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Original Article

The clinical impact of the novel tumor marker DR-70 in unresectable gastric cancer patients

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Abstract

Background: Gastric cancer tumor markers, such as carcinoembryonic antigen (CEA) and cancer antigen 19-9 (CA 19-9), have been applied in clinical practice to screen or monitor treatment responses. However, their sensitivity and specificity are unsatisfactory. Therefore, we assessed the novel tumor marker DR-70 and evaluated its performance in screening and response monitoring.
Methods: The study included newly diagnosed patients with advanced gastric cancer from March 2012 to October 2015. We measured the DR-70, CEA, and CA 19-9 levels at the time of enrollment. The patients subsequently underwent chemotherapy. We followed-up the patients every 3 months; DR-70 levels and abdominal computed tomography scans were re-evaluated and repeated, respectively, at each follow-up. The correlation between treatment response and DR-70 level after chemotherapy was analyzed. The overall survival and progression-free survival rates were also evaluated.
Results: A total of 51 patients with gastric cancer were enrolled. Most (82.4%) had metastatic disease. At enrollment, the sensitivity of DR-70 in our study group was 78.4%, compared with 52.9% and 43.1% for CEA and CA 19-9, respectively. When we used the three tumor markers together, the sensitivity increased to 80.4%. We observed a correlation between treatment response and DR-70 level after chemotherapy. No difference in either overall survival or progression-free survival was observed between the DR-70 positive and negative groups. However, a trend toward poorer overall survival was observed for the high DR-70 group, although this was not statistically significant.
Conclusion: DR-70 is a powerful tool not only for screening unresectable gastric cancer but also for treatment response evaluation.
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Keywords: Chemotherapy; Gastric cancer; Prognosis; Tumor markers

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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1. Introduction

Gastric cancer is one of the most common cancers worldwide, especially in high prevalence regions, such as China, Japan, Korea, and Taiwan. In 2011, 3824 patients were newly diagnosed with gastric cancer in Taiwan, and it ranks seventh among the causes of cancer-related mortality. Some tumor markers, such as carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA 19-9), and carbohydrate antigen 72-4 (CA 72-4), have been used to detect cancer.[1,2] However, the sensitivity was unsatisfactory. The value of these markers for survival prediction and treatment response evaluation was

Table 1
Characteristics of study population.

	All Patients (n = 51)	DR-70 > 1 (−) (n = 40)	DR-70 < 1 (+) (n = 11)	*p*
Age, years				
Median (interquartile range)	63 (32−83)	62.5 (32−79)	63 (55−83)	0.4495
Gender, n (%)				
Male	37 (72.5%)	27 (67.5%)	10 (90.9%)	0.2508
Female	14 (27.5%)	13 (32.5%)	1 (9.1%)	
Performance Status (ECOG), n (%)				
0	15 (29.4%)	9 (22.5%)	6 (54.5%)	0.1039
1	35 (68.6%)	30 (75.0%)	5 (45.5%)	
2	1 (2.0%)	1 (2.5%)	0 (0%)	
Previously Underwent Surgery for Gastric Adenocarcinoma, n (%)				
No	35 (68.6%)	29 (72.5%)	6 (54.5%)	0.2884
Yes	16 (31.4%)	11 (27.5%)	5 (45.5%)	
Disease-Free after Primary Surgery, n (%)				
No	2 (12.5%)	2 (18.2%)	0 (0%)	1.0000
Yes	14 (87.5%)	9 (81.8%)	5 (100%)	
Previously Received Adjuvant Chemotherapy for Gastric Cancer, n (%)				
No	47 (92.2%)	39 (97.5%)	8 (72.7%)	0.0277
Yes	4 (7.8%)	1 (2.5%)	3 (27.3%)	
T Stage, n (%)				
1	2 (3.9%)	2 (5.0%)	0 (0%)	0.7306
2	2 (3.9%)	1 (2.5%)	1 (9.1%)	
3	19 (37.3%)	13 (32.5%)	6 (54.5%)	
4	19 (37.3%)	16 (40.0%)	3 (27.3%)	
4a	7 (13.7%)	6 (15.0%)	1 (9.1%)	
4b	1 (2.0%)	1 (2.5%)	0 (0%)	
x	1 (2.0%)	1 (2.5%)	0 (0%)	
N Stage, n (%)				
0	5 (9.8%)	4 (10.0%)	1 (9.1%)	0.5792
1	9 (17.6%)	5 (12.5%)	4 (36.4%)	
2	9 (17.6%)	7 (17.5%)	2 (18.2%)	
3	21 (41.2%)	17 (42.5%)	4 (36.4%)	
3a	4 (7.8%)	4 (10.0%)	0 (0%)	
3b	3 (5.9%)	3 (7.5%)	0 (0%)	
M Stage, n (%)				
0	9 (17.6%)	6 (15.0%)	3 (27.3%)	0.3849
1	42 (82.4%)	34 (85.0%)	8 (72.7%)	
Histology Type, n (%)				
Adenocarcinoma	46 (90.2%)	36 (90.0%)	10 (90.9%)	0.4631
Adenocarcinoma with Signet-Ring Cell Carcinoma	3 (5.9%)	3 (7.5%)	0 (0%)	
Unspecified	2 (3.9%)	1 (2.5%)	1 (9.1%)	
CEA, n (%)				
Negative	24 (47.1%)	17 (42.5%)	7 (63.6%)	0.3095
Positive	27 (52.9%)	23 (57.5%)	4 (36.4%)	
CA 19-9, n (%)				
Negative	29 (56.9%)	21 (52.5%)	8 (72.7%)	0.3116
Positive	22 (43.1%)	19 (47.5%)	3 (27.3%)	
Site(s) of involvement at registration, n (%)				
Primary Site/Stomach	40 (78.4%)	32 (80.0%)	8 (72.7%)	0.6842
Regional Lymph Nodes	36 (70.6%)	29 (72.5%)	7 (63.6%)	0.7109
Distant Lymph Nodes	32 (62.7%)	25 (62.5%)	7 (63.6%)	1.0000
Peritoneum	17 (33.3%)	16 (40.0%)	1 (9.1%)	0.0751
Lungs	9 (17.6%)	9 (22.5%)	0 (0%)	0.1767
Bone	2 (3.9%)	2 (5.0%)	0 (0%)	1.0000
Liver	20 (39.2%)	16 (40.0%)	4 (36.4%)	1.0000
Others	17 (33.3%)	12 (30%)	5 (45.5%)	0.4719

controversial. Therefore, novel tumor markers are being investigated.

The activity of cancer cells may increase serum proteolytic activity and activate parts of the coagulation cascade. A DR-70 immunoassay was designed to evaluate levels of fibrinogen degradation products. Some previous studies have reported its value in screening cancer of various cancer types.[3–7] The prognostic value and its role in monitoring chemotherapy response have also been discussed in other cancers.[3–7] However, few studies have reported the value of DR-70 in screening for gastric cancer in comparison with well-known markers such as CEA and CA-199. Neither of them focused on the power of monitoring treatment response.

To clarify the clinical power of DR-70, we compared levels of DR-70 and other tumor markers in metastatic gastric patients at the time of diagnosis. We also assessed the ability of DR-70 to predict treatment response after chemotherapy.

2. Methods

2.1. Study population

We studied patients from the investigator-initiated clinical trial TCOG 3211[8]; this included patients with biopsy-proven advanced adenocarcinoma of the stomach in multiple medical centers in Taiwan from March 2012 to October 2015. The initially staging was based on the AJCC staging system. The patient was considered advanced stage either with metastatic disease or with advanced N stage. Patients who received curative surgery with tumor recurrence were also eligible. DR-70 was sampled and examined at the time of enrollment. The AMDL DR-70 assay was performed by enzyme-linked immunosorbent assay. CEA and CA 19-9 were also checked at the same time. These patients then received identical chemotherapy regimens, which began with capecitabine and oxaliplatin. The regimen was shifted to docetaxel and capecitabine if disease progression was observed by imaging studies. Restaging was performed with abdominal CT scan every 3 months. DR-70 was examined again at that time. If the disease had progressed in 3 months, DR-70 was analyzed when disease progression was confirmed by imaging. Docetaxel and capecitabine were then administered. The study was approved by the Institutional Review Board of each participating center or the competent authority and their Ethics Committee. The study was conducted in full accordance with the International Conference on Harmonization Good Clinical Practice guidelines and the Declaration of Helsinki. Written informed consent was obtained from all patients before enrollment.

Table 2
Baseline CEA, CA 19-9, and DR-70 status.

	N	(%)
No. of Enrolled Patients	51	
CEA		
CEA (−)	24	47.1
CEA (+)	27	52.9
CA 19-9		
CA 19-9 (−)	29	56.9
CA 19-9 (+)	22	43.1
DR-70		
> 1 (+)	40	78.4
< 1 (−)	11	21.6
Status of DR-70, CEA, and CA 19-9		
DR-70 (−)+CEA (−)+CA 19-9 (−)	10	19.6
DR-70 (+)/CEA (+)/CA 19-9 (+)	41	80.4
DR-70 (−)/CEA (+)/CA 19-9 (+)	35	68.6

2.2. Method of DR-70 examination

DR-70 was evaluated using the AMDL DR-70 kit, which was developed by Super Religare Laboratories, Kolkata. This enzyme-linked immunosorbent assay utilizes affinity purified rabbit anti-DR-70 polyclonal antibodies. The DR-70 antigen in diluted patient serum (1:200) was captured by the antibodies, and after a wash step, horseradish peroxidase-conjugated antibodies were added to the wells. In the presence of the DR-70 antigen, the horseradish peroxidase-labeled anti-DR-70 antibodies would bind to the captured tumor marker to form an immunological sandwich with the immobilized antibodies. After a second wash step, the enzyme substrate 3,3′,5,5′-tetramethylbenzidine was added to the well. The end-point of the test was read in a micro-plate reader at 450 nm, when the reaction was stopped with 0.1 N HCl. The intensity of color formed during the test proportionally reflects the DR-70 level in serum. Levels were quantified by interpolation from a standard curve using the calibrators provided with the kit.

2.3. Statistical analyses

By observing the results of DR-70, CEA, and CA 19-9 data, we compared the sensitivity of these tumor marker tests for screening gastric cancer patients. We defined disease progression as when the second DR-70 value was elevated by more than 20% from the value measured at the time of enrollment. If the DR-70 was elevated by less than 20% or decreased by less than 20%, we defined it as stable disease. Conversely, if the DR-70 value decreased by more than 20%, it was defined as partial remission. These data were only analyzed if the DR-70 level was above the normal limit at the time of enrollment. Fisher's exact test was used to clarify the correlation between tumor marker response and clinical image response. Overall survival of these patients, stratified by tumor marker levels at the time of enrollment, was also analyzed. All statistical analyses were performed using IBM SPSS statistical software (version 19.0 for Windows; IBM Corp., New York, NY, USA). p values < 0.05 were considered statistically significant.

3. Results

3.1. Clinical characteristics of the study population

During the study period, a total of 51 patients with advanced gastric cancer were enrolled. The patients ranged in age from 32 to 83 years, with a median age of 63 years; 14

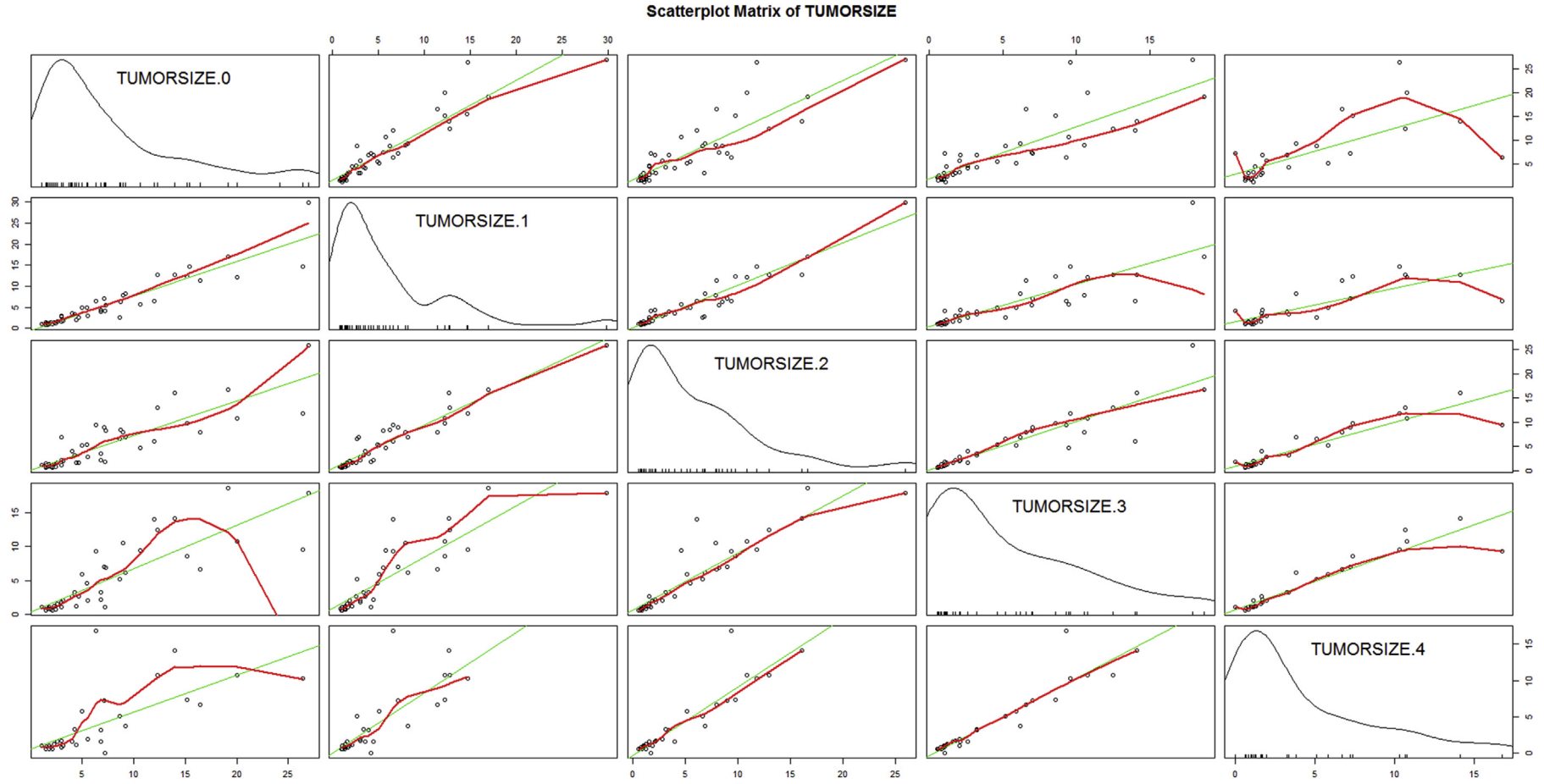

Fig. 1. Scatterplot matrix which showed the trend of positive correlation in DR-70 and tumor size, either in initial visit or every visit.

patients were women, and 37 were men. Four (7.8%) had T1 and T2 stage disease, and 42 (82.4%) had metastatic disease. Most patients (n = 46, 96.1%) were diagnosed with adenocarcinoma. Among them, thirty-five (68.6%) did not undergo surgery. The most common metastatic sites were lymph nodes. The demographic data and characteristics of these gastric cancer patients are shown in Table 1.

3.2. Baseline tumor markers comparison

Table 2 presents the comparison of overall positive rates of each tumor marker (DR-70, CEA, CA 19-9). The overall sensitivity of DR-70, CEA, and CA 19-9 were 78.4%, 52.9%, and 43.1%, respectively. When we integrated all the three tumor markers, the overall positive rate was as high as 80.4%.

3.3. DR-70 level and tumor size

We analyzed the correlation of DR-70 level and the tumor size. There was a trend of positive correlation. The detailed analysis was shown on Fig. 1.

3.4. Treatment response evaluation

The comparison of tumor marker response and clinical image response after treatment is shown in Table 2. Twenty-six patients were both DR-70 responders and RECIST responders. In contrast, four were not responders by either DR-70 or clinical imaging evaluation. Fisher's exact test was performed, which revealed a significant correlation between DR-70 response and clinical RECIST response criteria ($p = 0.0260$).

3.5. Overall survival prediction by tumor markers

Overall survival rates of these patients were stratified and analyzed by tumor marker levels at the time of enrollment. The Kaplan–Meier survival curves of these patients are shown in Figs. 2–4. There were no significant survival differences between positive or negative in these three tumor markers. However, there was a trend for patients with DR-70 > 1 μg/mL to have inferior survival ($p = 0.1029$).

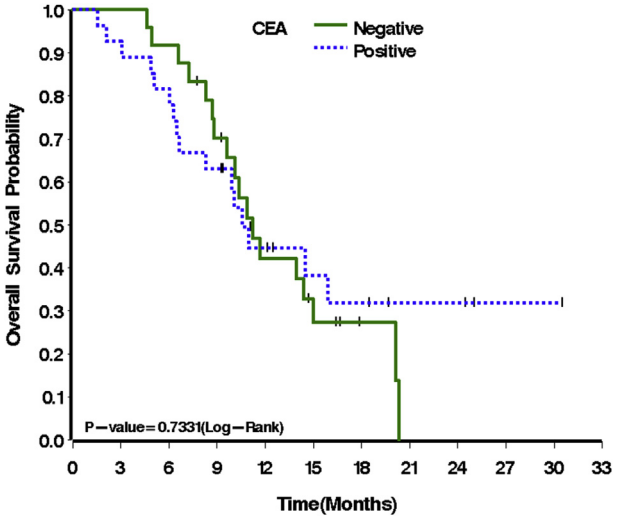

Fig. 3. Kaplan–Meier curves for overall survival and overall survival of 51 advanced gastric cancer patients who were stratified by CEA level.

4. Discussion

The present study is the first to discuss the application of DR-70 in gastric cancer. Our main findings were as follows: (1) compared to the most common tumor markers in gastric cancer, DR-70 had much better overall positive rates; (2) when evaluating treatment response, response based on DR-70 level showed a high correlation with clinical image response; (3) none of the current tumor markers at the time of diagnosis can successfully predict survival, although patients with DR-70 level > 1 tended to have inferior survival.

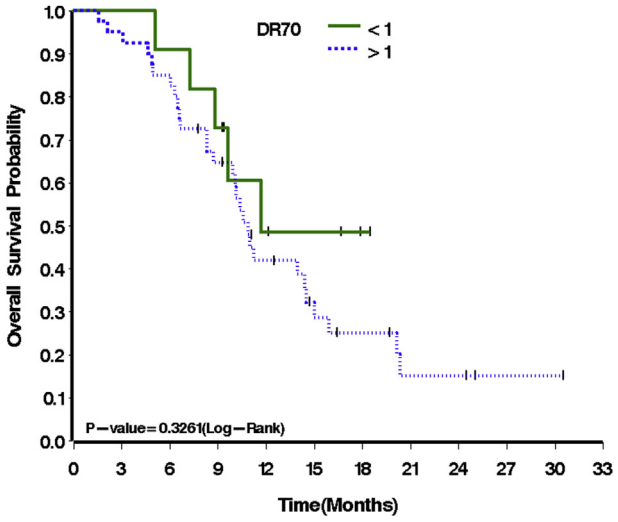

Fig. 2. Kaplan–Meier curves for overall survival and overall survival of 51 advanced gastric cancer patients who were stratified by DR-70 level.

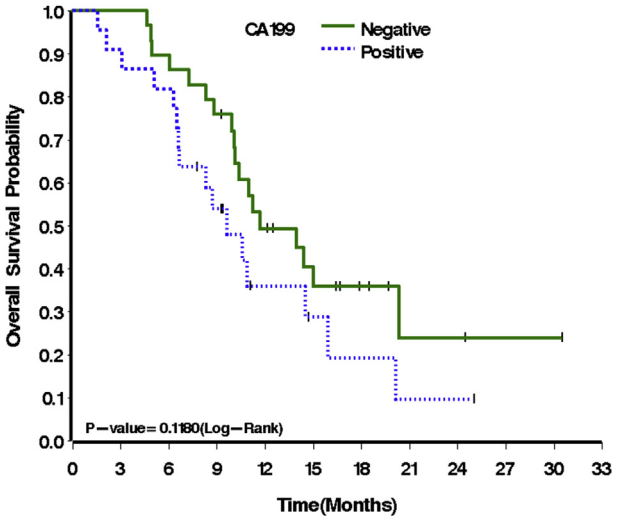

Fig. 4. Kaplan–Meier curves for overall survival and overall survival of 51 advanced gastric cancer patients who were stratified by CA 19-9 level.

Table 3
Correlation assessment of response to chemotherapy between DR-70 and Imaging.

	RECIST Responder, n	RECIST Non-responder, n	Total, n
DR-70 Responder	26	0	26
DR-70 Non-responder	15	4	19
Total	41	4	45

Fisher's exact test; the two-tailed p value equals 0.0260.

In a review of 46 studies by Shimada et al., the overall positive rates for CEA and CA 19-9 were 24.0% and 27.0%, respectively. When analyzing only the patients with stage IV disease, the overall positive rates were 39.5% and 44.7%, respectively.[1] Our positive rates were similar to these findings. Wu et al. proposed a clinical study to detect 13 different cancers in 136 cancer patients, and at a 95% specificity level, the sensitivity of the assay was as high as 92.6%.[3] Another small-scale study that enrolled 10 gastric cancer patients reported a sensitivity of 90.6% with a specificity of 92.08%.[7] However, the population of patients with gastric cancer in this study was limited. In our study, we enrolled 51 patients with advanced gastric cancer, and the overall DR-70 positive rate was much higher than those of conventional tumor markers CEA and CA 19-9.

Previous studies have not demonstrated that CEA and CA 19-9 are good tools to monitor chemotherapy response in advanced gastric cancer.[1,9] Therefore, these assays had minimal roles for response monitoring in advanced gastric cancer chemotherapy.[10−12] On the other hand, we found a significant correlation between the DR-70 response and the response observed by clinical imaging. DR-70 may be a useful tool to follow-up patients receiving chemotherapy and may prevent frequent imaging studies in these patients.

Shimada et al. initiated a study of 663 patients and concluded that preoperative CA 19-9 is a better prognostic factor than CEA in advanced gastric cancer. In contrast, Mihmanli et al. proposed that CEA level is a predictor of prognosis.[13] In the current study, we found only marginal predictive value of these tumor markers. Among them, DR-70 had a more distinct trend to predict the survival of patients before treatment.

Our study had some limitations. First, as this was a prospective trial, our study cohort was limited to patients with advanced gastric cancer and did not reflect a normal population. This may lessen the application for cancer screening in a normal population, which may include some early stage gastric cancer patients. Second, our population was limited, and thus, we observed marginal results in the survival analysis. Finally, CEA and CA 19-9 were not evaluated later in the study, so it was not possible to compare the value of response evaluation between these tumor markers.

In conclusion, compared to commonly used tumor markers of advanced gastric cancer, DR-70 has better overall positive predictive value when screening, has a good correlation with treatment response, and is of borderline prognostic value. Further large-scale studies may be warranted to confirm these findings (see Table 3).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jcma.2018.01.009.

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