RESEARCH COMMUNICATION

Predictive Value of Thymidylate Synthase Expression in Gastric Cancer: A Systematic Review with Meta-analysis

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Abstract

Purpose: The relationship between thymidylate synthase (TS) expression and outcomes in gastric cancer (GC) patients remains controversial, although most studies reported poor survival and reduced response to fluoropyrimidine were related to high TS in tumors. We carried out a systematic review of the literature with meta-analysis to estimate the predictive value of TS expression from published studies. Methods: We indentified 24 studies analysing the outcome data in gastric cancer stratified by TS expression. Effect measures of outcome were hazard ratios (HRs) for overall survival (OS) and event-free survival (EFS), or the odds ratio (OR) for overall response rate (ORR). HRs and ORs from these eligible studies were pooled using random-effects meta-analysis. Results: Fifteen studies investigated outcomes in a total of 844 patients with advanced GC, and nine studies investigated outcomes in a total of 1,235 patients with localized GC undergoing adjuvant therapy. Meta-analysis of estimates showed high TS expression was significantly associated with poor OS in the advanced setting (HR: 1.43, 95%CI: 1.08 - 1.90), and poor EFS in the adjuvant setting (HR: 1.53, 95%CI: 1.01 - 2.32). Subgroup analysis demonstrated TS expression to haves even greater value in predicting OS, EFS and ORR in advanced GC patients treated with fluoropyrimidine monotherapy (HR for OS: 2.32, 95%CI: 1.53 - 3.50; HR for EFS: 1.76, 95%CI: 1.19 - 2.60; OR for ORR: 0.32, 95%CI: 0.11 - 0.95). Conclusion: High levels of TS expression were asssociated with a poorer OS for advanced GC patients compared with low levels. In the adjuvant setting, high TS expression was also associated with a worse EFS. Additional studies with consistent methodology are needed to define the precise predictive value of TS.

Keywords: Thymidylate synthase - gastric cancer - fluoropyrimidine - meta-analysis

Asian Pacific J Cancer Prev, **13**, 261-267

Introduction

Gastric cancer (GC) is the 4th most common cancer and the 2nd most common cause of cancer mortality in the world (Jemal et al., 2011). It has been proven that fluoropyrimidine can significantly improve survival in GC patients. In the advanced GC, fluoropyrimidine has been widely used as the mainstay of chemortherapeutic agent (Van Cutsem et al., 2006; Koizumi et al., 2008). In localized disease, a large proportion of patients who were at risk of relapse after curative resection have benefited from adjuvant therapy. Adjuvant chemotherapy with fluoropyrimidine is an accepted standard of care in many parts of the world (Sakuramoto et al., 2007).

The antitumor effect of fluoropyrimidine mainly stems from its competitive inhibition of thymidylate synthase (TS). TS is a rate-limiting enzyme in the synthesis of 2'- deoythymidine-5'- monophosphate, which is an essential precursor for DNA biosynthesis (Santi et al., 1974). Intratumoral TS expression in vivo may be pivotal in predicting tumor sensitivity to fluoropyrimidine, as TS expression has been revealed to be determinant in such predictions in vitro (Berger et al., 1985; Johnston et al., 1992).

After over 10 years of research, although most studies reported poor survival and reduced response to fluoropyrimidine with high TS expressing in tumors, evidence is insufficient to conclude whether TS acts as a predictive marker in gastric cancer. The purpose of this article was to evaluate the scientific evidence for the effect of TS expression on GC outcome, using a standard meta-analysis of data from published studies. In fact, two major meta-analysis were performed separately, one in advanced GC and the other in localized disease undergoing adjuvant therapy.

Materials and Methods

Search Strategy and Study Selection

The search for studies was performed using the

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Table 1. Main Characteristics and Results of Individual Studies

First Author	Year	Treatment Setting	Chemotherapy	Method	Cutoff	No. of Pts	High TS (%)	HR for OS (95% CI)	HR for EFS (95% CI)	OR for ORR (95% CI)
Jeong	2011	Advanced	5-FU/Oxaliplatin	IHC	S: Median	72	49	1.18 (0.38-3.63)*	—	—
Yeh	1998	Advanced	5-FU	IHC	I: ≥ 2	30	53	2.50 (1.25-4.99)*	—	0.01 (0.00-0.14)
Miyamoto	2000	Advanced	S-1	IHC	I: ≥ 2	41	41	1.50 (0.23-9.89)*	—	1.05 (0.30-3.65)
Ichikawa	2006	Advanced	S-1	RTPCR	Median	59	53	4.75 (2.17-10.3)	—	0.10 (0.03-0.35)
Ichikawa	2004	Advanced	S-1/Irinotecan	RTPCR	Median	26	50	1.05 (0.90-1.22)*	—	2.56 (0.53-12.43)
Koizumi	2010	Advanced	S-1	RTPCR	$\chi 2$: > 4.46	66	30	2.71 (1.36-5.37)	—	—
Matsubara	2008	Advanced	S-1	RTPCR	$\chi 2$: > 3.67	66	36	—	2.11 (0.97-4.55)	0.32 (0.10-1.01)
Jeung	2011	Advanced	S-1	RTPCR	Median	75	51	1.31 (0.79-2.17)*	1.65 (1.04-2.59)*	0.68 (0.21-2.11)
Akamoto	2008	Advanced	S-1	RTPCR	Median	21	48	2.34 (0.92-5.94)*	—	—
Choi	2011	Advanced	S-1/Cisplatin	IHC	S: ≥ 3	40	33	0.88 (0.50-1.54)*	0.96 (0.89-1.04)*	1.26 (0.33-4.73)
Kwon	2007	Advanced	5-FU/Oxaliplatin	IHC	I: ≥ 2 and E: ≥ 2	64	30	1.48 (0.53-4.15)*	1.45 (0.52-4.07)*	0.88 (0.29-2.65)
Wei	2008	Advanced	5-FU/Oxaliplatin	RTPCR	$\chi 2$: > 6.06	76	72	0.83 (0.72-0.96)*	—	—
Tahara	2004	Advanced	5-FU/Methotrexate	IHC	P: > 25%	38	76	0.45 (0.02-8.36)*	—	0.67 (0.12-3.71)
Boku	1998	Advanced	5-FU/Cisplatin	IHC	I: ≥ 1	39	46	—	—	0.38 (0.09-1.56)
Boku†	2007	Advanced	5-FU	IHC	P: ≥ 20%	65	57	—	—	1.30 (0.28-5.98)
Boku‡	2007	Advanced	5-FU/Cisplatin	IHC	P: ≥ 20%	66	32	—	—	0.94 (0.33-2.67)
Choi	2001	Adjuvant	5-FU/Doxorubicin	IHC	P: ≥ 25%	103	63	1.09 (0.58-2.04)*	1.22 (0.63-2.37)*	—
Suda	1999	Adjuvant	Fluorouracil/ Mitomycin C	IHC	Positve signal	66	45	2.14 (1.07-4.27)	—	—
Yeh CN	2010	Adjuvant	5-FU based regimen	IHC	P: ≥ 20%	124	66	2.20 (1.29-3.83)	2.06 (1.18-3.58)*	—
Lee	2008	Adjuvant	5-FU	IHC	P: > 25%	463	19	0.87 (0.59-1.27)	—	—
Kim	2011	Adjuvant	5-FU/Cisplatin	IHC	I: ≥ 2 and E: ≥ 2	149	77	0.56 (0.32-0.99)*	—	—
Hua	2007	Adjuvant	5-FU based regimen	RTPCR	Median	51	49	2.52 (1.30-4.86)	1.73 (1.10-2.71)*	—
Ishido	2009	Adjuvant	S-1	RTPCR	Median	39	51	4.65 (1.00-21.66)	5.68 (1.22-26.50)	—
Cho	2006	Adjuvant	Doxifluridine/ Epirubicin/Mitomycin	IHC	S: ≥ 6	89	36	0.72 (0.31-1.66)*	—	—
Kim	2009	Adjuvant	5-FU/Cisplatin	IHC	S: ≥ 25	151	49	0.73 (0.47-1.13)	0.93 (0.62-1.39)	—

TS, thymidylate synthase; 5-FU, 5-fluorouracil; IHC, immunohistochemistry; RTPCR, reverse transcriptase polymerase chain reaction; HR, hazard ratio; OR, odd ratio; OS, overall survival; EFS, event-free survival; ORR, overall respond rate; Cutoff: I, grades of staning intensity; E, grades of staning extent; P, percentage of stained cells; S: score from multiplying the grades of staining intensity by either the grades of staining extent or the stained cell percentage; χ^2, the maximal χ^2 method; †patients received 5-FU; ‡patients received 5-FU/Cisplatin. —, not performed; * calculated result from published data

electronic database PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez?myncbishare=xysmlibrary) until July 15, 2011. The following Medical Subject Heading (MeSH) terms and/or text words were used: ("thymidylate synthase" [MeSH Terms] OR ("thymidylate" [All Fields] AND "synthase" [All Fields]) OR "thymidylate synthase" [All Fields]) AND ("stomach neoplasms" [MeSH Terms] OR ("stomach" [All Fields] AND "neoplasms" [All Fields]) OR "stomach neoplasms" [All Fields] OR ("gastric" [All Fields] AND "cancer" [All Fields]) OR "gastric cancer" [All Fields]). We also reviewed the references reported in the relevant studies to identify additional studies.

Studies that met the following criteria were eligible for inclusion: (1) patients had a diagnosis of gastric cancer; (2) all patients received fluoropyrimidine-containing chemotherapy; (3) overall survival, event-free survival, or treatment response to chemotherapy were analyzed stratified by TS expression; (4) the results are part of an original analysis; (5) when results reported by the same author were acquired from the same patient population in more than one publication, only the study involving the highest number of patients was included.

Data Extraction

Two investigators (HB-H and LK) extracted data from the eligible studies independently and reached consensus to all items. Data retrieved from each report included the first author, year of publication, treatment setting, chemoterapy rigemens, TS evaluation method, cutoff value used to dichotomize TS as "high" and "low", number of patients analyzed, proportion of high TS expression. If data from any of the above categories were not reported in the primary study, items were treated as "not applicable." We did not contact the author of the primary study to request the information.

Statistical Methods

For the quantitative aggregation of the results, statistical analysis of the overall hazard ratio (HR) for overall survival (OS) and event-free survival (EFS) (classified as progression-free survival, disease-free survival, time to progression), the odds ratio (OR) for overall response rate (ORR). By convention, for the high TS expression group, an observed HR >1 implied a worse prognosis, and OR <1 indicated a poor response to fluoropyrimidine-containing regimens. The impact of TS expression was considered to be statistically significant if their 95% CI did not overlap 1. If these statistical variables were not reported explicitly in the individual study, they were estimated by the methods of Parmar et al. (Parmar et al., 1998).

Heterogeneity test based on I^2 statistic was performed in all meta-analysis. I^2 is measured from 0-100% with increasing I^2 values indicating a larger impact of between-study heterogeneity (Higgins et al., 2002). A random-effects model was applied to pool study results in all meta-analysis reported below (DerSimonian et al., 1986).

Evidence of publication bias was obtained using the Begg's test ($p < 0.05$ was considered to represent

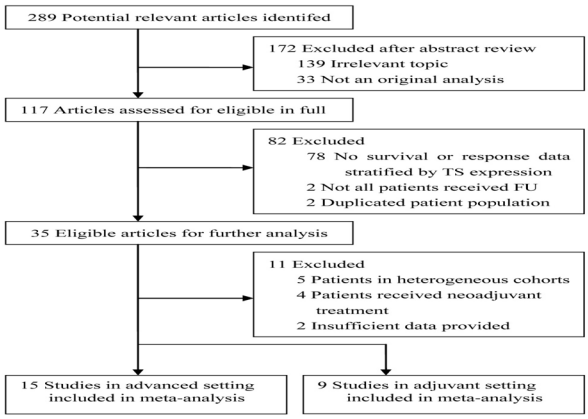

Figure 1. The Flow Diagram of Search Strategy

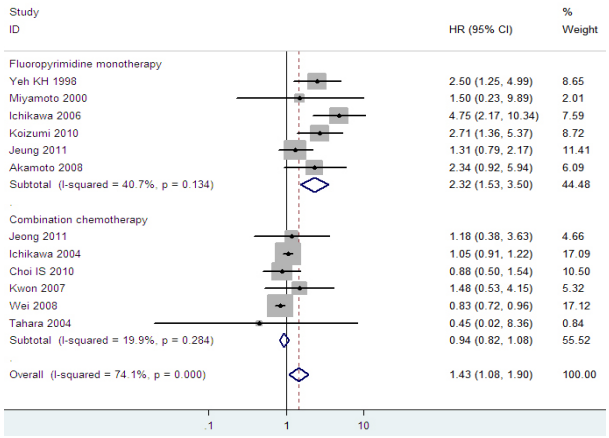

Figure 2. Forest Plot of Hazard Ratios for Overall Survival in Advanced Disease Setting, and Subgrouped by Chemotherapy Regimens

statistically significant publication bias) (Begg et al., 1994).

All calculations were performed using the program STATA version 11.0 (Stata Corporation, College Station, TX) and the modules METAN, and METABIAS.

Results

Eligible studies and Characteristics

Thirty-five studies that met the inclusion criteria were identified. Eleven studies were excluded from further analysis (Figure 1). Five were excluded because survival was assessed in heterogeneous patient cohorts (Kuniyasu et al., 1998; Ishikawa et al., 1999; Tsujitani et al., 2000; Terashima et al., 2003; Chung-Kang et al., 2006), comprising both advanced and localized GC patients, and extraction of separate risk estimates of outcome for patients treated in the advanced or adjuvant disease setting was not possible from data available. In four studies (Lenz et al., 1996; Metzger et al., 1998; Napieralski et al., 2005; Fukuda et al., 2006), patients have received neoadjuvant treatment, which may have altered TS expression, and a treatment-related effect cannot be entirely discounted. Two studies provided insufficient outcome data for effect estimation thus were excluded (Liu et al., 2004; Ishizone et al., 2006). Hence, a total of twenty-four studies with 2,079 patients remained eligible for meta- analysis (Table 1).

Fifteen eligible studies assessed survival or treatment

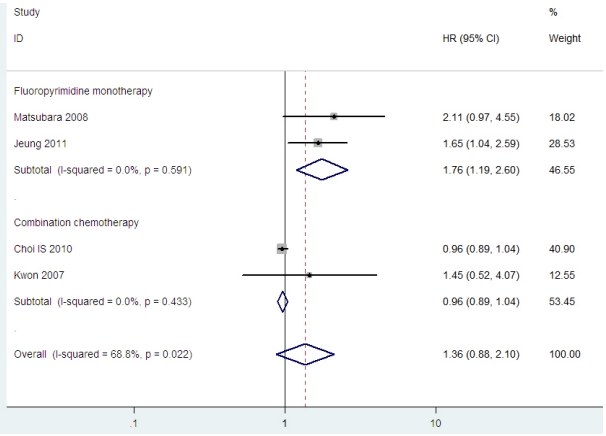

Figure 3. Forest Plot of Hazard Ratios for Event-free Survival in Advanced Disease Setting, and Subgrouped by Chemotherapy Regimens

response in the advanced disease setting (Boku et al., 1998; Yeh et al., 1998; Miyamoto et al., 2000; Ichikawa et al., 2004; Tahara et al., 2004; Ichikawa et al., 2006; Boku et al., 2007; Kwon et al., 2007; Akamoto et al., 2008; Matsubara et al., 2008; Wei et al., 2008; Koizumi et al., 2010; Choi et al., 2011; Jeong et al., 2011; Jeung et al., 2011), with a total of 844 patients available for pooling (median: 62, range: 21-76). All studieds used fluoropyrimidine-containing regimens, either combination chemotherapy or monotherapy. In the study by Boku et al (Boku et al., 2007), ORR data were presented separately for patients who received 5-fluorouracil (5-FU) or 5-FU/ Cisplatin, therefore two patient cohorts were considered separately for pooling.

In the adjuvant disease setting, nine studies that included survival data of total 1,235 patients available for pooling (median: 103, range: 39- 463) were eligible (Suda et al., 1999; Choi et al., 2001; Cho et al., 2006; Hua et al., 2007; Lee et al., 2008; Ishido et al., 2009;Kim et al., 2009; Yeh et al., 2010; Kim et al., 2011). Adjuvant fluoropyrimidine chemotherapy was given postoperatively to all patients.

Evaluation of TS Methodologies

The most widely-adopted technique to determine TS expression for survival analysis was Immunohistochemistry (IHC) (15 of 24 studies). A number of semiquantitative methods were used to dichotomize TS expression. In three studies (Boku et al., 1998; Yeh et al., 1998; Miyamoto et al., 2000), staining intensity grades lower than 1 or 2 represented low levels of TS expression. In five studies (Choi et al., 2001; Tahara et al., 2004; Boku et al., 2007; Lee et al., 2008; Yeh et al., 2010), expression was dichotomized by quantifying the proportion of stained cells using arbitrary thresholds of 20% or 25%. In two studies (Kwon HC et al., 2007; Kim KH et al., 2011), cases were defined as high expression on the condition that the grades of intensity and extent are both 2 or higher. In four studies (Cho et al., 2006; Kim et al., 2009; Choi et al., 2011; Jeong et al., 2011), from multiplying the grades of intensity by either the grades of extent or the percentage of stained cells, a IHC score was derived so as to dichotomize the levels of TS. In the remaining one study (Suda et al., 1999), the high expression were judged when

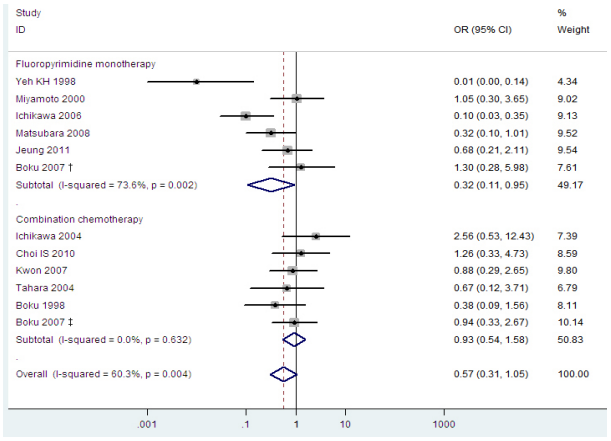

Figure 4. Forest Plot of Odds Ratios Overall Response Rates in the Advanced Disease Setting, Subgrouped by Chemotherapy Regimens

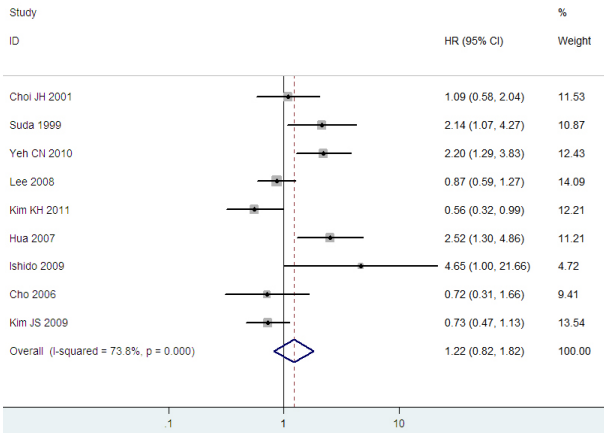

Figure 5. Forest Plot of Hazard Ratios for Overall Survival in the Adjuvant Disease Setting

the cytoplasm of cancer cells showed positive signals compared with stromal inflammatory cells.

In six studies assigned TS expression by reverse transcriptase polymerase chain reaction (RTPCR) (Ichikawa et al., 2004; Ichikawa et al., 2006; Hua et al., 2007; Akamoto et al., 2008; Ichikawa Ishido et al., 2009; Jeung et al., 2011), threshold was defined as the median observed ratio, while in the remaining three studies a maximal χ^2 method determined the optimal cut-off value (Matsubara et al., 2008; Wei et al., 2008; Koizumi et al., 2010).

Results of Meta-Analysis in the Advanced Disease Setting

The pooled HR for OS across twelve advanced studies was 1.43 (95%CI: 1.08 - 1.90), indicating that patients with high TS expression had a risk of death 1.43 times greater than patients with low TS expression (Figure 2). However, large heterogeneity was found among these studies (I^2 =74.1%). The analysis of chemotherapy regimen subgroup were performed (Figure 2). HR pooled from studies in which all patients received fluoropyrimidine monotherapy was 2.32 (95%CI: 1.53 - 3.50, I^2 =40.7%), indicating that the statistical link between high TS expression and poor OS was rather stronger. Unfortunately, limiting analysis to the studies in which fluoropyrimidine based combination chemotherapy was administrated, TS status is not significantly correlated with OS (HR: 0.94, 95%CI: 0.82 - 1.08, I^2 =19.9%). Heterogeneity was not detected

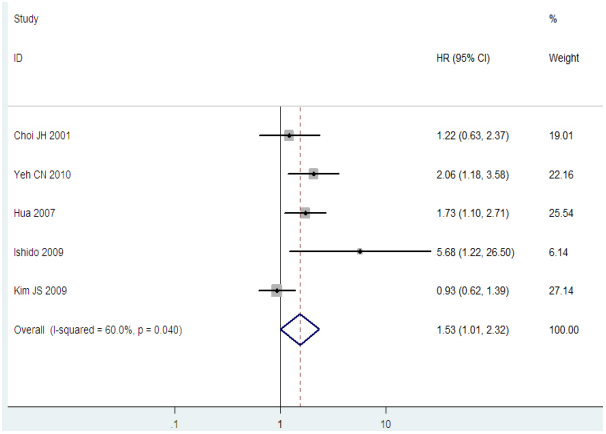

Figure 6. Forest Plot of Hazard Ratios for Event-free Survival in the Adjuvant Disease Setting

in both above subgroups. When grouped according to the method of TS assessment used, the pooled HR was 1.34 (95%CI: 0.88 - 2.05, I^2 =14.7%) for IHC and 1.49 (95%CI: 1.05 - 2.13, I^2 =85.5%) for RTPCR.

No statistically significant effect of TS on EFS was observed (Figure 3), the pooled HR from four studies was 1.36 (95%CI: 0.88 - 2.10), with evidence of study heterogeneity (I^2 =68.8%). When the analysis was limited to the studies in which patients received fluoropyrimidine monotherapy, there was a significant association between high TS expression and poor EFS (HR: 1.76, 95%CI: 1.19 - 2.60, I^2 =0%). However, these results should be interpreted with caution due to the small number of contributing studies.

Overall response rate stratified by TS expression was reported by evelen studies (Figure 4). There was evidence of a trend towards reduced response to fluoropyrimidine -containing chemotherapy with high TS expressing (OR: 0.57, 95%CI: 0.31 - 1.05, I^2 =60.3%), although this was not statistically significant. When we restrict analysis to the studies in which patients received fluoropyrimidine monotherapy, there was statistical evidence that high TS status indicated poorer response (OR: 0.32, 95%CI: 0.11 - 0.95, I^2 =73.6%).

Results of Meta-Analysis in the Adjuvant Disease Setting

In the adjuvant disease setting, no significant effect on OS was observed (Figure 5). The pooled HR from nine adjuvant studies was 1.22 (95%CI 0.82 - 1.82), with evidence of study heterogeneity (I^2 =73.8%). The result indicated that high TS expression was not significantly associated with OS in adjuvant disease setting. Seven studies used IHC to test the TS expression, in which the pooled HR was 1.03 (95%CI: 0.70 - 1.51, I^2 =69.8%). In the remaining two studies by RTPCR, the pooled HR was 2.77 (95%CI: 1.51 - 5.08, I^2 =0%).

Interestingly, we observed a significant association between high TS expression and poor EFS (Figure 6). The pooled HR from five studies was 1.53 (95%CI: 1.01 - 2.32, I^2 =60.0%).

Discussion

The results of this systematic review and meta-analysis demonstrate the predictive significance of TS expression

level in GC patients treated with fluoropyrimidine-containing chemotherapy. In the advanced setting including 844 patients, the results suggested that high TS expression was an indicator of poor OS in advanced GC patients. Especially in the subgroup of fluoropyrimidine monotherapy administrated, TS expression has even stronger value in predicting OS, EFS and ORR. Thus, for the elder and the patients who can not tolarance for multi-drug chemotherapy, the predictive value of TS expression may help clinicians choose the optimal single agent. However, in the subgroup of fluoropyrimidine based combination chemotherapy used, TS expression did not significantly predict the treatment outcomes. This may account for that the tumours with high TS expression might respond to other drugs, whereas those tumours were refractory to fluoropyrimidine alone. Therefore accordingly, it may contribute to more accurate prediction of treatment outcomes if we evaluate the interaction between TS and other known predictive factors.

In the adjuvant setting including 1,235 patients, high TS expression was not associated with OS. To localized GC who have received curative surgery, OS may be subject to other more important factors, for instance extent of gastric resection and lymphadenectomy. Interestingly, our results showed that high TS expression was significantly correlated with poor EFS in ajuvant studies.

The value of TS expression in predicting poor OS seems stronger in studies using RTPCR than IHC in both advanced and adjuvant settings. This is partially attributable to the thresholds used in TS status assignment, as in many RTPCR studies the dichotomizations were defined by the maximal χ^2 method and dependent on likely response. This may indicate a source of bias (Altman et al., 1994).

In all meta-analysis reported above, no siginificant publication bias was detected according to Begg's test. However, it should be kept in mind that this methodology is not completely bias-free, because there might have been rejection or even non-submission of negative data existed. In addition, another potential source of bias could be introduced and need to be paid attention as inadequate blinding of survival data from assessors of TS expression. Of all the fifteen studies using IHC, three did not point that their evalution of TS expression was done by assessors who were blind to clinical data.

A statistically significant heterogeneity must be addressed in our report. Firstly, some of the heterogeneity observed might account for different thresholds to define TS status and the wide variation in the proportion of high TS expression in each study. Secondly, varied antibodies for IHC and housekeeping genes for RTPCR were used with no consistent criteria. Thirdly, inadequate sample size was also a frequent problem in the studies included in our analysis, with only five of the twenty-four studies reporting outcomes from over 100 patients. Whilst pooling data may in part address deficiencies in individual study sample sizes, smaller studies are more likely to generate heterogeneity. Thus, a random-effects model was used to estimate the effect of TS high expression on outcomes due to these evidences of methodological heterogeneity across studies. This assumes that the studies were random samples from a hypothetical population of studies taking into account variability within and among studies.

We did no attempt to weigh each study by a quality score, since quality assessment tools for examining prognostic and predictive biomarker studies do not currently exist, and are only beginning to be discussed for prognosis studies in general (Hayden et al., 2006). Evidently the design of some studies is not optimal. For example, dissimilar methodologies and no criteria of threshold used in TS status assignment. Moreover, the majority of survival data were based on small-sized sample and retrospective analysis. In the future research, large multi-centre prospective studies should be conducted with the use of standard unbiased methods, with assessors blinded to the clinical data, and include more homogeneous GC patients, to investigate the precise predictive effect of TS expression in GC.

Acknowledgements

The authors declare that they have no competing interests.

References

Akamoto S, Usuki H, Haba R, et al (2008). The discrepancy in thymidylate synthase and dihydropyrimidine dehydrogenase expression depending on measurement methodologies in stage 4 gastric cancer. *Hepatogastroenterology*, **55**, 1882-6.

Altman DG, Lausen B, Sauerbrei W, et al (1994). Dangers of using "optimal" cutpoints in the evaluation of prognostic factors. *J Natl Cancer Inst*, **86**, 829-35.

Begg CB, Mazumdar M (1994). Operating characteristics of a rank correlation test for publication bias. *Biometrics*, **50**, 1088-101.

Berger SH, Jenh CH, Johnson LF, et al (1985). Thymidylate synthase overproduction and gene amplification in fluorodeoxyuridine-resistant human cells. *Mol Pharmacol*, **28**, 461-7.

Boku N, Chin K, Hosokawa K, et al (1998). Biological markers as a predictor for response and prognosis of unresectable gastric cancer patients treated with 5-fluorouracil and cis-platinum. *Clin Cancer Res*, **4**, 1469-74.

Boku N, Ohtsu A, Yoshida S, et al (2007). Significance of biological markers for predicting prognosis and selecting chemotherapy regimens of advanced gastric cancer patients between continuous infusion of 5-FU and a combination of 5-FU and cisplatin. *Jpn J Clin Oncol*, **37**, 275-81.

Cho MY, Yi SY, Eom M, et al (2006). A high thymidylate synthase expression is related to better outcome for advanced gastric cancer patients treated with 5-FU chemotherapy after curative resection. *Korean J Pathol*, **40**, 128-36.

Choi IS, Lee HS, Lee KW, et al (2011). Biomarker analysis in patients with advanced gastric cancer treated with S-1 plus cisplatin chemotherapy: orotate phosphoribosyltransferase expression is associated with treatment outcomes. *Med Oncol*, **28**, 991-8.

Choi J, Lim H, Nam DK, et al (2001). Expression of thymidylate synthase in gastric cancer patients treated with 5-fluorouracil and doxorubicin-based adjuvant chemotherapy after curative resection. *Br J Cancer*, **84**, 186-92.

Chung-Kang C, Ohno M, Nishimura K, et al (2006). Intratumoral heterogeneous expression of thymidylate synthase in gastric cancer. *Hepatogastroenterology*, **53**, 458-62.

DerSimonian R, Laird N (1986). Meta-analysis in clinical trials. *Control Clin Trials*, **7**, 177-88.

Fukuda H, Takiguchi N, Koda K, et al (2006). Thymidylate synthase and dihydropyrimidine dehydrogenase are related to histological effects of 5-fluorouracil and cisplatin neoadjuvant chemotherapy for primary gastric cancer patients. *Cancer Invest,* **24**, 235-41.

Hayden JA, Cote P, Bombardier C (2006). Evaluation of the quality of prognosis studies in systematic reviews. *Ann Intern Med*, **144**, 427-37.

Higgins JP, Thompson SG (2002). Quantifying heterogeneity in a meta-analysis. *Stat Med,* **21**, 1539-58.

Hua D, Huang ZH, Mao Y, et al (2007). Thymidylate synthase and thymidine phosphorylase gene expression as predictive parameters for the efficacy of 5-fluorouracil-based adjuvant chemotherapy for gastric cancer. *World J Gastroenterol*, **13**, 5030-4.

Ichikawa W, Takahashi T, Suto K, et al (2004). Thymidylate synthase predictive power is overcome by irinotecan combination therapy with S-1 for gastric cancer. *Br J Cancer*, **91**, 1245-50.

Ichikawa W, Takahashi T, Suto K, et al (2006). Simple combinations of 5-FU pathway genes predict the outcome of metastatic gastric cancer patients treated by S-1. *Int J Cancer,* **119**, 1927-33.

Ishido K, Azuma M, Koizumi W, et al (2009). Evaluation of prognostic factors for the response to S-1 in patients with stage II or III advanced gastric cancer who underwent gastrectomy. *Pharmacogenet Genomics*, 2009, Nov 5.

Ishikawa Y, Kubota T, Otani Y, et al (1999). Thymidylate synthetase and dihydropyrimidine dehydrogenase levels in gastric cancer. *Anticancer Res*, **19**, 5635-40.

Ishizone S, Maruta F, Saito H, et al (2006). Efficacy of S-1 for patients with peritoneal metastasis of gastric cancer. *Chemotherapy*, **52**, 301-7.

Jemal A, Bray F, Center MM, et al (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.

Jeung HC, Rha SY, Shin SJ, et al (2011). Predictive values of 5-fluorouracil pathway genes for S-1 treatment in patients with advanced gastric cancer. *Anticancer Drugs*, **22**, 801-10.

Jeong SH, Han JH, Kim JH, et al (2011). Bax predicts outcome in gastric cancer patients treated with 5-fluorouracil, leucovorin, and oxaliplatin palliative chemotherapy. *Dig Dis Sci*, **56**, 131-8.

Johnston PG, Drake JC, Trepel J, et al (1992). Immunological quantitation of thymidylate synthase using the monoclonal antibody TS 106 in 5-fluorouracil-sensitive and -resistant human cancer cell lines. *Cancer Res*, **52**, 4306-12.

Kim KH, Kwon HC, Oh SY, et al (2011). Clinicopathologic significance of ERCC1, thymidylate synthase and glutathione S-transferase P1 expression for advanced gastric cancer patients receiving adjuvant 5-FU and cisplatin chemotherapy. *Biomarkers*, **16**, 74-82.

Kim JS, Kim MA, Kim TM, et al (2009). Biomarker analysis in stage III-IV (M0) gastric cancer patients who received curative surgery followed by adjuvant 5-fluorouracil and cisplatin chemotherapy: epidermal growth factor receptor (EGFR) associated with favourable survival. *Br J Cancer*, **100**, 732-8.

Koizumi W, Narahara H, Hara T, et al (2008). S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. Lancet Oncol, 9, 215-21.

Koizumi W, Tanabe S, Azuma M, et al (2010). Impacts of fluorouracil-metabolizing enzymes on the outcomes of patients treated with S-1 alone or S-1 plus cisplatin for first-line treatment of advanced gastric cancer. *Int J Cancer*, **126**, 162-70.

Kuniyasu T, Nakamura T, Tabuchi Y, et al (1998). Immunohistochemical evaluation of thymidylate synthase in gastric carcinoma using a new polyclonal antibody: the clinical role of thymidylate synthase as a prognostic indicator and its therapeutic usefulness. *CANCER*, **83**, 1300-6.

Kwon HC, Roh MS, Oh SY, et al (2007). Prognostic value of expression of ERCC1, thymidylate synthase, and glutathione S-transferase P1 for 5-fluorouracil/oxaliplatin chemotherapy in advanced gastric cancer. *Ann Oncol*, **18**, 504-9.

Lenz HJ, Leichman CG, Danenberg KD, et al (1996). Thymidylate synthase mRNA level in adenocarcinoma of the stomach: a predictor for primary tumor response and overall survival. *J Clin Oncol*, **14**, 176-82.

Lee J, Park CK, Park JO, et al (2008). Impact of E2F-1 expression on clinical outcome of gastric adenocarcinoma patients with adjuvant chemoradiation therapy. *Clin Cancer Res*, **14**, 82-8.

Liu JM, Chen LT, Li AF, et al (2004). Prognostic implications of the expression of erbB2, topoisomerase II alpha and thymidylate synthase in metastatic gastric cancer after fluorouracil-based therapy. *Jpn J Clin Oncol*, **34**, 727-32.

Matsubara J, Nishina T, Yamada Y, et al (2008). Impacts of excision repair cross-complementing gene 1 (ERCC1), dihydropyrimidine dehydrogenase, and epidermal growth factor receptor on the outcomes of patients with advanced gastric cancer. *Br J Cancer,* **98**, 832-9.

Metzger R, Leichman CG, Danenberg KD, et al (1998). ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. *J Clin Oncol*, **16**, 309-16.

Miyamoto S, Boku N, Ohtsu A, et al (2000). Clinical implications of immunoreactivity of thymidylate synthase and dihydropyrimidine dehydrogenase in gastric cancer treated with oral fluoropyrimidine (S-1). Study Group of S-1 for Gastric Cancer. *Int J Oncol*, **17**, 653-8.

Napieralski R, Ott K, Kremer M, et al (2005). Combined GADD45A and thymidine phosphorylase expression levels predict response and survival of neoadjuvant-treated gastric cancer patients. *Clin Cancer Res*, **11**, 3025-31.

Parmar MK, Torri V, Stewart L (1998). Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med*, **17**, 2815-34.

Sakuramoto S, Sasako M, Yamaguchi T, et al (2007). Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. *N ENGL J MED*, **357**, 1810-20.

Santi DV, McHenry CS, Sommer H (1974). Mechanism of interaction of thymidylate synthetase with 5-fluorodeoxyuridylate. *Biochemistry*, **13**, 471-81.

Suda Y, Kuwashima Y, Tanaka Y, et al (1999). Expression of thymidylate synthase and thymidine phosphorylase in recurrence and survival rates of advanced gastric cancer. *Gastric Cancer*, **2**, 165-72.

Tahara M, Ochiai A, Fujimoto J, et al (2004). Expression of thymidylate synthase, thymidine phosphorylase, dihydropyrimidine dehydrogenase, E2F-1, Bak, Bcl-X, and Bcl-2, and clinical outcomes for gastric cancer patients treated with bolus 5-fluorouracil. *Oncol Rep*, **11**, 9-15.

Terashima M, Fujiwara H, Takagane A, et al (2003). Prediction of sensitivity to fluoropyrimidines by metabolic and target enzyme activities in gastric cancer. *Gastric Cancer*, **1**, 71-81.

Tsujitani S, Konishi I, Suzuki K, et al (2000). Expression of thymidylate synthase in relation to survival and chemosensitivity in gastric cancer patients. *J Exp Clin Cancer Res*, **19**, 189-95.

Van Cutsem E, Moiseyenko VM, Tjulandin S, et al (2006). Phase III study of docetaxel and cisplatin plus fluorouracil

compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. *J Clin Oncol*, **24**, 4991-7

Wei J, Zou Z, Qian X, et al (2008). ERCC1 mRNA levels and survival of advanced gastric cancer patients treated with a modified FOLFOX regimen. *Br J Cancer*, **98**, 1398-402.

Yeh CN, Jung SM, Chen TW, et al (2010). Expression of thymidylate synthase determines the response of gastric cancer patients undergoing gastrectomy to 5-fluorouracil-based adjuvant chemotherapy. *Langenbecks Arch Surg*, **395**, 217-25.

Yeh KH, Shun CT, Chen CL, et al (1998). High expression of thymidylate synthase is associated with the drug resistance of gastric carcinoma to high dose 5-fluorouracil-based systemic chemotherapy. *Cancer*, **82**, 1626-31.