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Original Article

Prognostic and clinicopathological significance of HER2 overexpression in pancreatic cancer: a meta-analysis

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Abstract: Human epidermal growth factor receptor 2 (HER2), if activated abnormally, can promote tumor progression. The correlation of overexpression of HER2 and the prognosis of breast cancer is clear, but the effect of HER2 overexpression on the prognosis of pancreatic cancer is still controversial. The present study aimed to evaluate the prognostic role of HER2 in pancreatic cancer. PubMed, EMBASE, Ovid, CNKI (China National Knowledge Infrastructure) and Web of Science (Jan 2001 to Jun 2015) were searched to identify eligible studies assessing the correlation between expression of HER2 and pancreatic cancer. Data were extracted from studies and computed into hazard ratios (HRs) with 95% confidence intervals (CIs). A total of 19 eligible studies comprising 2,123 patients were included in the analysis. The univariate analysis results showed that HER2 overexpression was not significantly associated with patients' overall survival (pooled HRs, 1.09, 95% CI, 0.88-1.34, P=0.44), which are maintained in three studies of multivariate analysis (HR 0.93, 95% CI, 0.49-1.78, P=0.823). It also had no correlation with clinicopathological factors such as gender, tumor size, lymph node metastasis, and tumor stage. Our results indicated that overexpression of HER2 may not predict poor outcomes in pancreatic cancer.

Keywords: Pancreatic neoplasms, HER2, meta analysis

Introduction

Pancreatic cancer is one of the most common malignancies and its incidence is currently increasing around the world [1]. The morbidity rate of pancreatic cancer has gradually increased in recent decades despite the extensive use of systematic therapies such as radiotherapy and chemotherapy [2]. Therefore, factors to effectively evaluate the patients' survival outcome are needed urgently. To date, there are a number of independent prognostic factors identified in clinical management of pancreatic cancer, including age, tumor size, differentiation and lymph node metastasis. Molecular genetic factors indicated the various structural and functional genetic alterations may also play an important role in the development and progression of pancreatic cancer. However, biomarkers available such as CA199 could not reflect the whole prognostic significance for pancreatic cancer patients. It is important to find out new prognostic biomarkers for patients with pancreatic cancer [3].

Nowadays, a variety of potential prognostic biomarkers are being studied and applied in basic and clinical research. Recent studies have revealed that HER2 is overexpressed in different kinds of cancer, including breast cancer, lung cancer, gastric cancer [4-6]. Many studies have shown that overexpression of HER2 could increase cell growth, inhibit apoptosis and may play a role in cancer invasion and metastasis. HER2, an important biomarker and target of therapy of breast cancer, is also found to overexpressed in 5%-30% of pancreatic cancer.

Studies have shown that overexpression of HER2 is involved in the development of pancreatic cancer. However, in contrast to breast cancer, the association between HER2 expression and the clinicopathological characteristics of PC remains controversial, with some studies suggesting that HER2 expression is associated with highly invasive phenotype and tolerance to conventional treatment and others showing that it is not an independent prognostic factor of patient outcome [7, 8].

In this study, we aim to perform a meta-analysis to assess the correlation of HER2 expression and prognosis in pancreatic cancer.

Materials and methods

Data sources and searches

This meta-analysis was carried out in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses [9]. PubMed, EMBASE, Ovid, CNKI and Web of Science were searched for studies published between Jan 1990 to Jun 2015, which assessed the expression of HER2 in pancreatic cancer. We used the MeSH terms "pancreatic neoplasms" and the most common form of HER2 described in pancreatic cancer including "Human Epidermal Growth Factor Receptor 2" or "HER2" or "erbB-2" or "HER2/neu". Additional studies were identified by a manual review of the references of relevant publications.

Study selection

The title of all the documents identified by the search strategy were assessed by two reviewers (LXP, ZHJ). All possible relevant literature were also evaluated for eligibility and the results were then pooled. Different views were resolved by consultations.

Inclusion criteria for studies were: (1) reporting of differential expression of HER2 in pancreatic cancer by immunohistochemistry (IHC), reverse transcription polymerase chain reaction (RT-PCR) and in situ hybridization (ISH) approaches; (2) they had to detect the expression of HER2 in pancreatic cancer tissues; (3) prevalence of HER2 in PC was reported; (4) availability of overall surviva (OS) and/or relapse-free survival (RFS); (5) The language used in publications is English or Chinese. Articles were excluded based on any of the following reasons: (1) review articles,laboratory articles or letters; (2) the relevant data couldn't be extracted; (3) the articles from one author and the studies brought into the repeated samples from the same patients when a study already included.

Data extraction and synthesis

The quality of all the studies included was systematically evaluated according to a critical review checklist of PRISMA. Most articles compiled in this study had a good quality score. Staging of pancreatic cancer was based on the UICC classification revised in 2012 [10]. Data retrieved from the publications included: year of publication, first author's name, country, sample size, gender, age, rate of HER2-positive expression, tumor stage, survival data, methods and cut-off used for the evaluation of HER2 overexpression, multivariate analysis, univariate analysis, kaplan-meier survival analysis. The references from the relevant literatures, including all the identified studies, reviews and editorials, were also reviewed manually. Disagreements were resolved by discussion among all authors in this paper. The software GetData Graph Digitizer 2.24 (http://getdata-graph-digitizer.com/) was applied to digitize and extract the data from the Kaplan-Meier curve in some articles.

Statistical analysis

All statistical analyses were performed with Review Manager 5.3 (The Cochrane Collaboration, Oxford, United Kingdom) and Stata 12.0 (http://www.stata.com/; Stata Corporation, College Station, Texas, USA). Pooled odds ratios (ORs) and 95% CI were calculated for the the strength of association between HER2 expression and clinicopathological parameters in subjects with PC. For the pooled analysis of HER2 expression on survival outcome,HRs and its 95% CIs were the recommended summary statistics for meta-analysis of OS. Heterogeneity assumption was checked by a chi-square based Q-test. Cochran's Q ($P<0.05$) and the I2 index (>50%) were used to define inter-study heterogeneity. The pooled OR estimate of each study was calculated by the fixed-effects mode if there was not significant heterogeneity. Otherwise, the random-effects model was used. Sensitivity analyses were carried out to examine the impact of methodological quality, statistical models and types of study design on the results. The potential for publication bias was carried out by a Begg's test (funnel plot method) and Egger's linear regression test ($P<0.05$ considered representative of statistical significance). All statistical tests were two sided, and statistical significance was defined as $P<0.05$.

Results

Description of studies

As shown in **Figure 1**, 350 records for HER2 and pancreatic cancer were first identified.

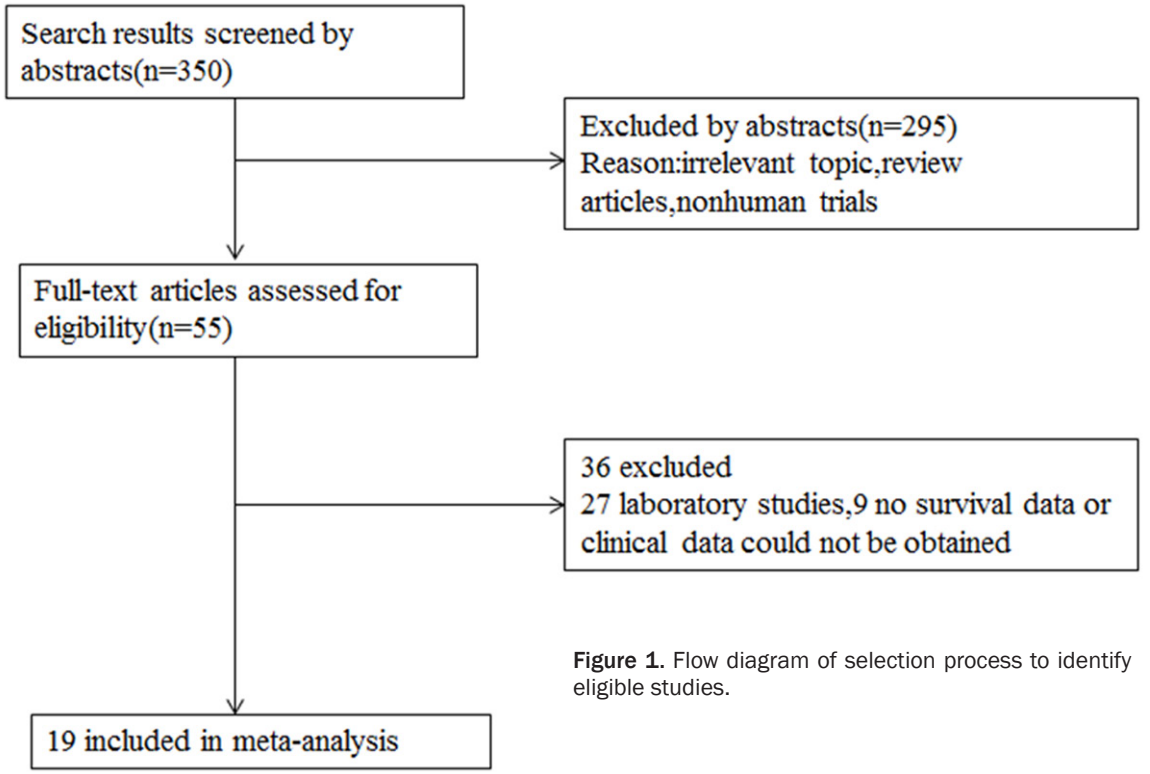

Figure 1. Flow diagram of selection process to identify eligible studies.

After reviewing these abstracts, 295 studies were excluded due to their irrelevance to the current analysis, letters, reviews and duplicate studies. Furthermore, 36 potential studies were excluded, due to laboratory studies or records without sufficient survival data for calculation. Therefore, 19 eligible articles including 491 cases and 1,632 controls were enrolled in this meta-analysis [7, 8, 11-27].

The studies represented a variety of geographical regions. Six evaluated patients from the United States of America, four evaluated patients from Japan, three studies evaluated patients from China, two evaluated patients from Australia, one evaluated patients from Austria, one evaluated patients from Germany, one evaluated patients from Greece and one evaluated patients from Czech. Sample sizes ranged from 21 to 469 in PC studies.

These eligible studies were all retrospective cohort studies. The method of HER2 detection were IHC, fluorescence in situ hybridization (FISH), dual-color in situ hybridization (DISH), chromogenic in situ hybridization (CISH) and RT-PCR. HER2 expression levels were measured in tumor tissue. The mean or medium length of follow-up ranged from 13 to 20 months. Characteristics of the eligible studies are summarized in **Table 1**. Twelve studies provided OS data and one study provided RFS data.

Methodological quality of the studies

We evaluated the qualities of 19 studies according to the Newcastle-Ottawa scale (NOS) [28]. Most of these included in this study were scored more than 5 scores, which indicated that they were of relatively high quality (**Table 1**).

Correlation between HER2 expression and clinical characteristics

The studies which referred the association between HER2 expression and some clinical characteristics (sex, age, differentiation, tumor location, tumor size, venous invasion, lymph node metastasis, T stage and TNM stage) have been combined to calculate the Odds ratios (ORs). Eight studies evaluated the correlation between HER2 expression and age. The pooled ORs were 1.49 (95% CI, 1.04-2.15, P=0.03) with no heterogeneity (I^2=8%, P=0.36), indicat-

Table 1. Main characteristics of all studies included in the meta-analysis

Study	Country	No. of patients (M/F)	Age (years)	Method	HER2 positive rate (%)	Follow-up period	Quality score
Liang 2007	China	26 (16/10)	Median 58 (35-72)	IHC and FISH	42.3	NA	6
Komoto 2009	Japan	129 (NA)	Mean 66.2 (33-85)	IHC	61.2	Median 18.3 months (3-129)	5
Dancer 2007	USA	38 (3/25)	Median 62 (34-82)	IHC and FISH	14.3	NA	5
Stoecklein 2004	Germany	50 (28/22)	NA	FISH	24	Median 13 months (3-99)	6
Zhang 2002	China	36 (24/12)	NA	IHC	41.7	NA	4
Aumayr 2014	Austria	87 (49/38)	Mean 66	IHC and DISH	8.8	Mean 21 months	6
Luo 2013	China	114 (49/65)	NA	IHC and FISH	27.2	NA	4
Tsiambas 2006	Greece	50 (29/21)	Median 62.5 (58-67)	IHC and CISH	29.0	NA	5
Sharif 2008	USA	63 (NA)	Mean 67 (28-85)	IHC and FISH	23.8	NA	6
Ueda 2004	Japan	76 (57/19)	Mean 62.9	IHC	48.7	NA	5
Yamanaka 1992	Japan	24 (17/7)	NA	IHC	25.0	NA	3
Lei 1995	USA	21 (14/7)	Mean 62.2 (51-81)	IHC	38.1	NA	4
Saxby 2005	Australia	30 (17/13)	Median 68 (39-82)	IHC, RT-PCR and FISH	23.3	Median 1.7 years	5
Dugan 1997	USA	79 (NA)	NA	IHC	58.2	NA	3
Safran 2001	USA	154 (83/71)	Median 66	IHC and FISH	20.8	NA	4
Chou 2013	Australia	469 (241/228)	Median 68 (28-88)	IHC and FISH	4.7	NA	6
Satoh 1993	Japan	14 (5/9)	Mean 63.8 (41-85)	IHC	7.1	NA	5
Novotny 2001	Czech	51 (NA)	Mean 55 (16-83)	IHC	19.6	NA	5
Koka 2002	USA	308 (160/148)	NA	IHC	15.6	NA	5

Abbreviations: M/F: male/female; NA: unknown; IHC: immunohistochemistry; FISH: fluorescence in situ hybridization; DISH: dual-color in situ hybridization; CISH: chromogenic in situ hybridization.

Table 2. Meta-analysis of HER2 overexpression in patients with pancreatic cancer

Clinicopathological parameters	No. of studies	cases	Model	OR	95% CI	*P* value for OR	*P* value for heterogeneity
Age (young/old)	8	805	F	1.49	1.04-2.15	0.03	0.36
Gender (male/female)	12	1565	F	1.27	0.95-1.70	0.11	0.08
Location (head/other)	8	1295	R	1.96	0.96-4.02	0.07	0.01
Differentiation (well+moderate/poorly)	14	1672	R	1.35	0.84-2.15	0.21	0.008
Tumor size (≤2/>2)	3	650	F	0.85	0.41-1.78	0.67	0.62
Lymph node metastasis (yes/no)	10	1127	F	1.32	0.92-21.89	0.13	0.23
Venous invasion (yes/no)	2	598	F	0.45	0.20-1.01	0.05	0.41
T stage (T1+T2/T3+T4)	4	329	F	1.17	0.65-2.13	0.60	0.43
TNM (I+II/III+IV)	10	1220	R	0.79	0.36-1.75	0.56	<0.001

Abbreviation: OR: odds ratio; CI: confidence interval; Fixed-effect model; R: random-effect model.

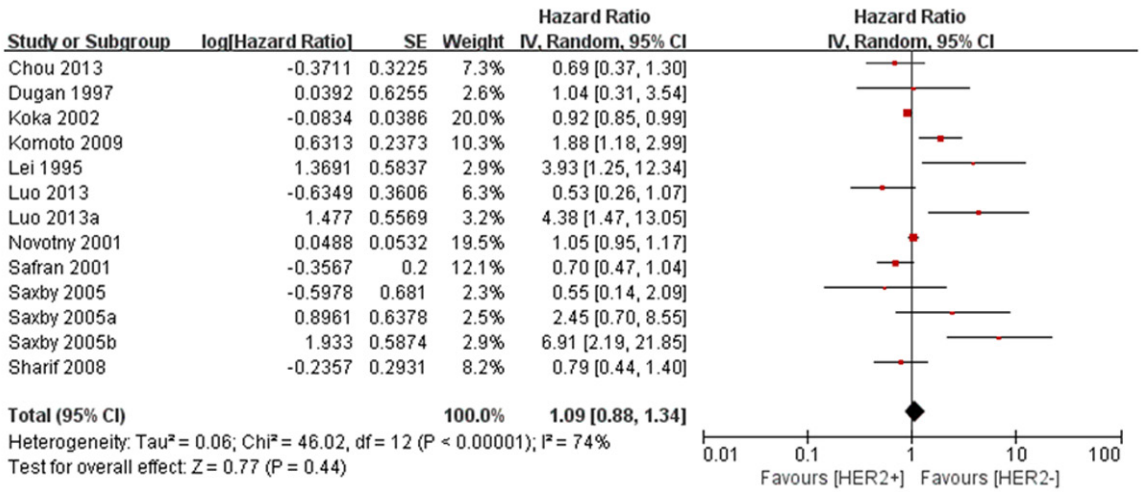

Figure 2. Forest plot of the association between HER2 expression and OS of pancreatic cancer in univariate analysis.

ing that HER2 expression was negatively related to age (**Table 2**). Furthermore, twelve studies reported data on gender, eight studies reported data on tumor location, three studies reported data on tumor size, fourteen studies reported data on tumor differentiation, ten studies reported data on lymph node metastasis, two studies reported data on venous invasion, four studies reported data on T stage, ten studies reported data on TNM classification and their relationship with HER2 overexpression. When the data was pooled respectively, there were no significant associations between HER2 expression and gender (P=0.11), tumor size (P=0.67), tumor location (P=0.07), differentiation (P=0.21), lymph node metastasis (P=0.13), venous invasion (P=0.05), T stage (P=0.60), and TNM stage (P=0.56). All these results could be reviewed in **Table 2**.

Correlation between HER2 expression and survival outcome (OS and RFS)

The meta-analysis was performed on 11 studies assessing the association of HER2 expression with OS in univariate analysis, including three study reporting on OS both in univariate and multivariate analysis. One study reported RFS data in univariate analysis. Meta-analysis of the ten univariate analysis showed that expression of HER2 was not associated with OS (HR 1.09, 95% CI, 0.88-1.34, P=0.44) (**Figure 2**). However, there was significant heterogeneity between the four univariate studies (P<0.0001, I^2=74%). In the case of heterogeneity, a random-effects model was used. One study reported no significant correlation between HER2-positive expression and RFS (HR 0.93, 95% CI, 0.49-1.78, P=0.823). Compared

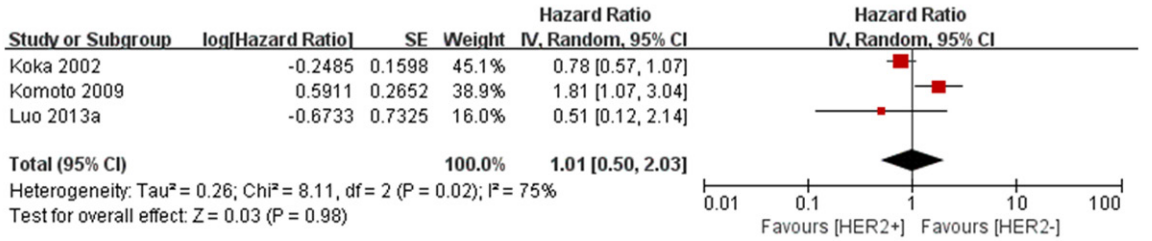

Figure 3. Forest plot of the association between HER2 expression and OS of pancreatic cancer in multivariate analysis.

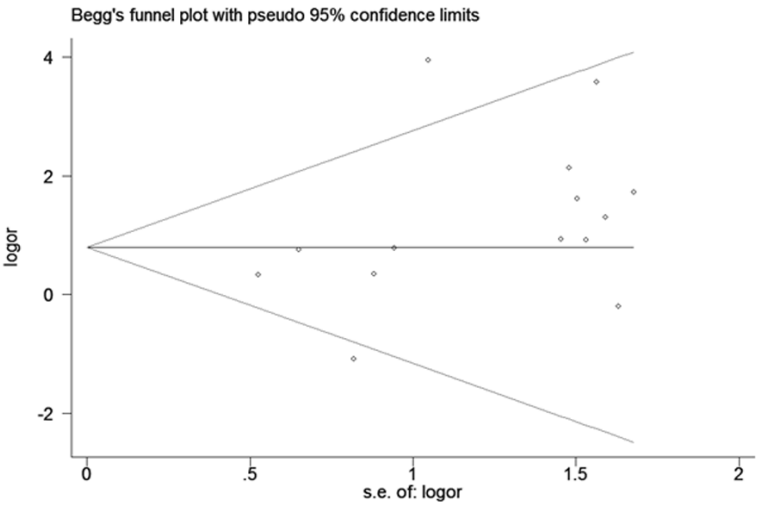

Figure 4. Begg's funnel plot for visual assessment of publication bias for HER2 expression and OS of pancreatic cancer.

with tumors without HER2 expression,the pooled HR of three studies for OS by multivariate analysis was 1.01 (95% CI, 0.50-2.03, P=0.98) (**Figure 3**) with heterogeneity (I^2=75%, P=0.02), and the random-effects model was used. It showed that there was little association between the overexpression of HER2 and poor overall survival of pancreatic cancer.

Sensitivity analysis

We performed sensitivity analyses to investigate clinicopathological parameters if there were differences in results with respect to OS. One factor was included: IHC versus in situ hybridization. We observed that HER2 expression was not associated with worse OS rate (HR=1.00, 95% CI, 0.82-1.21, P=0.97) when IHC carried on the tumor tissues. No significant difference was observed when ISH was carried out (HR=1.36, 95% CI, 0.62-2.98, P=0.45). The conclusions remained similar when a single study involved in the meta-analysis was removed each time to reflect the influence of the rest data-set on the pooled HRs, but the potential heterogeneity was observed again. We further limit the scope of our analysis, quality scores for 6 or higher were included for meta analysis. The results showed that HER2 wasn't significantly associated with OS, and no significant heterogeneity existed (P=0.76).

Publication bias

Funnel plot, Egger's test and the Begg's test were done to estimate the publication bias of literatures. In the funnel plot analysis, the shape of the funnel plot seemed symmetrical for OS. There were no significant publication biases in the meta-analysis of HER2 prediction value for OS (Begg's test, P=0.124; Egger's test, P=0.08) (**Figures 4** and **5**).

Discussion

Recently, numbers of studies are emerging that HER2 could be considered as revolutionary sources of biomarkers for cancer prognosis [29]. HER2 is a key factor in the development of human cancer and an important target for treatment. In pancreatic cancer, some studies found that HER2 was significantly associated with patients' survival. However, insignificant results of HER2 were also observed in other studies. In this meta-analysis, we aimed to clarify the effects of HER2 overexpression on the prognosis of patients with pancreatic cancer.

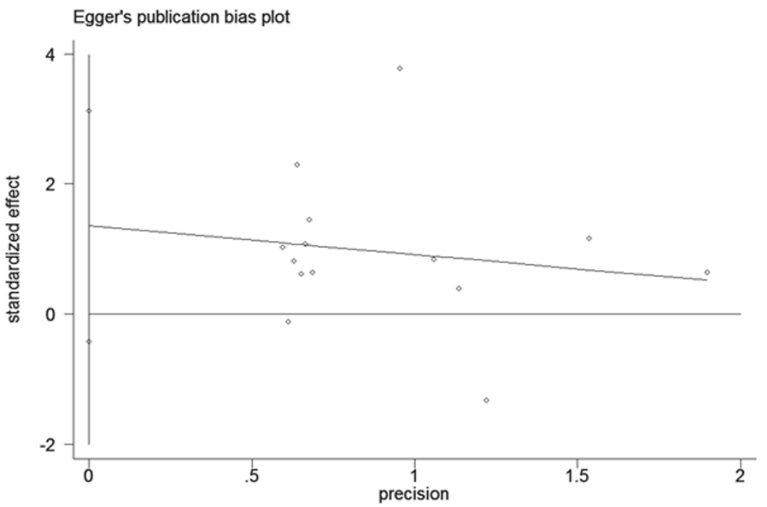

Figure 5. Egger's publication bias plot showed no publication bias for studies regarding HER2 and OS of pancreatic cancer.

We found 19 articles including 2123 patients that evaluated HER2.

In contrast to previous reports, our results show that HER2 expression is not a marker related to poor prognosis of pancreatic cancer. Our meta-analysis showed that the expression of HER2 in pancreatic cancer was relatively high, ranged from 4.7% to 61.2%. The prevalence rate of HER2+ in PC is similar to breast cancer. Studies have shown that old patients had the highest percentage of HER2 expression. The significance of tumour staging in HER2+ is still not clear. In a series of 30 pancreatic cancers, Saxby et al. [8] reported a general trend of increased HER2 amplification with tumor stage. On the other hand, Chou et al. [24] has shown that there was no correlation between HER2+ and tumour staging among PC patients. Our results showed that there is no statistically significant relationship between HER2 overexpression and gender, tumor size, grade of differentiation, T stage, and TNM stage, while ORs for age was significant. The prognosis of PC is depended on many clinicopathological factors such as tumor size, location, lymph node metastasis, stage, differentiation degree, etc. Of these factors, tumor size, location, lymph node metastasis and tumor stage seem to be most important. We found that there is not much evidence indicating HER2 as a prognostic marker for worse outcome. The reasons resulting in this contrary mainly lie in the different criteria for publication selection and enrollment. This study enrolled the publications that define HER2 status with various methods for HER2 detection and scoring such as IHC and ISH. This also supports the putative role of HER2 in tumor cell aggressiveness but only as a secondary event. We hope to get more information from a larger sample of studies in order to better understand the accuracy of the treatment in the future.

This meta-analysis also revealed that elevated HER2 expression didn't predict poor survival in patients of pancreatic cancer. The pooled HR for OS was 1.09 (95% CI, 0.88-1.34, P=0.44) with heterogeneity (I^2=74.0%, P<0.0001). Although the pooled results showed that HER2 positive may not correlate with a poor prognosis, some studies such as Komoto et al. [12] have suggested that HER2 positive patients had shorter survival compared with HER2 negative. Eight studies have showed that there was no difference between the survival rate and HER2 expression, while four studies reported that HER2 positive decreased the survival rate. The difference between these results may be due to several factors such as the diagnostic method, selection of diagnostic reagents and cut-off values. The differences of HRs were found to be no statistically significant, but significant heterogeneity was observed among the studies. Then random effects model was used to analyze the data, however, heterogeneity was still existed. The result remained similar in a sensitivity analysis when a single study was removed each time. After subgroup analysis by the articles' quality score, the heterogeneity was eliminated. Combining the results for OS and PFS, it may suggest that HER2 expression in pancreatic cancer patients couldn't predict their prognosis practically.

Nowadays, IHC is a common method for the determination of HER2 in pancreatic cancer tissues. The other technique is ISH, which is mainly to detect the level of HER2 gene amplification [16]. Precise and accurate detection of HER2

gene expression is crucial in pancreatic cancer to determine the future course of treatment. The decision of how best to evaluate HER-2 status has been controversial in pancreatic cancer, mainly because of the convenience and low cost of IHC as opposed to the accuracy of FISH. Although it has a high sensitivity and accuracy for the detection of HER2 gene amplification, FISH is often used together with IHC because of the good good correlation between HER2 gene amplification and protein overexpression. Dancer et al. [13] found that better correlation between HER2 overexpression by IHC and HER2 gene amplification by ISH in PC. Most of the included studies in this meta-anylysis used IHC to detect HER2 status. We found that the clinical relationship between HER2 expression and pancreatic cancer are not obvious by either IHC or ISH method.

Using anti-HER2 drugs for HER2 positive patients can greatly improve the efficacy of conventional chemotherapy. To choose suitable patients for trastuzumab treatment by IHC method is not enough. Patients may therefore be overtreated or unable to receive targeted therapy. Harder et al. [30] carried a phase II trial to assess the efficacy and safety of Xeloda and trastuzumab as first-line therapy in patients with HER2 positive metastatic pancreatic cancer. The results showed that the treatment was well tolerated, however, PFS and OS had no obvious advantages compared with standard chemotherapy. The definition for HER2 positivity has changed from previous IHC 2+ or 3+ or HER2 amplification to IHC 3+ or IHC 2+ with HER2 amplification. The principle of this method was first applied to the detection of gastric cancer tissues by Hofmann [31]. Among different HER2 testing methods, IHC for preliminary screening and then further confirmed by FISH is a good option.

There are some limitations of this study that should be considered. First, as it is a meta-analysis of the literature, neither Egger's test nor Begger's test showed evidence of publication bias, however, it should be noted that it is prone to selection bias. Second, the studies included are not the highest quality evidence, and meta-analysis of such data might lead to less powerful results. Third, lack of uniform standards among different studies, different race of included patients should be considered. The significant variability of HER2 expression definition makes the results incomparable from one study to another. The sample quality and antibody might be important for the difference in HER2 positivity. The prognostic significance changes even in the same population if different criteria are used to distinguish HER2+ and HER2- patients, which limits their clinical application, strengthening the need for generally accepted diagnostic criteria. Despite the use of different statistical methods and models to reduce the impact of heterogeneity, there is still uncertainty about the accuracy of meta-analysis. Four, some studies didn't provide hazard ratios and therefore we extracted the data from survival curves. This is might be of less credibility than direct analysis on HRs.

Approximately 25% of pancreatic adenocarcinomas overexpress HER-2. However, our meta-analysis showed that elevated HER2 expression was not significantly associated with poor survival in pancreatic cancer patients and might not potentially predict the poor survival in patients with pancreatic cancer. In preclinical models, the combination of anti-HER2 has provided encouraging results. But these results have not yet been reproduced clinically. Further studies with larger sample size should be conducted with all relevant diagnostic methods and classification systems used to further confirm these results.

Disclosure of conflict of interest

None.

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