RESEARCH COMMUNICATION

Effect of HER-2/neu Over-expression on Prognosis in Gastric Cancer: A Meta-analysis

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Abstract

Background: Her-2/neu is the most frequently studied molecular target in gastric cancer but its prognostic impact is still equivocal. We therefore conducted a meta-analysis to more precisely estimate its prognostic significance. Methods: Published studies that investigated between Her-2/neu status and survival were identified. Meta-analysis was performed by Dersimonian-Laird model. Pooled hazard ratio (HR) and its 95% confidence interval (95%CI) were calculated to evaluate the risk of disease. Results: A total of 19 studies were analyzed by meta-analysis method, cumulative 4342 cases were included. Pooled data of 15 studies using univariate analysis showed worse survival of patient with her-2/neu (+) (pooled HR=1.59, 95%CI: 1.20-2.12), which maintained in 7 studies of multivariate analysis (pooled HR=1.58, 95%CI: 1.18-2.12). The Q statistic test for 15 studies of univariate analysis and for 7 studies of multivariate analysis showed they had heterogeneity (Q=26.98, p=0.019, Q=17.76, p=0.007, respectively). Conclusion: HER-2/neu over-expression is related to poor prognosis of gastric cancer but has a modest effect on survival in gastric cancer as an independent prognosis factor.

Keywords: Gastric neoplasia - HER2/neu - gene - relative risk - prognosis - meta-analysis

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Introduction

Gastric cancer is one of the most common tumors and the second leading cause of cancer mortality in the world (Kamangar et al., 2006) . In China, it is the leading cause of morbidity and mortality of neoplasm in digestive system. Until now, clinical surgery is the mainstay of gastric cancer treatment and only can cure parts of patients in the early-stage, but for the patients in the advanced stage, even though surgery combined with postoperative chemoradiotherapy, their survival rate is still low (Cunningham et al., 2006). TNM staging is admitted as the most important factor which effects on the prognosis of gastric cancer, but patients with similar TNM-stages have different clinical outcomes; thus, except the traditional method of staging, we need other standards to identify individual case of patients with gastric cancer especially at advanced stage.

Over the last twenty years, the most frequently studied molecular biological prognostic factors in gastric cancer is human epidermal growth factor receptor 2 (HER-2/neu). The HER-2 protein (p185, HER-2/neu, ErbB-2) is a 185-kDa transmembrane tyrosine kinase (TK) receptor and a member of the epidermal growth factor receptors (EGFRs) family, binding of different ligands to the extracellular domain initiates a signal transduction cascade that can influence many aspects of tumor cell biology, including cell proliferation, apoptosis, adhesion, migration, and differentiation (Gravalos et al., 2008). As HER-2/neu has various kinds of cell functions, it holds the considerable promise as therapeutic targets (Cunningham et al., 2006) and its over-expression may affect on gastric cancer prognosis. However, evidence regarding its prognostic with respect is still inconclusive (Yasui et al., 2005; Gravalos et al., 2008; Bang et al., 2010). The identification of these methodological weaknesses and sources of heterogeneity is important to improve the quality of future prognostic and predictive factor studies in gastric cancer. The aim of this study is to more precisely estimate the prognostic factor of HER-2/neu over-expression. We design our study following the standard of Meta–analysis of observational studies established by MOOSE group (Stroup et al., 2000), using statistical methods to indirectly estimate hazard ratios from Cox regression analyses and P values from log-rank tests (Parmar et al., 1998), and quantitatively evaluate former findings. Moreover we perform in-depth analysis of our study quality, from publication bias to the extent and sources of heterogeneity between published studies.

Materials and Methods

Search strategy and selection criteria

A MEDLINE, PubMed, EMBASE and Chinese database of National Knowledge Infrastructure (CNKI) and WANFANG DATA search for studies investigating

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the prognostic significance of HER-2/neu was performed. Studies published between January 1st, 1990, and December 12th, 2010, were examined. MESH words used were 'stomach neoplasm', 'epidermal growth factor receptor', 'receptor erbB-2', 'prognosis', 'HER-2/neu', 'C-erbB-2'. In order to prevent missing relevant publications, the references of publication and reviews were hand-searched.

Studies were included in the meta-analysis if they met the following criteria: (1) patients included had surgery and their disease was identified as gastric cancer by postoperative pathological check; (2) the endpoint investigated was disease specific or overall survival; (3) the study reported a hazard ratio (HR) survival rate or data sufficient to estimate the HR; (4) all publications are limited to using English and Chinese. We excluded the studies that was of repetition, poor quality or had little information.

Two investigators (SY Wang and G Zheng) independently examined abstracts of articles (n=369) to decide whether full-text articles should be obtained (Fig1). Cases of disagreement were resolved by discussing the title and abstract. Full-text articles (n =37) were examined and 18 were excluded following the criteria below.

Data Extraction

Data were extracted independently by two researchers (SY Wang and G Zheng) by means of a predefined form. Topics in this form were year of publication, country, number of patients, years of patient inclusion, method of case selection (retrospective or prospective cohort of patients), tumor stage, tumor type and differentiation grade, treatment, primary surgery and postoperative treatment, time of follow-up (median, mean, minimum and maximum), HER-2 examination method and scoring protocol used, number of patients with positive and negative tumors, numbers of (disease specific and overall) death, and results of univariate and multivariate survival analyses.

Assessment of study quality

Study quality was assessed independently by two researchers (SY Wang and G Zheng) by means of a predefined form. This form was derived from the work of McShane et al (2005). In summary, the following criteria were included: whether (1) the study reported inclusion and exclusion criteria; (2) study data were prospectively or retrospectively gathered; (3) patient and tumor characteristics were sufficiently described; (4) the method used to measure HER-2/neu over-expression was sufficiently described; (5) the start point and endpoint of the study was provided; (6) the follow-up time of patients in the study was described; (7) the study reported how many patients were lost to follow-up and the percentage should be below 10%. Studies with a total score of 8 were considered to show the highest study quality, whereas a zero score indicated the lowest quality.

Statistical analysis

Statistical analyses were carried out using Stata version 11.0 (Statacorp LP. Texas. USA.).The first step of our meta-analysis was to obtain a log-hazard ratio and its standard error for each study according to methods previously described by Parmar et al (1998). If the study reported results of a univariate Cox regression analysis, log-hazard and its standard error were directly included in the meta-analysis. When the study did not report the standard error, it was estimated from the 95% confidence interval (CI) or P value of univariate Cox regression analyses. If results of univariate Cox regression analyses were not presented in the paper, the log-hazard ratio and its standard error were estimated indirectly from P values of the log- rank test. Q statistic test was used to estimate the heterogeneity of these studies, if P>0.05, it mean studies had little heterogeneity and fixed-effect model (inverse variance)could be used in statistic analysis; if these studies had great heterogeneity, the cause of heterogeneity was analyzed. And these studies were divided into several sub-groups and took statistic analysis, further, sub-groups still had heterogeneity. Meta-analysis was performed by using the DerSimonian – Laird random effects model (DerSimonian and Laird, 1986), the inverse of variance as a weighing factor was applied.

Heterogeneity was investigated by use of the I2 statistic, which takes values from 0 to 100% (Higgins and Thompson, 2002). An I2 value>50% was considered to represent substantial heterogeneity between studies, I2 value <70% was considered that heterogeneity between studies could accept (Alderson et al., 2004). Publication and selection bias were investigated through a funnel plot by Egger's and Begg's test (Begg et al., 1994; Egger et al., 1997).

Results

Study characteristics

For HER-2/neu, 19 studies were subjected to final analysis, a number of 4342 patients were included, and among these, 2571 patients were Asian. All patient accepted R0 or R1 resection of gastric cancer, and HER-2/neu over-expression was examined through tumor specimen, postoperative follow-up time were no less than 5 years. The median quality score was 4 (rang 2-7) for included studies (Table 1). Five studies prospectively collected patient data (Yonemura et al., 1991; Nakajima et al., 1999; Allgayer et al., 2000; Barros-Silva et al, 2009; Grabsch et al, 2010). Methods to determine HER-2/neu status included immunohistochemistry (IHC) with 3 studies additionally performing fluorescence in situ hybridization (FISH) or Southern blot. One of the studies using FISH only had the survival data of patients; other 18 studies all abstracted the data of IHC. Five different cut-off values for positive HER-2/neu protein expression were used. The median percentage of positive tumors was 16.4% (range 3.9 %– 51.1%). Six studies reported that HER-2/neu was a worse parameter of overall survival in 15 univariate analysis, three of seven multivariate analysis considered that HER-2/neu was insignificant with prognosis of gastric cancer. The characteristics of the included studies are showed in (Table 1.)

Meta-analysis

Table 1. Characteristics of Included Studies with Effects on Survival

Author (year)	Case source	No. analysed	HER2 method	+ve(%)	TNM staging	Score Protocol	Treatment	HER2 affect	Other influential factor
Yonemura et al, 1991	Japan	197	IHC	12.1	I-IV	4	Operation + PC	S	—
Hilton et al, 1991	England	87	IHC	9.2	—	4	Operation	NS	—
Jain et al, 1991	England	68	IHC	10.3	I-IV	3	Operation	NS	—
Sasano et al, 1992	Japan	35	IHC	20.7	I-IV	5	Operation	NS	—
Li Ning et al, 1994	China	73	IHC	15.1	II-IV	3	Operation	S	—
Gong et al, 1996	China	97	IHC	30.9	I-IV	4	Operation	S	Age, tumor size/ differentiation, TN-stage
Nakajima et al, 1998	Japan	128	IHC	16.4	I-IV	4	Operation + PC	S	—
Lin et al, 1998	China	106	IHC	18.9	I-IV\	2	Operation	NS	—
Shao et al, 2000	China	149	IHC	47.0	II-IV	2	Operation + PC	S	P53, EGFR, E-CAD
Allgayer 2000	Germany	189	IHC	51.1	I-IV	7	Operation + PC	S	PAI, TN-stage
Pinto-de-Sousa et al, 2002	Portugal	157	IHC	15.3	I-IV	5	Operation	S	—
Kubicka et al, 2002	Germany	42	IHC	38.0	—	3	Operation	NS	—
Lee et al, 2003	Korea	841	IHC	16.4	I-IV	4	Operation + PC	NS	Tumor size, TNM-stage, Borrman 's type
Garcia I et al, 2003	Spain	63	IHC/South	50.8	I-IV	5	Operation	S	—
Seock AI et al, 2005	Korea	94	IHC	43.6	I-IV	6	Operation	S	PTEN
Park DII et al, 2006	Korea	182	IHC/FISH	14.8	I-IV	5	Operation + PC	S	Age, TNM-stage
Barros-Silva et al, 2009	Portugal	256	IHC/FISH	10.7	I-IV	4	Operation	NS	—
Yu et al, 2009	China	669	IHC	28.0	I-IV	5	Operation + PC	NS	Gender, age, TNM-stage, Grb-2 etc.
Grabsch et al, 2010	Eng/Ger	909	IHC	3.9	I-IV	4	Operation	NS	—

S, significant; NS, insignificant; —: not mentioned; PC, postoperative chemotherapy

Meta-analysis, using the Dersimonian-Laird model, of 15 univariate analysis on the prognostic value of HER-2/neu showed that over-expression of HER-2/neu is associated with poor overall survival (HR: 1.58, 95%CI 1.20 – 2.12)(Figure 1). Using Q statistic test analyzed 15 univariate articles, the result showed that Q value = 26.98, p value =0.019 and I2 = 48.1%, that meant the heterogeneity between those articles had statistic significance.

Influential factors related to the prognosis of gastric cancer not only were the characteristics of tumor itself, such as tumor stage, tumor type, differentiation grade etc., but also included the individual conditions of patients and treatment method. However, univariate analysis did not take those confounding factors into consideration, thus combined analysis of multivariate studies was of necessary. 7 multivariate articles analyzed with Q-test showed that they had accepted heterogeneity (p =0.007, I2 =66.2%), combined analysis showed HR=1.58 (95%CI:1.18-2.12) (Figure 2). Those studies were reviewed, and the covariant variables of Cox-model in each of them were matched, we found that the covariant variables of three studies have complete TNM-stage (Lee et al., 2003; Park et al., 2006; Yu et al., 2009), other two studies set TN-stage and modus operandi as covariant variables (Gong et al., 1996; Allgayer et al., 2000); the remaining two studies (Shao et al., 2000; Seock et al., 2005) did neither show complete TNM-stage nor modus operandi. So, five studies were analyzed which had similar covariant variables of Cox-model, Q-test showed no statistic significance of heterogeneity (p value= 0.401, I2 =0.9%). Finally, meta-analysis, using Fixed-model, of five

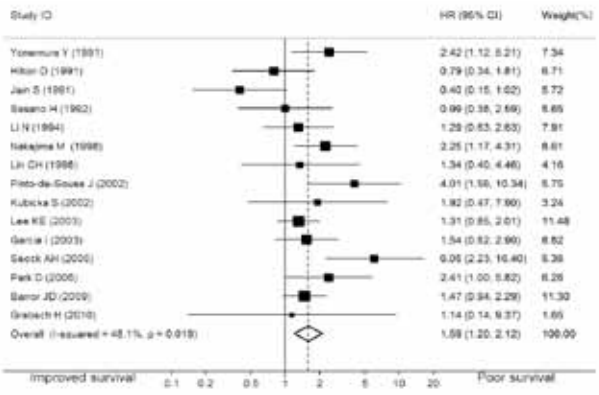

Figure 1. Forest Plot of Results of Univariate Studies on the Prognostic Value of Her-2/neu Over-expression. Hazard ratios and 95% CI (confidence interval) of individual studies for patients with Her-2/neu positive tumours. Each study is shown by the name of the lead author, year of publication. Hazard ratios: squares stand for weights whose are from random effect analysis, and their respective confidence intervals (horizontal lines). Summary hazard ratio: diamond with horizontal limits at the confidence limits and width inversely related to its variance. Hazard ratios higher than 1 indicate an increased risk of death for patients with a tumor with aberrant Her-2/neu status

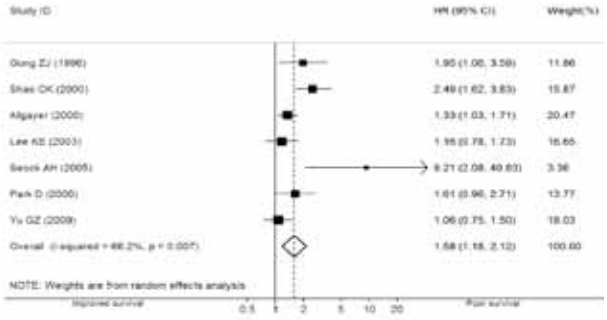

Figure 2. Forest Plot of Results of Multivariate Studies on the Prognostic Value of Her-2/neu Over-expression. Hazard ratios and 95% CI (confidence intervals) for patients with HER-2/neu positive tumors (symbols as in Figure 1).

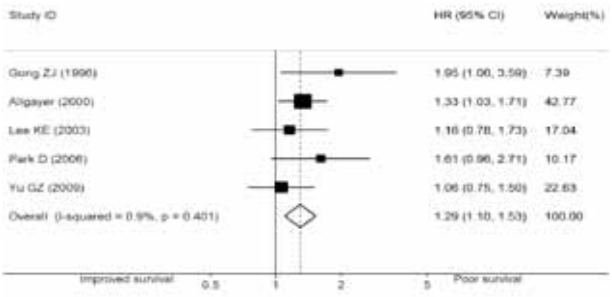

Figure 3. Forest Plot of Results of 5 Multivariate Studies on the Prognostic Value of Her-2/neu Over-expression. Hazard ratios and 95% CI (confidence intervals) for patients with HER-2/neu positive tumors (symbols as in Figure 1).

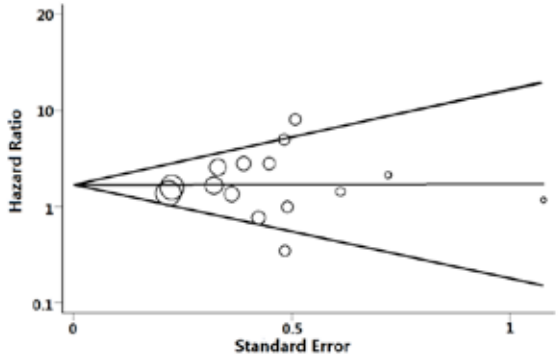

Figure 4. Funnel Plots for Univariate Studies. Funnel plots showing the relationship between the effect size of individual studies (standard error, horizontal axis) and the precision of the study estimate (hazard ratios for overall survival, vertical axis) for HER-2/neu

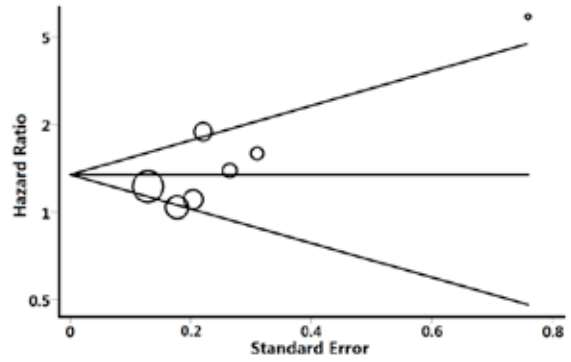

Figure 5. Funnel Plots for Multivariate Studies. Details as in Figure 5

multivariate studies on the prognostic value of HER-2/neu showed that over-expression is associated with poor overall survival (HR: 1.30, 95%CI 1.09-1.52). (Figure 3)

Publication bias and susceptivity analysis

Investigation of bias by funnel plot showed 15 univariate studies were scattered symmetrically, two plots were too far away (HR: 0.396 and 6.05), the rest of the plots were funnel-formed distribution. The p values of Begg's and Egger's test of 15 univariate studies were 0.692 and 0.711, respectively, suggesting the publication bias was insignificant (Figure 4). The value of HR was ranging from 0.396-6.05, we excluded the study with the maximum value of HR (Seock et al., 2005)and analyzed the rest 14 studies: Q = 19.57, P value = 0.106>0.05, that meant the selected studies had homogeneity; comparing the calculated result(HR = 1.479, 95%CI 1.145-1.910) with the result before (HR: 1.57, 95%CI 1.20 – 2.12), the two were similar, suggested that combined analysis of 15 univariate studies was stable.

The Begg's and Egger's test for 7 multivariate studies showed that P values were 0.135 and 0.081, respectively, both were greater than 0.05, suggested the publication bias or selection bias was insignificant. When we reviewed the funnel graph, there were two plots that scarred out of the funnel, they both had a one-to-one relationship with two studies (Shao et al., 2000; Seock et al., 2005). These two studies were excluded at the time we carried out subgroup analysis of 7 multivariate articles, the rest of plots were all inside the funnel graph (Figure 5).The result of Begg's and Egger's test indicated that subgroup analysis was much less susceptible to publication bias.

Discussion

In this study, we presented a pooled estimate of the prognostic value of HER-2/neu in gastric cancer. Our results show that HER-2/neu over-expression is an independent factor related to poor prognosis of gastric cancer, and its effect was modest. The calculated pooled HR was 1.59 and 1.58 for univariate and multivariate studies, respectively, their CIs were exclusive of 1, all of these illustrated that pooled HR had statistical significance. Simultaneously, For univariate studies, the heterogeneity (I2=48.1%)of them was indistinctive the result of combined analysis showed that HER-2/neu over-expression meant poor prognosis of gastric cancer patients; for multivariate studies, appreciable heterogeneity appeared, comparative analysis of COX-models in 7 studies showed that 2 studies failed to take into TNM-stage as one covariate variable. Considering that it is publicly accepted TNM-stage is an important parameter of gastric cancer prognosis for the last twenty years, in order to reduce the impact of confounding factors on our result, we excluded two studies(Shao et al., 2000; Seock et al., 2005) without complete TNM-stage or modus operandi, the pooled HR (HR=1.30, 95%CI 1.09-1.52) still showed that HER-2/neu was an independent prognostic factor of gastric cancer, but its effect was modest.

As is known to all, the prognosis of gastric cancer is related to synergy effect of several factors. For gastric cancer after curative surgery, prognosis factors, such as age of patient, tumor location, tumor size, tumor differentiation degree, TNM stage etc., are familiar to us; among all of those factors, TNM system (7thUICC) is accepted as a golden standard to evaluate gastric cancer prognosis. Recently, Chae et al (2011), according to 7th UICC staging system, conducted a study to analyze the significant prognostic factors of gastric cancer after curative surgery, found that depth of primary tumor infiltration (T classification) and number of metastatic lymph nodes (N classification) were the most important prognostic factors, especially the extent of lymph nodes metastasis was considered to be the most important independent prognostic factor. In other words, studies aim at illustrating the relationship between biomarkers

and gastric cancer prognosis, when multi-factors model designs, it is necessary to make TNM-stage one of the covariates to analyze. Naturally, if we carry on subgroup analysis using confounding factors such as TNM-stage, age of patient, modus operandi, a more accurate pooled HR can be calculated and comparatively clear relationship between HER-2/neu expression and gastric cancer can be illustrated, however, because of the limitation of meta-analysis of observational studies, namely it is hard to get primary materials or data, and then to get rid of related confounding factors, we may overestimate the power of influence that HER-2/neu expression impact gastric cancer prognosis. Thus, our conclusion needs to be verified by studies of prospective, large sample size and well- designed multifactor analysis.

Nowadays, the testing methodologies commonly used for HER-2/neu positivity are IHC and FISH, IHC for protein level and FISH for gene level. FISH is considered as a golden standard for HER-2/neu testing, its sensitivity and specificity are 96.5% and 100%, respectively, reported by Pauletti (1996). Comparing with FISH, IHC testing Her-2/neu positivity has the following advantages: saving times, operating much easily and requiring less equipment, also it has the disadvantage that its result is susceptible to differences of tissue fixation or choice of antibody (Seidal et al., 2001). In our included studies, most of them used IHC to test Her-2/neu, the rang of Her-2/neu positivity rate were relatively wide, from 3.9% to 51.1%, similar to the reported result (6%-35%) by Vita et al (2010). Using IHC only scarcely influenced the detection rate of HER-2/neu positivity, one study showed that IHC and FISH had a concordance of 93.5% when they were used to detect HER-2/neu positivity of gastric cancer specimens (Van Cutsem et al., 2009); To a certain degree, no matter which method are used to detect HER-2/neu it should get similar result in HER-2/neu test, but why Her-2/neu positivity rate had a wide range? Three important reasons emerge as follows. First of all, there isn't a unified, scientific and widely-accepted scoring system to determine HER-2/neu positivity of gastric cancer specimen, in our meta-analysis, included studies adopted several standard to identify her-2/neu positivity, and the existing scoring systems for breast cancer samples (HercepTestTM) were mostly applied, but biological origin of the two has significant difference, study conducted by Van Cutsem et al (2009) made it one reason that when using HercepTestTM necessary modifications should be adopted. At present, Hofmann et al (2008) had already recommended a scoring system for Her-2/neu of gastric cancer, they suggested: Moderate to strong complete or basolateral membranous reactivity in >10% of tumor cells could be considered as IHC3+ (HER-2/neu positive) . Second, gastric cancer is of great heterogeneity, within the same tumor, different cancer cell lines show differences in histology, antigen, immunity, hormone receptors and so on. This characteristic brings in the discrepancy of her-2 positive rate, besides, relevant studies showed that intestinal type and better differentiated gastric cancer had a higher HER-2/neu positivity rate than diffused type (Grabsch et al., 2010; Vita et al., 2010), thus heterogeneity of gastric cancer is one reason that interferes with positive rate of Her-2/neu test. Thirdly, it should be noted that different studies have different experimental designs, various antibodies (CB-11,MLG/7S,NB3,21N etc.) are used, samples vary in size, also the subjectivity of the pathologists' interpretations exist, these all contribute to the occurrence of Her-2/neu positivity rate difference.

There are some limitations of this meta-analysis. Firstly, literatures may not fully integrated, especially for 7 multivariate studies, considerable heterogeneity was observed, one reason for this is that different covariates of multifactor models were applied, when we excluded 2 studies which didn't use TNM-stage as one of the covariates, Q-test showed heterogeneity between studies was indistinctive. However, prognosis factors of gastric cancer were complicated, our data for meta-analysis was from the articles and primary data was hard to get, we were unable to exclude every possible confounding factors. This indicates our results should be interpreted with caution and it requires more detailed and accurate data to verify. And languages selection brings another bias, we have restricted our analysis to published studies written in English or Chinese, other language such as Japanese, German were excluded based on language criteria. This may result in language bias leading to an overestimation of effect sizes (Egger et al, 1997; Pham et al, 2005). Secondly, technique bias is an important bias that needs to be mentioned. FISH and IHC were two methods that use for testing HER-2 in our included literatures, two used FISH plus IHC and the others only used IHC for testing, usage of IHC held a high proportion, and most of them used breast cancer scoring system for Her-2/neu (HercepTestTM), And, compared to other kinds of tumors, gastric cancer has the characteristic of great heterogeneity as we talked about in the last paragraph, in addition, when using IHC to detect Her-2/neu positivity, the phenomenon that incomplete staining of gastric cancer cells are more common than of breast cancer cells (Vita et al., 2010). Thirdly, when we carried out combined analysis of multivariate studies, as we mentioned above, studies were chosen if they set TNM-stage as one covariate. We after all could not conduct an all-around stratified analysis according to every other confounding factors, for example age of patient, modus operandi, adjuvant therapy etc. This may overestimate the effect of Her-2/neu positivity on gastric cancer prognosis.

In advanced-stage gastric cancer, adjuvant chemotherapy had a beneficial effect on survival and quality of life (Paoletti et al., 2010). At present, several particular molecular targeted drugs has been introduced to clinical trials, for example treatments directly aims at HER-2/neu and vascular endothelial growth factor. Recently, ToGA (Bang et al., 2010), a phase 3, open-label, randomized controlled trial showed that trastuzumab plus chemotherapy substantially improved overall survival in patients in advanced stage with high expression of HER2 protein. It was significant that studies on HER-2/neu over-expression were useful to evaluate clinical outcomes of individual gastric cancer patient, and was the key point to choose proper targeted therapy for gastric cancer.

In conclusion, our study shows that HER-2/neu over-expression has a modest effect on survival in gastric cancer as an independent prognosis factor and relates to poor prognosis of gastric cancer. Identification of various

methodological flaws and sources of heterogeneity in currently available prognostic factor studies should contribute to improve design and reporting of future prognostic and predictive factor studies. Our results also offer a hint that additional studies should use standardized testing method, unified scoring system, complete analysis and report of results; in this way can we derive clearer and more accurate prognostic significance of her-2 over-expression in gastric cancer patients.

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