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

Hua-Bin Hu1&, Lei Kuang2&, Xiao-Min Zeng3, Bin Li1, En-Yi Liu1, Mei-Zuo Zhong1*  

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 have 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 associated 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

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 chemotherapeutic 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'-deoxythymidine-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

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Treatment Setting</th>
<th>Chemotherapy</th>
<th>Method</th>
<th>Cutoff</th>
<th>No. of Pts TS (%)</th>
<th>HR for OS (95% CI)</th>
<th>HR for EFS (95% CI)</th>
<th>OR for ORR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jeong</td>
<td>2011</td>
<td>Advanced 5-FU/Oxaliplatin</td>
<td>IHC</td>
<td>S: Median</td>
<td>72</td>
<td>49</td>
<td>1.18 (0.38-3.63)*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Yeh</td>
<td>1998</td>
<td>Advanced 5-FU</td>
<td>IHC</td>
<td>I: ≥ 2</td>
<td>30</td>
<td>53</td>
<td>2.50 (1.25-4.99)*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Miyamoto</td>
<td>2000</td>
<td>Advanced S-1</td>
<td>IHC</td>
<td>I: ≥ 2</td>
<td>41</td>
<td>15</td>
<td>1.50 (0.23-9.89)*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ichikawa</td>
<td>2006</td>
<td>Advanced S-1</td>
<td>RTPCR</td>
<td>Median</td>
<td>59</td>
<td>53</td>
<td>4.75 (2.17-10.3)</td>
<td>—</td>
<td>0.10 (0.03-0.35)</td>
</tr>
<tr>
<td>Ichikawa</td>
<td>2004</td>
<td>Advanced S-1/Irinotecan</td>
<td>RTPCR</td>
<td>Median</td>
<td>26</td>
<td>50</td>
<td>1.05 (0.90-1.22)*</td>
<td>—</td>
<td>2.56 (0.53-12.43)</td>
</tr>
<tr>
<td>Koizumi</td>
<td>2010</td>
<td>Advanced S-1</td>
<td>RTPCR</td>
<td>χ2: &gt; 4.46</td>
<td>66</td>
<td>30</td>
<td>2.71 (1.36-5.37)</td>
<td>—</td>
<td>0.32 (1.0-1.10)</td>
</tr>
<tr>
<td>Matsubara</td>
<td>2008</td>
<td>Advanced S-1</td>
<td>RTPCR</td>
<td>χ2: &gt; 3.67</td>
<td>66</td>
<td>36</td>
<td>2.11 (0.97-4.55)</td>
<td>—</td>
<td>0.68 (0.21-2.11)</td>
</tr>
<tr>
<td>Jeong</td>
<td>2011</td>
<td>Advanced S-1</td>
<td>RTPCR</td>
<td>Median</td>
<td>75</td>
<td>51</td>
<td>1.31 (0.79-2.17)*</td>
<td>1.65 (1.04-2.59)*</td>
<td>0.68 (0.21-2.11)</td>
</tr>
<tr>
<td>Akamota</td>
<td>2008</td>
<td>Advanced S-1</td>
<td>RTPCR</td>
<td>Median</td>
<td>21</td>
<td>48</td>
<td>2.34 (0.92-5.94)*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Choi</td>
<td>2011</td>
<td>Advanced S-1/Cisplatin</td>
<td>IHC</td>
<td>S: ≥ 3</td>
<td>40</td>
<td>33</td>
<td>0.88 (0.50-1.54)*</td>
<td>0.96 (0.89-1.04)*</td>
<td>1.26 (0.33-4.73)</td>
</tr>
<tr>
<td>Kwon</td>
<td>2007</td>
<td>Advanced 5-FU</td>
<td>IHC</td>
<td>I: ≥ 2 and E: ≥ 2</td>
<td>64</td>
<td>30</td>
<td>1.48 (0.53-4.15)</td>
<td>1.45 (0.52-4.07)*</td>
<td>0.88 (0.29-2.65)</td>
</tr>
<tr>
<td>Wei</td>
<td>2008</td>
<td>Advanced 5-FU/Oxaliplatin</td>
<td>RTPCR</td>
<td>χ2: &gt; 0.66</td>
<td>76</td>
<td>72</td>
<td>0.83 (0.72-0.96)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tahara</td>
<td>2004</td>
<td>Advanced 5-FU/Methotrexate</td>
<td>IHC</td>
<td>P: &gt; 25%</td>
<td>38</td>
<td>76</td>
<td>0.45 (0.02-8.36)*</td>
<td>—</td>
<td>0.67 (0.12-3.71)</td>
</tr>
<tr>
<td>Boku</td>
<td>1998</td>
<td>Advanced S-1/Cisplatin</td>
<td>IHC</td>
<td>I: ≥ 1</td>
<td>39</td>
<td>46</td>
<td>—</td>
<td>—</td>
<td>0.38 (0.09-1.56)</td>
</tr>
<tr>
<td>Boku†</td>
<td>2007</td>
<td>Advanced 5-FU</td>
<td>IHC</td>
<td>P: ≥ 20%</td>
<td>65</td>
<td>57</td>
<td>—</td>
<td>—</td>
<td>1.30 (0.28-5.98)</td>
</tr>
<tr>
<td>Boku‡</td>
<td>2007</td>
<td>Advanced 5-FU/Cisplatin</td>
<td>IHC</td>
<td>P: ≥ 20%</td>
<td>66</td>
<td>32</td>
<td>—</td>
<td>—</td>
<td>0.94 (0.33-2.67)</td>
</tr>
<tr>
<td>Choi</td>
<td>2001</td>
<td>Adjuvant 5-FU/Doxorubicin</td>
<td>IHC</td>
<td>P: ≥ 25%</td>
<td>103</td>
<td>63</td>
<td>1.09 (0.58-2.04)</td>
<td>1.22 (0.63-2.37)*</td>
<td>—</td>
</tr>
<tr>
<td>Suda</td>
<td>1999</td>
<td>Adjuvant Fluorouracil/Mitomycin C</td>
<td>IHC</td>
<td>Positive signal</td>
<td>66</td>
<td>45</td>
<td>2.14 (1.07-4.27)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Yeh CN</td>
<td>2010</td>
<td>Adjuvant 5-FU based regimen</td>
<td>IHC</td>
<td>P: ≥ 20%</td>
<td>124</td>
<td>66</td>
<td>2.20 (1.29-3.83)</td>
<td>2.06 (1.18-3.58)*</td>
<td>—</td>
</tr>
<tr>
<td>Lee</td>
<td>2008</td>
<td>Adjuvant 5-FU</td>
<td>IHC</td>
<td>P: &gt; 25%</td>
<td>463</td>
<td>19</td>
<td>0.87 (0.59-1.27)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Kim</td>
<td>2011</td>
<td>Adjuvant 5-FU/Cisplatin</td>
<td>IHC</td>
<td>I: ≥ 2 and E: ≥ 2</td>
<td>124</td>
<td>77</td>
<td>0.56 (0.32-0.99)*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hua</td>
<td>2007</td>
<td>Advanced 5-FU based regimen</td>
<td>RTPCR</td>
<td>Median</td>
<td>51</td>
<td>49</td>
<td>2.52 (1.30-4.86)</td>
<td>1.73 (1.10-2.71)*</td>
<td>—</td>
</tr>
<tr>
<td>Ishido</td>
<td>2009</td>
<td>Adjuvant S-1</td>
<td>RTPCR</td>
<td>Median</td>
<td>39</td>
<td>51</td>
<td>4.65 (1.00-21.66)</td>
<td>5.68 (1.22-26.50)</td>
<td>—</td>
</tr>
<tr>
<td>Cho</td>
<td>2006</td>
<td>Adjuvant Doxifluridine/Epirubicin/Mitomycin C</td>
<td>IHC</td>
<td>S: ≥ 6</td>
<td>89</td>
<td>36</td>
<td>0.72 (0.31-1.66)*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Kim</td>
<td>2009</td>
<td>Adjuvant 5-FU/Cisplatin</td>
<td>IHC</td>
<td>S: ≥ 25</td>
<td>151</td>
<td>49</td>
<td>0.73 (0.47-1.13)</td>
<td>0.93 (0.62-1.39)</td>
<td>—</td>
</tr>
</tbody>
</table>

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 staining intensity; E, grades of staining 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; χ², the maximal χ² method; ‘patients received 5-FU; †patients received 5-FU/Cisplatin. ‡, not performed; *calculated result from published data.

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 I² statistic was performed in all meta-analysis. I² is measured from 0-100% with increasing I² 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
Fifteen eligible studies assessed survival or treatment-related effect cannot be entirely discounted. In four studies (Cho et al., 2006; Kim et al., 2009; Choi et al., 2011; Jeong et al., 2011), expression was dichotomized by quantifying the proportion of stained cells using arbitrary thresholds of 20% or 25%. In two 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 (Boku et al., 1998; Yeh et al., 1998; Miyamoto et al., 2000), the high expression were judged when the grades of intensity and extent are both 2 or higher.

In the adjuvant disease setting, nine studies that included survival data of total 1,235 patients available for pooling (median: 62, range: 21-76). All studies 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 percentages 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...
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 eleven studies (Figure 4). There was evidence of a trend towards reduced response to fluoropyrimidine-containing chemotherapy with high TS expression (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 administered, TS expression has even stronger value in predicting OS, EFS and ORR. Thus, for the elder and the patients who can not tolerate 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 adjuvant 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 χ² method and dependent on likely response. This may indicate a source of bias (Altman et al., 1994).

In all meta-analysis reported above, no significant 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 evaluation of TS expression was done by 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


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*Control Clin Trials,* 7, 177-88.


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