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DCC and TS protein expression in resected gastric cancer: A Hellenic Cooperative Oncology Group Study

Research article

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Abstract: There is a high risk of relapse after resection of gastric cancer. We studied the prognostic significance of the deleted colorectal cancer (DCC) gene and thymidylate synthase (TS) protein expression after resection of gastric cancer. Protein expression in the primary tumor of 146 patients with serosal and/or lymph node involvement was studied immunohistochemically by using anti-DCC and anti-TS monoclonal antibodies. DCC expression was found in 69.9%, while low TS staining intensity (0+,1+) and focal staining (<25% of tumor cells stained) were found in 44.6% and 33.8%, respectively. Overall survival (OS) was significantly longer in patients with DCC (p=0.014) negative tumors. TS expression was not an independent prognostic factor. Lack of DCC expression was associated with significantly longer cause-specific survival (CSS) (p=0.040) after curative resection. In conclusion, DCC expression is an independent prognostic factor in patients undergoing resection of gastric cancer while TS expression was not associated with the prognosis in our study.

Keywords: *DCC • TS • Gastric cancer*

1. Introduction

Gastric cancer is a frequent cause of death from cancer [1] and its prognosis has remained poor over the last 20 years. Complete surgical resection of the tumor with tumor-free resection margins offers the only potential for cure. Depth of invasion and lymph node metastases represent the most important prognostic factors for relapse and survival [2]. Nevertheless, these factors have only limited value for most patients undergoing curative surgery in USA and Europe, since serosal or adjacent organ invasion or lymph node metastases (T3, T4, N1-3, in TNM classification) are found in 80-85% of cases [3]. These patients have at least a 60% chance of dying from their disease within 5 years from surgery [3]. Therefore, the majority of patients belong to a "high-risk" group and it is not yet possible to further predict their prognosis using clinical or pathological features. Hence, there is a need for other prognostic factors for patients with gastric cancer undergoing surgery with a curative intent.

The role of the DCC gene and the enzyme TS in the carcinogenesis as well as their prognostic and predictive value have been the focus of recent research. The DCC gene is located on chromosome 18 encoding for a transmembrane protein and is considered a candidate tumor suppressor gene [4]. 18q loss of heterozygosity (LOH) and loss of expression of DCC mRNA have

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been described in gastric carcinoma [5,6] as well as in other malignancies [7-9] but information regarding its prognostic significance is limited. LOH at the 18q region was shown to be an adverse prognostic factor in patients with stage II but not stage III colorectal tumors [10]. Nevertheless, this finding did not reach statistical significance in another study [11], while another study showed prognostic significance only in stage III tumors [12]. Recently, DCC protein expression was shown to be a favorable prognostic factor in patients with stages II and III colorectal carcinoma [13]. Nevertheless, this was shown only for diploid and low S-phase fraction tumors and not for individual Duke's stages in another study [14]. On the contrary, no association of LOH at 18q21 with prognosis was found in patients with gastric carcinoma [15]. We have recently reported that lack of expression of DCC protein was a favorable prognostic factor in a small series of 66 patients with high risk gastric cancer (serosal invasion and/or lymph node involvement) undergoing gastrectomy with curative intent [16]. Since the number of patients was small we performed a larger confirmatory multicenter study.

TS is essential for DNA synthesis and a critical target of 5FU [17]. The relationship of TS levels with response to 5FU has been demonstrated in clinical studies [18-20]. In addition, the prognostic significance of TS in breast and colorectal cancer, regardless of 5FU administration, has also been shown [21-23] but was not confirmed by a recent study [24]. Reports of the correlation of TS expression with prognosis in patients with gastric cancer are limited. One study included 47 patients who received preoperative 5FU/Cisplatin and 18 patients with inoperable disease receiving the same regimen [25]. A survival advantage for patients with low TS mRNA levels was shown. Two studies included patients who received adjuvant 5FU based chemotherapy after curative resection. One study showed a significant 5-year survival benefit in patients with stage IIIb with low TS expression [26], while the other study showed no benefit [27]. Finally, two studies including both treated and untreated patients suggested a survival benefit for patients with low TS expression [28,29]. Nevertheless, statistical significance was reached only in one of them [28].

The aim of this study was the evaluation of the prognostic value of DCC and TS protein expression in patients with serosal and/or lymph node involvement following resection of gastric cancer with curative intent. Moreover, since both DCC and TS have been associated with cell proliferation [4,30], we also studied the proliferative activity of our material using a monoclonal antibody against proliferating cell nuclear antigen (PCNA) [31,32].

2. Material and Methods

2.1. Patients

Blocks of paraffin-embedded tissue were obtained from 146 patients who had undergone surgery for gastric carcinoma between 1988 and 1998 and were followed up at the Oncology Departments of the following Greek Hospitals: AHEPA Hospital of Thessaloniki, Ioannina University Hospital and Ippokration Hospital in Athens. Patients who had been lost to follow up or died within 1 month from surgery were not included in the study. Histopathological slides from surgery from all patients were reviewed by the Pathology Department of the University of Ioannina. All patients had been operated with curative intent, meaning that preoperative staging revealed no distant metastases and primary tumor was deemed resectable after endoscopy and computerized tomography assessment. All patients were at high risk for relapse since they had at least one of the following adverse prognostic factors: a) serosal or adjacent organ involvement (T3 or T4 according to TNM classification); b) lymph node involvement (N1-3 according to TNM classification).

2.2. Immunohistochemistry

Immunostaining was performed on formalin-fixed, paraffin-embedded sections of the primary tumor using the labelled streptavidin avidin biotin (LSAB) method (LSAB kit Dako, Glostrup; Denmark) as described previously [33]. Monoclonal antibodies directed against DCC protein (clone: G97-449, Pharmigen; San Diego; dilution 1:300), TS (TS106, Chemicon, overnight incubation, dilution 1:80) and PCNA (clone: PC10, Dako, dilution 1:100) were applied. Antigen retrieval by microwave heating (3 x 5 min, 750-W in 10mM citrate buffer pH 6) was used in all cases. Sections

Figure 1. Immunohistochemical cytoplasmic expression of DCC protein in gastric adenocarcinoma (x 270).

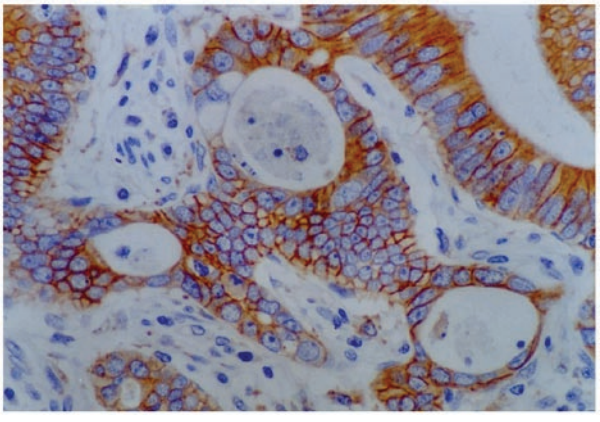


Table 1. Patients' characteristics and correlation with DCC expression.

Characteristic	Pts	No expression		p
		Pts	%	
Gender				
Male/Female	89/57	22/22	24.7/38.6	0.096
Age		DCC-ve	DCC+ve	0.036
Median	60	56,5	61	
Range	20-88	29-84	20-88	
Lauren's classification				
Intestinal	62	9	14.5	0.004
Diffuse	72	31	43	
Mixed	12	4	33.3	
Differentiation				
Good	9	0	0	<0.001
Moderate	36	3	8.3	
Poor	71	23	32.4	
Signet ring	30	18	60	
Depth of invasion				
T1	5	3	60	0.297
T2	20	4	20	
T3	115	36	31.3	
T4	6	1	16.7	
Lymph node involvement				
Yes/No	130/16	39/5	30/31.3	1.0
Metastases				
Yes/No	17/129	5/39	29.4/30.2	1.0
Margins				
Positive	11	2	18.2	0.505
Negative	135	42	31.4	
PCNA LI (n=121)				
Mean	69%			
Range	25%-97%			
Low score (n=61)		16	26.2	0.840
High score (n=60)		17	28.3	

PCNA: proliferating cell nuclear antigen; LI: labeling index.

stained by omitting the primary antibody were used as negative controls. Positive control slides were included in all cases. They consisted of colon adenocarcinomas known to have high DCC and TS protein expression and reactive lymph nodes with follicular hyperplasia for PCNA. Moreover, TS immunostaining in the proliferative zone of gastric epithelia and the germinal center of secondary mucosa-associated tissues in the normal gastric mucosa as well as DCC immunostaining of normal gastric epithelia were used as internal positive controls.

A continuous score system using the x40 objective lens and counting at least five fields was adopted for the counting of positive cells. DCC expression was classified as positive if any tumour cells were stained regardless of the intensity of staining. TS expression grading system was based on the intensity of staining (low intensity: 0,1+; high intensity: 2+,3+) and the extent (focal: <25% of positive tumor cells or diffuse >25% of positive tumor cells). For analysis purposes, tumors with no TS staining were included in the focal group. Only nuclear staining with PC10 antibody was considered positive. The PCNA labelling index (LI) was calculated as the percentage of positive cell nuclei. Values above or equal to the mean were considered high PCNA LI and the rest were considered low LI.

All cases were examined independently by two investigators (M.B. and M.M.). There was a close agreement (>90%) in the evaluation of immunostaining between the two investigators.

Table 2. Correlation of TS expression with baseline characteristics.

	Intensity			Extent		
	0,1 (%)	2,3 (%)	p	Focal(%)	Diffuse(%)	p
Gender			0.104			0.059
Male (n=80)	31(38.7)	49(61.3)		22(27.5)	58(72.5)	
Female (n=50)	27(54)	23(46)		22(44)	28(56)	
Age			0.195		63,5	<0.001
Median	59	61		55	40-88	
Range	36-79	20-88		20-78		
Lauren's classification			0.128			0.314
Intestinal(n=59)	22(37.3)	37(62.7)		17(28.8)	42(71.2)	
Diffuse(n=59)	32(54.2)	27(45.5)		24(40.7)	35(59.3)	
Mixed(n=12)	4(33.3)	8(66.7)		3(25)	9(75)	
Differentiation			0.252			0.138
Good(n=8)	4(50)	4(50)		1(12.5)	7(87.5)	
Moderate(n=36)	14(38.9)	22(61.1)		10(27.8)	26(72.2)	
Poor(n=58)	23(39.7)	35(60.3)		19(32.8)	39(67.2)	
Signet ring(n=28)	17(60.7)	11(39.3)		14(50)	14(50)	
Depth of invasion			0.539			0.138
T1(n=4)	3(75)	1(25)		3(75)	1(25)	
T2(n=15)	6(40)	9(60)		5(33.3)	10(66.7)	
T3(n=106)	46(43.4)	60(56.6)		35(33)	71(67)	
T4(n=5)	3(60)	2(40)		1(20)	4(80)	
Lymph node involvement			0.601			0.260
Yes(n=114)	52(45.6)	62(54.4)		41(36)	73(64)	
No(n=16)	6(37.5)	10(72.5)		3(18.8)	13(81.2)	
Metastases			0.271			0.773
Yes (n=16)	10(62.5)	6(37.5)		4(25)	12(75)	
No (n=114)	48(42.1)	66(57.9)		40(35)	74(65)	
PCNA LI (n=107)			0.078			0.005
Low score(n=56)	29(51.8)	27(48.2)		23(41)	33(59)	
High score(n=51)	17(33.3)	34(66.7)		8(15.7)	43(84.3)	

PCNA: proliferating cell nuclear antigen; LI: labeling index.

2.3. Statistical Analysis

Chi-square test [34] was used to test correlation of DCC and TS expression with: sex; differentiation; tumor type; T classification; lymph node involvement; metastases at the time of surgery; positive surgical margins; and, PCNA LI. Yate's correction and Fischer exact test were applied when appropriate. The median ages of each group were compared using the Mann-Whitney test. OS was estimated from the date of surgery. CSS was calculated by censoring deaths not due to gastric cancer. Cumulative survival curves were computed according to the method of Kaplan-Meier [35]. Log-rank tests were used to test survival differences, while Cox's proportional hazard models were used to assess hazard ratios [36]. All p-values were two-sided and 5% was chosen as the level of statistical significance. The SPSS software was used for statistical analysis (SPSS for Windows, version 10, SPSS Inc., Chicago, IL).

3. Results

3.1. Patients

The characteristics of the patients included in the study are shown in Table 1. During surgery metastatic disease was found in 17 cases: peritoneal metastases in 11 cases and liver metastases in 6. Seventy patients received treatment following surgery: 64 received chemotherapy; 5 radiotherapy; and, 1 combination of radiotherapy and chemotherapy. Chemotherapy consisted of combinations containing 5FU in all cases. There was no significant difference in the percentage of patients receiving treatment among DCC positive (53/102, 52%) and DCC negative (17/44, 38,6%) patients (Fisher's exact test p=0,153).

3.2. Correlation of DCC and TS expression with clinicopathological characteristics

DCC protein expression was evaluated in all 146 cases. It was detected in 102 cases (69.9%) and absent in 44 cases (30.1%). Immunostaining was cytoplasmic showing a microglanular rather than diffuse staining pattern. Heterogeneity of staining was observed in the majority of positive cases (Figure 1). Younger age, poor differentiation and diffuse type were associated with lower DCC expression (Table 1).

TS expression was evaluated in 130 cases due to insufficient archival tumor tissue in 16 cases. Immunostaining of cancer cells was cytoplasmic. No TS expression was found in 11 cases (8.5%), while intensity of 1+, 2+ and 3+ was observed in 47 (36.1%), 52 (40%) and 20 (15.4%) cases, respectively (Figure 2). Focal and diffuse staining were observed in 44 (33.8%) and 86 (66.2%) cases. There was a significant correlation between the results of the two grading methods (p<0.001): 33 of 44 (75%) cases with focal staining pattern showed low intensity, while 61 of the 86 cases with diffuse staining (71%) showed high intensity. Correlation of TS expression with baseline characteristics is shown in Table 2. TS staining intensity did not correlate with any clinicopathological baseline characteristic. In contrast, diffuse staining was correlated with older age and higher PCNA LI.

3.3. Correlation of DCC Protein Expression with Survival

The median follow-up of patients included in this study was 54 months (range: 16-183). One hundred and one patients died during follow-up: 99 due to gastric cancer, 1 due to heroin overdose and 1 due to myocardial infarction. 5-year OS of patients whose tumors showed DCC expression was 17.98% (95% confidence intervals [CI]: 8.84-27.12), compared to 34.06% in the group with no expression (95% CI: 15.26-52.86) (Figure 3). In univariate analysis, which included all characteristics listed in Table 1 and the following were associated with poor prognosis: administration of adjuvant treatment; presence of metastases at the time of surgery (p<0.0001); higher T classification (p=0.0003); lymph node metastases (p=0.03); positive resection margins (p=0.0367); and, presence of DCC expression (p=0.0137). Multivariate analysis showed that metastases at the time of surgery, lymph node metastases, T classification and DCC expression were independent prognostic factors for survival (Table 3). Univariate and multivariate analysis for CSS showed similar results.

Figure 2. High intensity TS staining of gastric adenocarcinoma detected by immunohistochemistry using TS106 antibody (x 270).

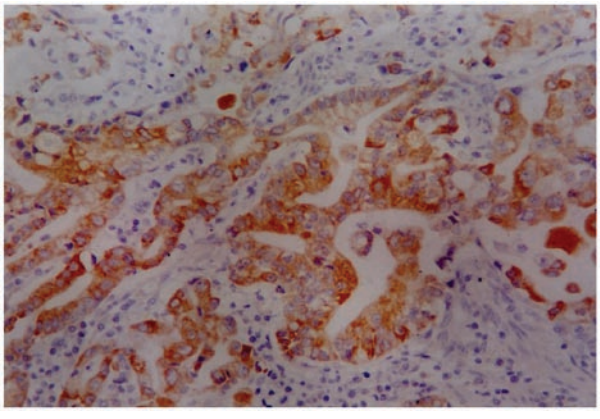


Table 3. Multivariate analysis in relation to overall survival in DCC studied patients.

Variable	Hazard ratio	95% C.I.	p
Depth of invasion			0.004
T1 (n=5)	0.052	0.006-0.447	
T2 (n=20)	0.204	0.076-0.551	
T3 (n=115)	0.305	0.130-0.712	
T4 (n=6)	1		
LN involvement	0.3661	0.159-0.841	0.006
No (n=16)			
Yes (n=130)			
Metastases			<0.001
No (n=17)	0.2931	0.168-0.514	
Yes (n=129)			
Positive margins			0.094
No (n=135)	0.532	0.251-1.127	
Yes (n=11)	1		
DCC staining			0.014
Negative (n=44)	0.532	0.358-0.912	
Positive (n=102)	1		

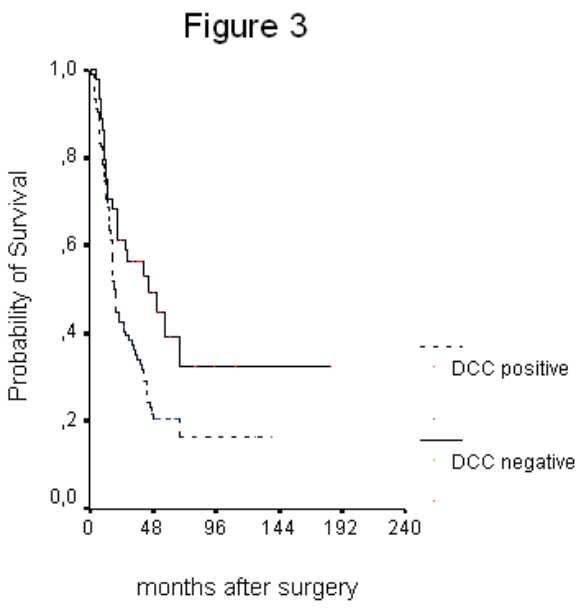
C.I.: confidence intervals; LN: lymph node.

In exploratory analysis, we investigated the interaction of DCC expression with PCNA LI. When patients were stratified according to PCNA LI, lack of DCC expression was a favorable prognostic factor only in the low PCNA LI group (p=0.0412). Nevertheless, when DCC-PCNA LI interaction was included in the multivariate analysis for OS, the interaction term was not significant (p=0.824). In addition, in a multivariate analysis of low PCNA LI group the survival difference between DCC expressors and non-expressors lost its significance (p=0.063).

3.4. Correlation of TS Expression with Survival

The median follow-up of patients included in TS analysis was 54 months (range: 16-183). During follow-up 91 patients died: 90 patients died due to gastric cancer and 1 patient due to heroin overdose. Univariate analysis

Figure 3. Overall survival (OS) after resection of gastric cancer according to DCC expression.



showed presence of metastases at the time of surgery (p<0.0001), T classification (p=0.0014), lymph node involvement (p=0.0208) and positive surgical margins (p=0.0472) were associated with poor prognosis. Five-year OS of patients was similar between patients with low and high intensity of staining (21.79% vs. 23.70%, p=0.4327) and focal and diffuse staining pattern (24.15% vs. 23.05%, p=0.3633) (Figure 4). Patients with no TS staining had significantly longer median OS (68 months) compared to patients who showed any TS expression (20 months) (p=0.0421). Multivariate analysis showed that T classification, lymph node involvement, metastases and absence of TS staining were independent prognostic factors for OS (Table 4). Univariate and multivariate analysis for CSS showed similar results.

When patients were stratified according to the extent of staining, patients with low PCNA LI survived significantly longer than those with high PCNA (p=0.0040) in the group of focal staining pattern while there was no such difference in the group of diffuse staining (p=0.5411). When TS-PCNA LI interaction was included in the multivariate analysis for OS, the interaction term was not significant (p=0.415). Multivariate analysis for the subgroup with focal staining pattern could not be performed because of the small number of cases.

3.5. Patients with curative resection

One hundred and twenty patients underwent curative resection (no macroscopic disease left unresected, negative surgical margins, no metastatic disease). Five-year OS of patients whose tumors showed DCC expression was 21.35% (95% CI: 11.21-31.49), compared to 36.54% (95% CI: 16-57.08) in the group with no expression (p=0.0414) (Figure 5). Multivariate analysis showed that lymph node involvement (p=0.001), T stage (p=0.025) and DCC expression (p=0.040) were independent prognostic factors for CSS. DCC expression showed a strong trend (p=0.058) of independent prognostic significance for OS.

Among the 130 patients who were tested for TS expression, 106 underwent curative resection. There was no correlation of survival with TS intensity (p=0.3489) or staining pattern (p=0.5938). Multivariate analysis for OS and CSS showed that absence of lymph node involvement and absence of TS staining were independent factors of better prognosis (p=0.008 and p=0.038, respectively).

3.6. Correlation of DCC and TS expression with outcome of 5FU treatment

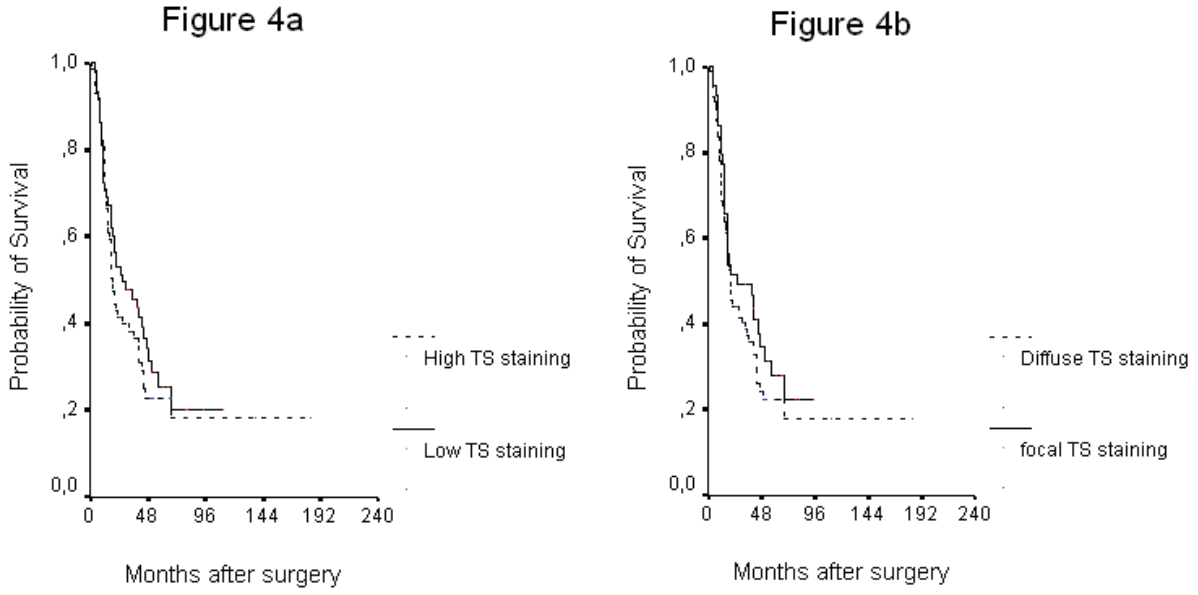
Subgroup analysis among patients who underwent curative resection did not show any significant interaction between DCC or TS expression and treatment with adjuvant 5FU. This was shown for the whole population as well as patients who underwent curative resection.

4. Discussion

This study suggests a prognostic significance of DCC immunohistochemical expression in gastric cancer patients with a high risk of relapse following gastrectomy. This was found not only for the entire population but also for patients who underwent curative resection. This group includes the only patients with any chance of cure and DCC expression might represent a means of selection for adjuvant treatment. Survival analysis showed that 3-year OS (57.5%) of DCC negative patients, who underwent curative resection, compare favorably with those reported recently in patients who received adjuvant chemoradiotherapy [37]. Although this is an indirect comparison, the inclusion criteria in both studies were similar and our results indicate that DCC positive patients is the group that urgently requires improvement of its prognosis by adjuvant treatment.

The reason for the improved survival in patients with loss of DCC expression is unclear. Functional studies of the DCC gene have shown that it is involved in the regulation of differentiation and proliferation of normal cells and it has been speculated that its inactivation may lead to aberrant differentiation and loss of growth control [4] resulting in worse prognosis. Nevertheless, these findings have not always been confirmed in clinical studies. We found higher DCC expression in well

Figure 4. Overall survival (OS) after resection of gastric cancer according to TS intensity of staining (a) and TS staining pattern (b).



differentiated tumors but there was no correlation with proliferative activity, while absence of DCC expression was related with low S-phase fraction in another study [14]. In addition, data regarding its prognostic significance is limited and contradicting, as mentioned in the Introduction [10-15]. These discrepancies may arise from methodological differences (molecular biology techniques vs. immunohistochemistry, polyclonal vs. monoclonal antibodies), but also from the lack of correlation between gene alteration (allelic loss, mutations) and expression of its product. Indeed no mutations of DCC were found in patients with 18q21 LOH, in spite of loss or reduction of protein expression by immunohistochemistry [38]. Furthermore, 18q LOH frequently results in allelic loss of other genes (Smad2 and Smad4) in addition to DCC, as shown in two recent studies [12,15]. The prognostic significance of 18q LOH reported in colorectal cancer may, thus, reflect the prognostic role of the other two genes and not only that of the DCC gene. Finally, the selection criteria may have played a role in our results. Studies in colorectal cancer patients showed that 18q LOH and loss of DCC expression were adverse prognostic factors only in stage II and diploid tumors or tumors with low proliferative activity [10,14]. Our patients were in more advanced stages since we did not include T1N0 or T2N0 tumors, while most of our patients had at least stage III disease (74%). In addition, most of our patients had a high LI compared to the results of other studies using the same method [31,32]. These findings might indicate that the prognostic role of DCC expression is different in more advanced stages or tumors with more aggressive behaviour.

Few studies have addressed the prognostic value of TS in patients undergoing resection of gastric carcinoma. Our results suggest that low TS expression might be a favorable prognostic factor. Focal TS staining was associated with low tumor proliferative activity while patients with no TS negative tumors had significantly longer survival than patients with any expression. Nevertheless, the latter finding should be viewed with caution because of the small number of patients in this group (11 patients, 8.5% of the total). For the same reason this significant difference in survival is of little clinical relevance. Improved survival in patients with low TS activity has been shown in patients with colorectal and breast cancer [21-23,40] and has been suggested for gastric cancer [26,28,29], although lack of correlation has also been reported [24,27]. Our study showed no difference in survival between patients with low and high TS expression. The difference of our results from those of the above mentioned studies could be due to a variety of reasons: different methodology, lack of statistical power of our study or real lack of prognostic significance in this population.

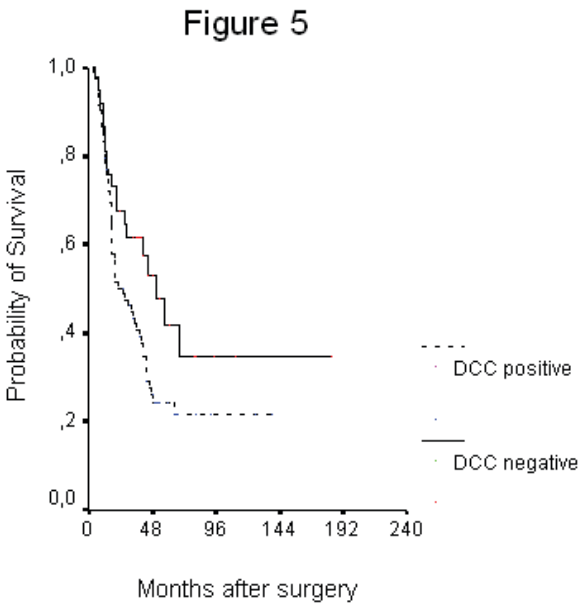
The best way to evaluate TS activity has not been resolved. Immunohistochemistry has been popular because it is easily applied and can be used in archive material. In addition, TS immunostaining using TS106 monoclonal antibody has been shown to correlate TSmRNA levels in gastrointestinal tumors [40]. Intensity of staining (0-3+) and percentage of positive tumor cells (<25% and >25%) have both been used to assess TS protein expression. In our study, both methods yielded

Table 4. Multivariate analysis in relation to overall survival in TS studied patients.

Variable	Hazard ratio	95% C.I.	p
Depth of invasion			0.001
T1 (n=4)	<0.001		
T2 (n=15)	0.250	0.086-0.722	
T3 (n=106)	0.294	0.117-0.738	
T4 (n=5)	1		
LN involvement		0.159-0.845	0.007
No (n=16)	0.367		
Yes (n=114)	1		
Metastases			<0.001
No (n=14)	0.295	0.168-0.514	
Yes (n=116)	1		
Positive margins			0.139
No (n=119)	0.551	0.261-1.161	
Yes (n=11)	1		
DCC staining			0.032
Negative (n=11)	0.448	0.185-0.892	
Positive (n=119)	1		

C.I.: confidence intervals; LN: lymph node.

Figure 5. Overall survival (OS) after curative resection according to DCC expression.



similar results. With identical methodology, our results are similar to those of Choi et al, who have published the only study with patients who had gastrectomy for gastric cancer. Three other studies, which included patients with gastric cancer, have reported survival benefit in patients with low TS expression, but different methods of assessment of TS expression were used [26,28,29]. Our sample may be inadequate to reach statistical significance, since studies, which showed prognostic significance of TS expression, included significantly higher number of patients [21-23,39]. In our study the hazard ratio for low vs. high TS staining intensity in patients who underwent curative resection was 0.738 (95%CI: 0.446-1.221). This could be translated in a clinically relevant survival benefit, which could be detected by a study with adequate power. Nevertheless, survival curves were almost identical, making this assumption unlikely.

Interactions of DCC and TS expression with tumor proliferative activity have been reported previously [14,24]. Our results suggest that DCC expression may be of prognostic significance only in patients with low proliferative activity. Such interaction has also been reported in patients with colorectal adenocarcinoma (14), although lack of DCC expression was an adverse prognostic factor in that study. Our finding should be viewed with caution, since the significance of the interaction was not confirmed by multivariate analysis. We also used a different method to assess proliferative activity. Proliferative tumor activity was higher than expected in our study, as already mentioned, indicating that our low PCNA LI group is not comparable to the <5% S-phase fraction group of the study of Sun et al. Nevertheless, our results along with those of their report further support our previous speculation that the prognostic role of DCC expression might be related to the behaviour of the tumor. We also found that patients with low PCNA LI survived significantly longer in the group with focal TS staining, while no such difference was found in the group with diffuse staining pattern. Low proliferative activity has been associated with better survival in some studies [41], while a different role of ki-67 staining according to TS expression has also been reported [24]. Nevertheless, the number of patients in this group was inadequate for multivariate analysis and therefore, the significance of this finding is not clear.

Our results should be viewed within the context of recent data regarding the role of adjuvant therapies in gastric cancer. We found no correlation between TS expression and outcome after 5FU adjuvant treatment. This is in agreement with a recent study [26] showing no correlation of TS activity with the effect of adjuvant 5FU/Adriamycin chemotherapy in patients with gastric cancer. Two other studies, using different methodology, showed better survival in patients with low TS expression [26,28], while studies in colorectal cancer have shown that patients with high TS expression benefited more than low expressors by adjuvant 5-FU [22,23,39]. It has to be noted that the retrospective non-randomized analysis used in all three mentioned studies as well as ours is not the ideal way of evaluating the prognostic

role of a marker. In addition, the number of patients who received adjuvant treatment in our study is probably too small to detect significant differences. Finally, there is no established benefit from adjuvant 5FU in patients with gastric cancer, as opposed to colorectal cancer, which might explain the lack of any prognostic role of TS expression. On the contrary, a recent randomized study showed a survival benefit by the administration of adjuvant chemoradiotherapy in resected gastric cancer [37]. Unfortunately, no patients in our study received such therapy and only 6 received adjuvant radiotherapy. Therefore, this study offers no information in this respect.

In conclusion, DCC expression seems to be a promising prognostic marker in patients with high risk of relapse after gastric cancer resection. The usefulness of this marker as a selection criterion for adjuvant therapies should be addressed in prospective randomized trials. In contrast, the prognostic value of TS expression in gastric cancer remains questionable. The prognostic value of this marker in certain subgroups of patients and its interaction with adjuvant therapies remains to be defined.

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