] 1.23–2.78) (Razis et al., 2011). However, this finding was not always supported by other studies. For example, in the study of Fabi et al. with 73 patients, no significant association between PTEN negativity and overall survival was found, despite a trend disfavoring PTEN-negative patients (Fabi et al., 2010). It is unclear whether the discrepancy between existing studies is due to the varying sample sizes or other reasons, leaving the prognostic value of PTEN negativity controversial.

We thus conducted a comprehensive systematic review and meta-analysis to synthesize current evidence on this topic. The main objective is to quantify the association between PTEN negativity and the prognosis of breast cancer. The impact of PTEN negativity on overall survival and disease-free survival was evaluated.

2. Materials and methods

2.1. Search strategy

This meta-analysis was conducted and reported according to the PRISMA guidelines (Moher et al., 2009). We performed a systematic search of PubMed, EMBASE (including the conference proceedings of American Society of Clinical Oncology and European Society of Medical Oncology) and Chinese Biomedical Literature Database (in Chinese) from their respective inception through 2012. For the studies published as abstracts at the time of literature search, the full texts were tracked through July 2013. Keywords used to search relevant publications included: "breast cancer*", "breast carcinoma*", "breast tumor*", "breast

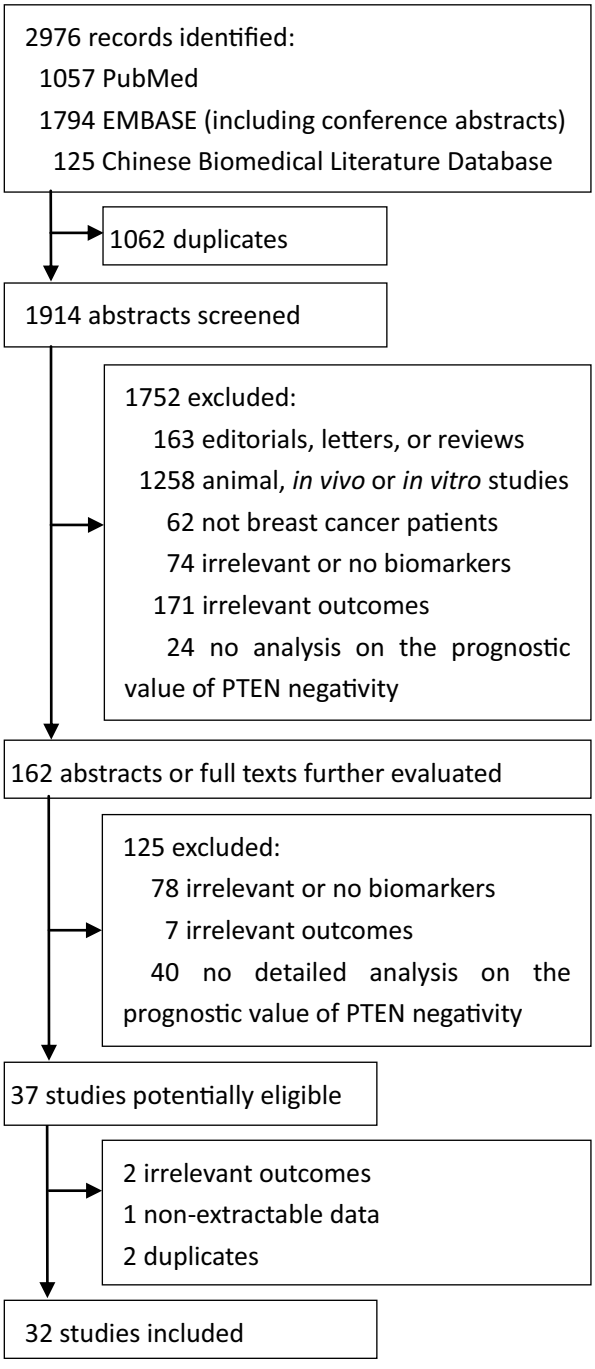

Fig. 1. Flow chart of study selection.

tumour*"; "phosphatase and tensin homolog", "PTEN", "P-TEN"; "prognos*", "outcome*", "progress*", "metasta*", "relapse*", "recurren*", "surviv*", "death*", "die*", "dead", "dying", "mortality". As the association of PTEN status with prognosis was often investigated by "supplementary" analysis in the studies that focused on Akt protein and/or *PIK3CA* gene, the following keywords related to the two biomarkers were also used in the literature search: "Akt*"; "pAkt"; "p-Akt"; "PIK3CA"; "PI3K*"; "PIK3*"; "phosphoinositide 3-kinase"; "phosphoinositide-3-kinase"; "phosphatidylinositol 3 kinase"; "phosphatidylinositol 3-kinase"; "PI 3-kinase"; "phosphatidylinositol-3 kinase". No restrictions were placed on language or publication status. Wherever possible; the searches were limited to "human studies". The reference lists of eligible studies and relevant reviews were also scrutinized for additional eligible studies.

2.2. Study selection

The titles and abstracts of all identified records were screened to judge their relevance. The full texts of the studies seemingly fulfilling the inclusion criteria were obtained for further examination. Cohort studies that met the following criteria were considered eligible: (1) the subjects were patients diagnosed with breast cancer; (2) the outcome events included death, disease recurrence, or both; and (3) PTEN status was tested and correlated with the outcomes. As with the usual practice of research in this field, PTEN negativity was defined as loss or impairment of PTEN expression. Duplicates and studies with non-extractable data were excluded.

2.3. Data extraction

The following data were extracted from eligible studies: (1) bibliographic information, such as first author, country and publication year; (2) data on clinical and pathological characteristics of patients, such as sample size, age, stage of disease, cancer histology, ER status, progesterone receptor (PR) status, HER2 status, and trastuzumab treatment status; (3) definition and rate of PTEN negativity; (4) main results of the study, such as HR and 95% CI (if available, multivariate results were preferable); (5) information on study quality (see below).

Authors of the original studies were contacted as needed to clarify the ambiguities in reported methods or results and to seek additional data not included in the published reports. If not explicitly reported in the original paper and still not available after contact with author, HR was estimated according to the method reported by Parmar et al. and recommended by the Cochrane Handbook for Systematic Reviews (Higgins and Deeks, 2011; Parmar et al., 1998). Where HR was not estimable, rate ratio (RR) was used as a substitute for it (JAMA). Data extraction was completed independently by two reviewers. Disagreements between the two were resolved by revisiting the original paper and discussion until consensus was reached.

2.4. Quality assessment

The quality of included studies was assessed according to the Newcastle-Ottawa scale (Wells et al., 2016), which was frequently employed by previous studies (Thosani et al., 2013). This scale was focused on three aspects of a study, including selection of patients, comparability of baseline characteristics and outcome assessment. For each aspect, there are up to 4 items for detailed evaluation. The overall study quality was denoted by a numerical score ranging from 0 to 9, with 9 representing the highest quality. Quality assessment was completed independently by two reviewers. Disagreements between the two were resolved by revisiting original papers and discussion. Unsettled disagreements were referred to a third researcher for final decision.

2.5. Statistical analysis

The basic characteristics of included studies were summarized descriptively. The rates of PTEN negativity reported by different studies were combined to obtain a summary estimate. The inter-relationship between PTEN negativity and the following factors were assessed by combining the 2×2 tables reported in relevant studies: age, menopausal status, tumor size, cancer histology, lymph node positivity, tumor-node-metastasis (TNM) stage, histological grade, ER status, PR status, and HER2 status. A summary RR was estimated for the inter-relationship between PTEN status and each factor.

The primary and secondary clinical outcomes of this meta-analysis were overall survival, defined as the time from diagnosis to death or end of follow-up, whichever earlier, and disease-free survival, defined as the time from diagnosis to recurrence, death or end of follow-up, whichever earlier, respectively. The effect of PTEN negativity on the outcomes was measured by HR with 95% CI, and the HRs from relevant studies were combined to produce a summary HR for each outcome. HR>1 means that the prognosis of patients with PTEN negativity is poorer than that of PTEN-positive patients, while HR<1 means the opposite.

The random-effects model was used for all meta-analyses. The heterogeneity among studies was assessed by the Cochran's *Q*-test and the I^2 statistic (Higgins et al., 2003). A p value ≤ 0.10 for the *Q*-test or an $I^2 > 50\%$ was suggestive of substantial between-study heterogeneity. If substantial, the heterogeneity in the meta-analyses on the two outcomes was investigated by subgroup and meta-regression analyses to see if it could be explained by clinicopathological characteristics such as study population, stage of cancer, the proportion of patients with HER2+ and trastuzumab treatment status and methodological characteristics such as sample size, length of follow-up, effect measure, analyzing method and study quality score. Sensitivity analyses were conducted by omitting one study each time, by limiting the meta-analysis to the studies reporting multivariate HRs, and by changing from random-effect model to fixed-effect model. Begg's funnel plot and Egger's regression test were used to examine the possibility of publication bias (Sterne et al., 2011). In presence of an asymmetric funnel plot, the Duval and Tweedie nonparametric trim-and-fill method was used to adjust for the potential publication bias and obtain an adjusted result of meta-analysis (Duval and Tweedie, 2000).The meta-analyses of rates of PTEN negativity were conducted by using the Meta-Analyst software (Wallace et al., 2009), while the meta-analyses of HRs or RRs and other related analyses wes were performed with STATA version 11.0 (StataCorp, College Station, TX, USA).

3. Results

3.1. Literature search and study selection

Initially, 2,976 records, including 1,062 duplicates, were identified by the literature search. Among the 1,914 unique references, 37 studies were considered potentially eligible, and 32 studies published between 2001 and 2012 were finally included (Table 1) (Razis et al., 2011; Fabi et al., 2010; Bose et al., 2006; Capodanno et al., 2009; Dai et al., 2007; Deng et al., 2008; Esteva et al., 2010; Giner et al., 2010; Iqbal et al., 2012; Janssen et al., 2007; Jensen et al., 2012; Kiatpanabhikul et al., 2012; Li et al., 2007; Lin et al., 2003; Lin et al., 2005; Liu et al., 2007; Lu and Xu, 2004; Milovanovic et al., 2011; Oliveria et al., 2011; Perez et al., 2013; Shoman et al.,

Table 1
Characteristics of included studies.

Study	Country	*N*	Age: mean (range) (year) at baseline	Stage of disease at baseline	IDC (%)	ER + (%)	PR+ (%)	HER2+ (%)	PTEN − (%)	Mean FU (year)	HR	NOS
Bose et al., 2006	USA	193	–	Non-metastatic	49	79	71	22	20	–	Uni	6
Capodanno et al., 2009	Italy	72	55(34–82)	LNN	96[a]	63	–	14	32	10	Multi	6
Dai et al., 2007	China	92	50(29–71)	1-4[b]	–	64	67	–	46	>5	Uni	7
Deng et al., 2008	China	72	– (26–75)	1-3	–	69	70	–	63	>5	Uni	6
Esteva et al., 2010	USA	137	44(20-73)	Metastatic	90	41	33	100	45	–	Uni	5
Fabi et al., 2010	Italy	73	47(24–67)	Metastatic	96[a]	38	36	100	52	–	Uni	5
Giner et al., 2010	USA	210	58(24–87)	Non-metastatic	–	–	–	100	16	6	Uni	5
Iqbal et al., 2012	Singapore	144	53(28–88)	Non-metastatic	94	0	0	0	27	3	Uni	5
Janssen et al., 2007	Norwegian	125	<55	LNN	–	53	62	4	55	11	Uni	6
Jensen et al., 2012	Denmark	236	–	Early	96[a]	50	–	100	24	6	Multi	6
Kiatpanabhikul et al., 2012	Thailand	82	50[c]	1-4[b]	–	0	0	0	29	–	Uni	5
Li et al., 2007	China	60	46(32–61)	1–4	65	69	62	–	45	5-8	Uni	6
Lin et al., 2003	China	61	45(28–75)	1–4[b]	–	59	48	–	47	6	Uni	6
Lin et al., 2005	China	81	48	LNN	100	78	–	–	37	–	Uni	6
Liu et al., 2007	China	130	55(41–68)	1–3	95	100	–	28	72	5	Multi	6
Lu et al., 2004	China	42	48(26–92)	1–3	67	62	–	–	57	–	Uni	7
Milovanovic et al., 2011	Serbia	78	–	1–2	55[a]	–	–	–	32	–	Uni	5
Oliveira et al., 2011	Spain	26	47(33–78)	Metastatic	–	0	0	0	38	–	Uni	5
Perez et al., 2013	USA	1802	50(22–80)	Early	95[a]	–	–	100	26	6	Multi	7
Razis et al., 2011	Greece	182	56(28–95)	Metastatic	–	68	48	61	55	6	Multi	8
Shoman et al., 2005	Canada	100	62(26–93)	1–4[b]	97	100	39	59	40	6	Multi	5
Sun et al., 2006	China	81	50	Non-metastatic	65	67	–	57	38	>10	Uni	7
Sun et al., 2008	China	260	52(28–78)	1–3	67	–	–	–	31	–	Uni	6
Tsutsui et al., 2005	Japan	236	58(23–86)	1–4[b]	100	44	–	–	31	7	Multi	6
Wang et al., 2011	China	81	48(25–67)	LNN	–	78	–	–	37	>5	Uni	5
Weng et al., 2008	China	83	46(32–68)	1–3	100	47	–	–	39	5	Uni	5
Winter et al., 2007	USA	146	<55[42%pts]	1–4[b]	100	55	45	–	34	7	Uni	5
Xiang et al., 2004	China	60	48(27–81)	Non-metastatic	–	52	–	–	57	5	Uni	6
Yang et al., 2008	China	95	44(22–74)	1–4[b]	–	–	–	–	34	6	Uni	6
Yi et al., 2005	China	62	48(34–71)	Non-metastatic	85	–	–	–	35	>5	Uni	6
Yonemori et al., 2009	Japan	27	57(33–78)	2–3	–	11	4	70	74	–	Uni	6
Zhang et al., 2006	China	95	45(22–73)	1–4[b]	100	52	–	–	42	–	Uni	6

[a] This is the percentage of ductal carcinoma rather than invasive ductal carcinoma.
[b] In these studies, the percentages of patients with metastatic (stage IV) cancer were all less than 5%.
[c] This is the mean age of PTEN negative patients.

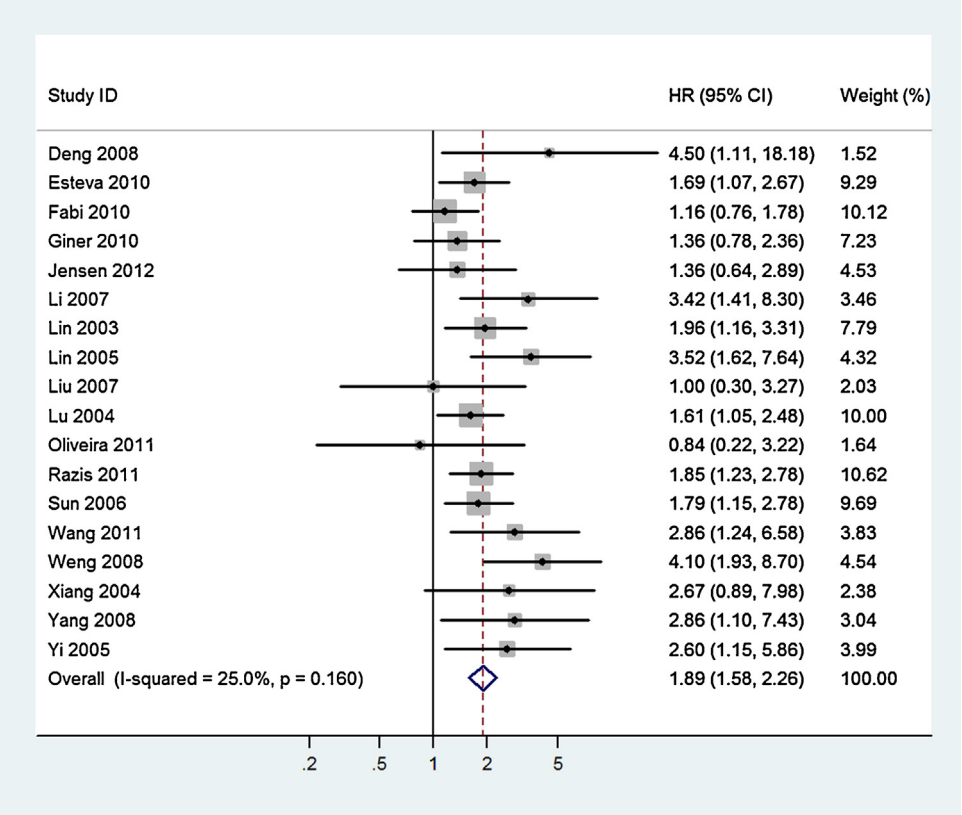

Fig. 2. Forest plot for the meta-analysis of the association between PTEN negativity and overall survival in breast cancer. Results are presented as individual and pooled hazard ratios (HRs) with corresponding 95% confidence intervals (CIs). HR>1 means that the overall survival of PTEN-negative patients is poorer than that of PTEN-positive ones, while HR<1 means the opposite.

2005; Sun et al., 2008; Sun et al., 2006; Tsutsui et al., 2005; Wang et al., 2011; Weng et al., 2008; Winter et al., 2007; Xiang et al., 2004; Yang et al., 2008; Yi et al., 2004; Yonemori et al., 2009; Zhang et al., 2006). The flow chart of study selection is shown in Fig. 1.

3.2. Characteristics of included studies

The basic characteristics of included studies are summarized in Table 1. Nineteen studies were conducted in Asia, six in North America and seven in Europe. The sample sizes ranged from 26 to 1,802, with a median of 88 and a total of 4,393. PTEN status was consistently tested by immunohistochemistry using stained breast tumor tissue slides across the included studies. The definitions of PTEN negativity in different studies are summarized in Supplementary Table 1. The rates of PTEN negativity ranged from 16% to 74%, with a total of 1,762 patients having PTEN negativity and a summary rate of 40% (95% CI 35%-45%; heterogeneity $I^2 = 48\%$, $P < 0.001$). Four studies were conducted in metastatic breast cancer only, while in other studies the stage of cancer varied. In five studies, all patients had invasive ductal carcinoma. Two, zero, five and three studies, respectively, were conducted in ER+, PR+, HER2+ and triple-negative (i.e. ER-, PR- and HER2-) breast cancer. The mean length of follow-up ranged from 3 to 11 years, with the majority being 5 years or more. HR was estimated by multivariate analysis in seven studies (Razis et al., 2011; Bose et al., 2006; Jensen et al., 2012; Liu et al., 2007; Perez et al., 2013; Shoman et al., 2005; Tsutsui et al., 2005) and univariate analysis in the others. The range, median and mean of study quality scores based on Newcastle-Ottawa scale was 5–8, 6 and 5.9, respectively.

Supplementry material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.critrevonc.2016.01.013.

3.3. Inter-relationship between PTEN Negativity and Selected Clinical or Pathological Factors

As shown in Supplementary Table 2, PTEN negativity was more frequent in breast cancer with lymph nodes metastasis, higher TNM stage, higher histological grade, or negative ER. The summary RR for the association between PTEN negativity and these factors varied from 1.26 to 1.69. No significant association was observed between PTEN negativity and other factors.

Supplementry material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.critrevonc.2016.01.013.

3.4. Meta-analyses for the Primary Outcome

Data on overall survival was available from 18 of the 34 included studies (Table 1) (Razis et al., 2011; Fabi et al., 2010; Deng et al., 2008; Esteva et al., 2010; Giner et al., 2010; Jensen et al., 2012; Li et al., 2007; Lin et al., 2003; Lin et al., 2005; Liu et al., 2007; Lu and Xu, 2004; Oliveria et al., 2011; Sun et al., 2006; Wang et al., 2011; Weng et al., 2008; Xiang et al., 2004; Yang et al., 2008; Yi et al., 2004). Meta-analysis of the 18 studies with 1,772 patients showed that PTEN negativity was significantly associated with unfavorable overall survival in breast cancer. The summary HR was 1.89 (95% CI 1.58–2.26), with low heterogeneity among the studies ($I^2 = 25\%$, $P = 0.160$) (Fig. 2). Sensitivity analyses omitting one study each time showed that individually Fabi 2010 and Weng 2008 had the largest influence on the overall results. The summary HR was 1.97 (95% CI 1.66–2.32; heterogeneity test $I^2 = 9.5\%$, $P = 0.343$) when Fabi 2010 was omitted and

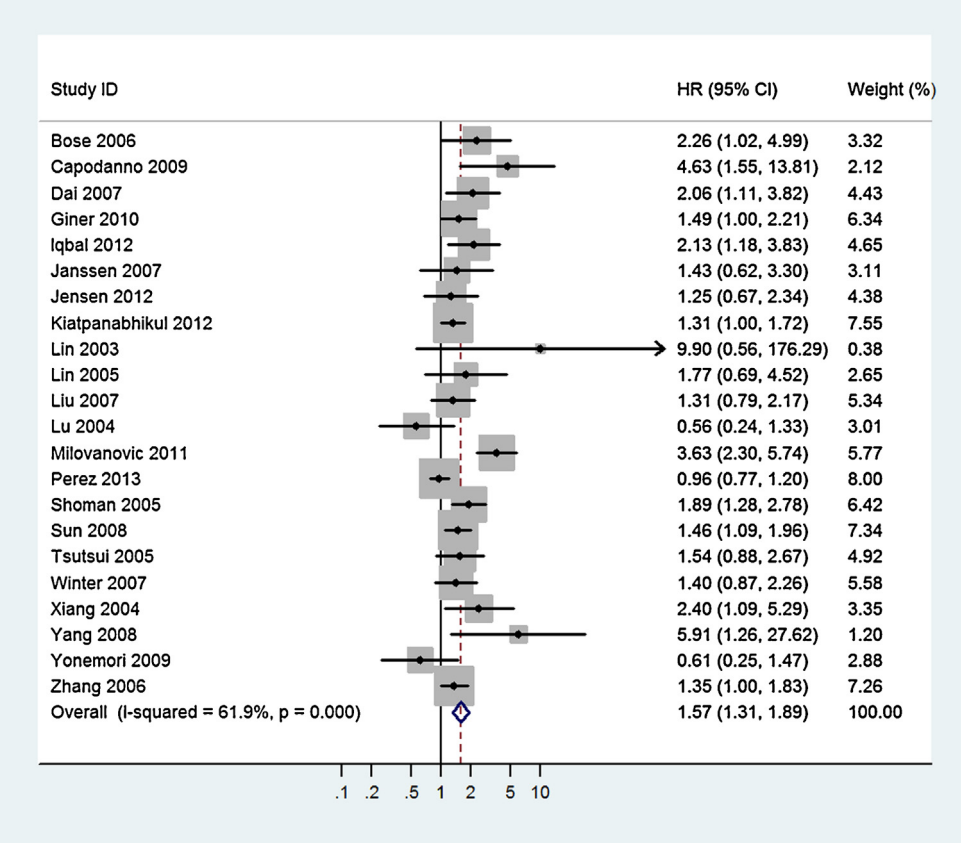

Fig. 3. Forest plot for the meta-analysis of the association between PTEN negativity and disease-free survival in breast cancer, stratified by trastuzumab treatment status. Results are presented as individual and pooled HRs with corresponding 95% CIs. HR>1 means that the disease-free survival of PTEN-negative patients is poorer than that of PTEN-positive ones, while HR<1 means the opposite.

1.79 (95% CI 1.52–2.11; heterogeneity test $I^2 = 11.6\%$, $P = 0.318$) when Weng 2008 was omitted, both of which were still statistically significant. When the meta-analysis was limited to the studies reporting multivariate HRs (Razis et al., 2011; Jensen et al., 2012; Liu et al., 2007), the summary HR was 1.65 (95% CI 1.17–2.33; heterogeneity test $I^2 = 0\%$, $P = 0.541$). Changing to fixed-effect model, the summary HR was almost unchanged (1.83, 95% CI 1.58–2.12).

3.5. Meta-analyses for the secondary outcome

Data on disease-free survival was available from 22 of the 32 included studies (Table 1) (Bose et al., 2006; Capodanno et al., 2009; Dai et al., 2007; Giner et al., 2010; Iqbal et al., 2012; Janssen et al., 2007; Jensen et al., 2012; Kiatpanabhikul et al., 2012; Lin et al., 2003; Lin et al., 2005; Liu et al., 2007; Lu and Xu, 2004; Milovanovic et al., 2011; Perez et al., 2013; Shoman et al., 2005; Sun et al., 2008; Tsutsui et al., 2005; Winter et al., 2007; Xiang et al., 2004; Yang et al., 2008; Yonemori et al., 2009; Zhang et al., 2006). Meta-analysis of the 22 studies with 4,367 patients showed that PTEN negativity was also significantly associated with unfavorable disease-free survival in breast cancer. The summary HR was 1.57 (95% CI 1.31–1.89), with substantial between-study heterogeneity ($I^2 = 61.9\%$, $P < 0.001$) (Fig. 3). However, none of the HRs was significantly smaller than 1. Sensitivity analyses omitting one study each time showed that individually Perez 2013 and Milovanovic 2011 had the largest influence on the overall results. The summary HR was 1.63 (95% CI 1.37–1.94; heterogeneity test $I^2 = 49.9\%$, $P = 0.005$) when Perez 2013 was omitted and 1.47 (95% CI 1.25–1.72; heterogeneity test $I^2 = 47.8\%$, $P = 0.008$) when Milovanovic 2011 was omitted, both of which were still statistically significant. When the meta-analysis was limited to the studies reporting multivariate HRs (Bose et al., 2006; Jensen et al., 2012; Liu et al., 2007; Perez et al., 2013; Shoman et al., 2005; Tsutsui et al., 2005), the summary HR was 1.46 (95% CI 1.03-2.07; heterogeneity test $I^2 = 68.4\%$, $P = 0.007$). Changing to fixed-effect model, the summary HR was 1.43 (95% CI 1.29–1.57).

3.6. Subgroup and meta-regression analyses

The results of subgroup and meta-regression analyses to explore the source of heterogeneity detected in Fig. 3 are summarized in Table 2, which shows that none of the pre-specified factors could account for the heterogeneity. Although the association of PTEN loss with disease-free survival was not observed in the subgroup in which all patients received trastuzumab treatment (HR = 1.01, 95% CI 0.70-1.46) and was significant in the subgroup with no trastuzumab treatment (HR = 1.68, 95% CI 1.34–2.10), the difference between the subgroups did not reach statistical significance (test for subgroup difference: $P = 0.117$).

3.7. Analysis of publication bias

The funnel plot (Fig. 4A) based on the data presented in Fig. 2 was symmetric (Egger's regression test: $P = 0.080$), whereas the one (Fig. 4B) based on Fig. 3 demonstrated some degree of asymmetry (Egger's regression tests: $P = 0.037$), suggesting the possibility of publication bias. After adjusting for the potential publication bias by trim-and-fill method, the summary HR based on the data in Fig. 3

Table 2
Results of subgroup and meta-regression analyses for the data on disease-free survival.

Factors and subgroups	No. of studies	No. of patients	Summary HR (95% CI)	Heterogeneity	Meta-regression *P*-value
1. Sample size					0.606
<88	8	503	1.73 (1.01-2.96)	I^2 = 77.4%, *P* = 0.000	
≥88	14	3864	1.47 (1.25–1.73)	I^2 = 40.9%, *P* = 0.056	
2. PTEN negativity rate					0.560
<40%	14	3730	1.63 (1.31–2.02)	I^2 = 67.5%, *P* = 0.000	
≥40%	8	637	1.43 (0.99–2.06)	I^2 = 52.4%, *P* = 0.040	
3. Follow-up length					0.810
<5 years, or unclear	9	1002	1.53 (1.13–2.06)	I^2 = 70.8%, *P* = 0.001	
≥5 years	13	3365	1.59 (1.26–2.02)	I^2 = 54.9%, *P* = 0.009	
4. Effect measure					0.492
Hazard ratio	10	3073	1.44 (1.16–1.79)	I^2 = 53.0%, *P* = 0.024	
Risk ratio	12	1294	1.70 (1.26–2.29)	I^2 = 64.6%, *P* = 0.001	
5. Analyzing method					0.587
Univariate	16	1791	1.63 (1.32–2.02)	I^2 = 56.2%, *P* = 0.003	
Multivariate	6	2576	1.46 (1.03-2.07)	I^2 = 68.4%, *P* = 0.007	
6. Study quality score					0.279
<6	6	760	1.81 (1.34–2.44)	I^2 = 68.8%, *P* = 0.007	
≥6	16	3607	1.44 (1.16–1.80)	I^2 = 53.5%, *P* = 0.006	
7. Population					0.518
Asian	13	1405	1.46 (1.20-1.78)	I^2 = 36.3%, *P* =0.093	
Non-Asian	9	2962	1.74 (1.23–2.46)	I^2 = 77.9%, *P* = 0.000	
8. Country of study					0.921
China	9	916	1.52 (1.17–1.98)	I^2 = 38.3%, *P* = 0.113	
Other countries	13	3451	1.60 (1.24–2.05)	I^2 = 71.4%, *P* = 0.000	
9. Invasive ductal carcinoma					0.585
<90%	4	573	1.69 (0.87–3.30)	I^2 = 83.7%, *P* = 0.000	
≥90	10	3042	1.47 (1.18–1.83)	I^2 = 54.0%, *P* = 0.021	
10. ER+ patients					0.591
0%	2	226	1.55 (0.99–2.45)	I^2 = 53.7%, *P* = 0.142	
1–99%	13	1466	1.50 (1.18–1.92)	I^2 = 37.2%, *P* = 0.086	
100%	2	230	1.63 (1.15–2.32)	I^2 = 21.4%, *P* = 0.259	
11. PR+ patients					0.759
0%	2	226	1.55 (0.99–2.45)	I^2 = 53.7%, *P* = 0.142	
1–99%	7	744	1.62 (1.20-2.20)	I^2 = 29.2%, *P* = 0.205	
100%	0	–	–	–	
12. HER2+ patients					0.346
0%	2	226	1.55 (0.99–2.45)	I^2 = 53.7%, *P* = 0.142	
1–99%	6	647	1.62 (1.09–2.42)	I^2 = 51.2%, *P* = 0.068	
100%	3	2248	1.16 (0.85–1.57)	I^2 = 47.7%, *P* = 0.148	
13. Trastuzumab					0.117
Yes	3	1464	1.01 (0.70–1.46)	I^2 = 0.0%, *P* = 0.427	
Not mentioned	3	436	1.46 (1.16–1.82)	I^2 = 8.9%, *P* = 0.334	
No	17	2467	1.68 (1.34–2.10)	I^2 = 62.0%, *P* < 0.001	

Abbreviations: HR: hazard ratio; CI: confidence interval; –: not applicable; ER: estrogen receptor positive; PR: progesterone receptor positive; HER2: human epidermal growth factor receptor 2 positive.

was 1.54 (95% CI 1.28–1.85), which was almost the same with the unadjusted estimate.

4. Discussion

Although heavily investigated, the prognostic value of PTEN negativity in breast cancer remained controversial due to the not always consistent results of previous studies. This meta-analysis included 32 studies with 4393 patients to assess the effect of PTEN negativity on the prognosis of breast cancer, representing the most comprehensive synthesis of existing evidence on this topic as of today. PTEN negativity was found to be significantly associated with unfavorable overall survival in breast cancer, with low heterogeneity among the studies and no evidence for publication bias. The results were robust in sensitivity analyses. In terms of disease-free survival, the prognostic effect of PTEN loss was heterogeneous across studies. Trastuzumab treatment status seemed to be responsible for part of the heterogeneity, but the subgroup difference did not reach statistical significance, precluding a firm conclusion to be drawn. Thus, the exact source of heterogeneity remains unclear.

Our findings about the adverse effect of PTEN negativity on overall survival are supported by studies looking at the prognostic value of PTEN negativity from other perspectives. For example, Wikman et al. and Piekarski and Biernat found that PTEN negativity was significantly associated with metastasis in breast cancer (Piekarski and Biernat, 2006; Wikman et al., 2012), while Shoman et al. found that it was associated with breast cancer-specific survival (Shoman et al., 2005). A meta-analysis by Ocana et al. found that PTEN negativity was associated with poor 5-year overall survival in colorectal, hepatocellular, prostate, and endometrial cancers (odds ratio 3.50, 95% CI 1.94–6.31) (Ocana et al., 2014). A meta-analysis by Chen et al. found that PTEN negativity was also associated with poor 5-year overall survival in gastric cancer (RR 1.64, 95% CI 1.45–1.85) (Chen et al., 2014). These evidences lend further support to the results of the present meta-analysis.

The prognostic effect of PTEN negativity on overall survival should inform the risk stratification of breast cancer patients in clinical practice. More importantly, this finding implies that PTEN negativity could be a potential predictor for sensitivity to PI3 K/Akt/mTOR inhibitors which are currently undergoing preclinical or clinical tests, because it takes effect through deregulation of the PI3 K/Akt/mTOR pathway (Courtney et al., 2010; Gonzalez-Angulo et al., 2011). In fact, some cell-line studies have already shown that PTEN-negative breast cancer cells are more sensitive than the PTEN-positive ones to PI3 K/Akt/mTOR inhibitors such as LY294002 and rapamycin (DeGraffenried et al., 2004). In the future,

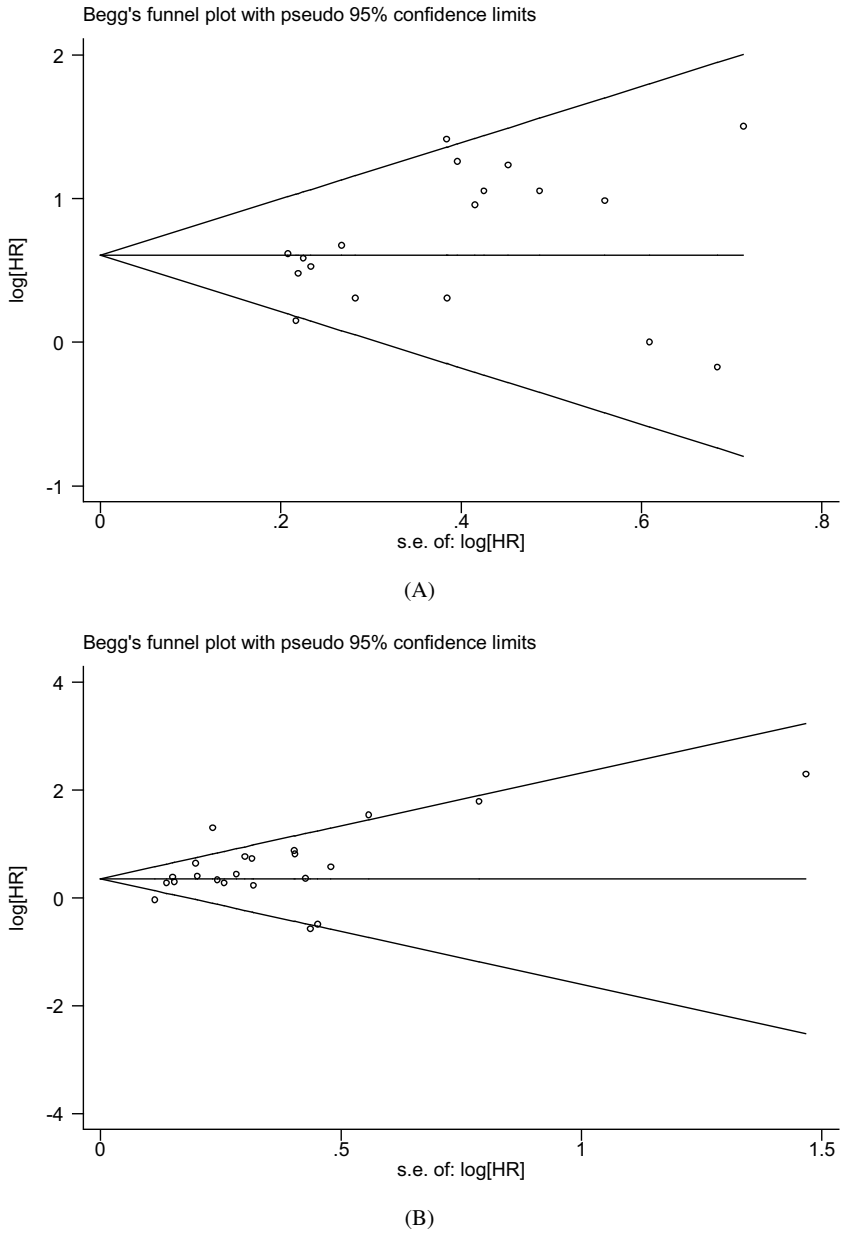

Fig. 4. Funnel plots to examine the possibility of publication bias in the data for overall survival (A) and that for disease-free survival (B). The standard error of log hazard ratio (S.E. of log HR) was plotted against log HR for each individual study as represented in a circle. Egger's tests were conducted to examine whether the funnel plots were symmetric or not (P = 0.080 for Fig. 4(A); P = 0.037 for Fig. 4(B)). Asymmetric funnel plot (i.e. Fig. 4(B)) indicates the potential for publication bias.

more clinical studies are warranted to further investigate this issue. If its predictive value was confirmed, PTEN negativity should contribute to the individualization of targeted treatment of breast cancer, similar to the situation of *KRAS* mutations in the treatment of metastatic colorectal cancer with cetuximab (Dahabreth et al., 2011).

There are some issues that should be considered in interpreting our results. First, many studies included in this systematic review were from China, which may raise people's concern about the quality and generalizability of evidence. However, we do not think this would represent a big problem, because the average quality score of China studies (6.06 out of 9) was better than that for other studies (5.72 out of 9), and there was no significant difference between China and non-China studies in their results (Fig. 2; Table 2).

The second issue has to do with missing data. In the process of study selection of this systematic review, one study was excluded due to non-extractable data (Noh et al., 2008). That study stated that PTEN loss was not associated with worse disease-free survival, with no HR and 95% CI provided. Although the study's conclusion seemingly contradicts with ours, its sample size is small, with only 5 PTEN-negative patients, which means that the 95% CI of HR must be wide, and adding that study to our meta-analysis of disease-free survival is likely to exert negligible influence on the summary results.

The third issue is that the scoring methods and definitions of PTEN negativity differed considerably across the included studies, which were reflected by the varying rates of PTEN negativity. On one hand, this implies that the prognostic value of PTEN negativity may be independent of the scoring methods and definitions, which is partly supported by the results of our subgroup analysis according to the rate of PTEN negativity. On the other hand, this highlights the need for a standardized methodology for testing and scoring PTEN negativity, which should facilitate future research on this biomarker as well as its application to clinical practice.

The fourth issue is that some studies used for the analysis of disease-free survival included some patients with metastatic breast cancer, for whom freedom from disease is rarely possible and thus calculation of disease-free survival may not be appropriate. However, such patients accounted for only a small fraction (<5%) of the patients in those studies (see footnote of Table 1). Therefore, this issue was unlikely to have had important impact on the overall results.

Lastly, as not all the eligible studies conducted multivariate analyses, confounding bias could be a concern, although the meta-analyses of studies that reported multivariate HRs yielded similar results to the overall meta-analyses. This is mainly due to two reasons. First, PTEN negativity was more frequent in some patients, such as those with lymph node metastasis, higher histological grade, higher TNM stage and negative ER, than in others (Supplementary Table 2). Second, biological events other than PTEN negativity in the PI3 K/Akt/mTOR signaling pathway, such as pAkt overexpression and *PIK3CA* mutations, were also associated with poor prognosis of breast cancer (Lerma et al., 2008; Li et al., 2006; Spears et al., 2012; Nagai et al., 2010). Thus, controlling for these factors is important for establishing the independent prognostic effect of PTEN negativity, and should be emphasized more in future studies.

In conclusion, this meta-analysis showed that PTEN negativity was significantly associated with unfavorable prognosis in terms of overall survival in breast cancer. Under some circumstances, PTEN negativity might be associated with unfavorable disease-free survival as well, but current studies do not agree well with each other on this point, and further studies are needed.

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Conflict of interest

None declared.

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