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The Prognostic and Predictive Value of DR-70 Immunoassay, A Novel Fibrin-Associated Biomarker, in Patients with Advanced Gastrointestinal Cancers

İleri Evre Gastrointestinal Kanserli Hastalarda Fibrin ile İlişkili Yeni Bir Biyobelirteç Olan Serum DR-70 Düzeyinin Prognostik ve Prediktif Değeri

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ABSTRACT

Aim: DR-70 is a newly developed immunoassay that detects fibrin degradation products in blood. We aimed to evaluate ability of DR-70 in monitoring treatment response in advanced gastrointestinal (GI) cancers.

Materials and Methods: We prospectively enrolled patients with advanced GI cancers treated with different lines of systemic therapies. Imaging studies, DR-70 and conventional tumor markers [carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9] were analyzed at baseline and on the third month of treatment.

Results: A total of 142 patients diagnosed with colorectal (52.1%), esophago-gastric (32.4%) and pancreaticobiliary cancer (15.5%) were enrolled. Most patients were getting first-line treatment (56.3%). Second blood sampling was performed in 57% of patients. Among patients with esophago-gastric cancer, DR-70 response correlated well with treatment response (p=0.007) and low baseline DR-70 level was significantly associated with longer overall survival (p=0.02). There was a positive but weak correlation between pre-treatment DR-70 and CEA levels (p=0.03, r=0.244) in patients with colorectal cancer, while a moderate positive correlation was present between pre-treatment DR-70 and CA 19-9 levels in esophago-gastric and pancreaticobiliary cancers (p=0.01, r=0.402 and p=0.04, r=0.515, respectively). More than 25% reduction in DR-70 concentration was associated with better overall and progression-free survival.

Conclusion: DR-70 is a strong predictor of treatment response and survival, particularly in esophago-gastric cancer.

Keywords: Tumor markers, gastrointestinal cancers, treatment response, biomarker, prognosis

ÖZ

Amaç: DR-70, kandaki fibrin yıkım ürünlerini tespit eden yeni geliştirilmiş bir testtir. Bu çalışmada ileri evre gastrointestinal (GI) kanserlerde DR-70'in tedavi yanıtını izlemedeki etkinliğini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Çalışmaya farklı serilerdeki sistemik tedaviler ile tedavi edilen ileri evre GI kanserli hastalar dahil edildi. Görüntüleme çalışmaları, DR-70 ve geleneksel tümör belirteçleri [karsinoembriyonik antijen (CEA), karbonhidrat antijeni (CA) 19-9] başlangıçta ve tedavinin üçüncü ayında tekrarlandı.

Bulgular: Çalışmaya kolorektal (%52,1), özofagogastrik (%32,4) ve pankreatikobiliyer kanser (%15,5) tanısı konan toplam 142 hasta alındı. Hastaların çoğu birinci basamak tedavi alıyordu (%56,3). Hastaların %57'sinde ikinci kan örneği alındı. Özofagogastrik kanseri olan hastalarda, DR-70 yanıtı tedavi yanıtı ile iyi korelasyon gösterdi (p=0,007) ve başlangıçta düşük serum DR-70 düzeyi, daha uzun genel sağkalım ile anlamlı şekilde ilişkiliydi (p=0,02). Kolorektal kanserli hastalarda tedavi öncesi DR-70 ile CEA düzeyleri arasında pozitif fakat zayıf bir korelasyon (p=0,03, r=0,244) varken,

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tedavi öncesi DR-70 ile CA 19-9 arasında özofagogastrik ve pankreatikobilier kanserlerde orta düzeyde pozitif bir korelasyon vardı (sırasıyla p=0,01, r=0,402 ve p=0,04, r=0,515). DR-70 konsantrasyonunda %25'ten fazla azalma, daha iyi genel ve progresyonsuz sağkalım ile ilişkiliydi.

Sonuç: DR-70, özellikle özofagogastrik kanserde tedaviye yanıtı ve sağkalımı ön gören güçlü bir belirteçtir.

Anahtar Kelimeler: Tümör belirteçleri, gastrointestinal kanserler, tedavi yanıtı, biyobelirteç, prognoz

INTRODUCTION

Recently updated Global Cancer Statistics revealed that gastrointestinal (GI) tract cancers, including colorectal, gastric, liver, pancreatic and esophageal cancers, represent one of the most important public health problems, with an estimated 5 million new cases worldwide[1]. Survival rates are unsatisfyingly low, particularly in advanced stages; thereby discovering effective tools to use in early detection and follow-up period has received much attention over the last years. Serum tumor markers are one of those tools that have screening, diagnostic and monitoring roles[2].

Carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9, CA 125 and alpha-fetoprotein are well-known and routinely used biomarkers shown in the literature with different diagnostic, prognostic and monitoring power[3]. However, controversies still exist regarding the value of these traditional markers for all above-mentioned roles. Therefore, development of new biomarkers that could be easily implemented in routine clinical practice is still of interest to many researchers.

In the presence of cancer, coagulation and fibrinolytic systems are known to be activated regardless of the type of tumor cells. DR-70 immunoassay was developed to detect fibrin and fibrin degradation products (FDPs) in human blood samples[4]. A growing body of literature has evaluated the relationship between FDPs and tumor growth and highlighted that patients with cancer have elevated FDPs in plasma[5-7]. Numerous studies have reported the diagnostic and screening performance of DR-70 immunoassay in different types of cancer[8-15], while only a few have also evaluated its role in prognosis[13,14]. However, only one study focused on the clinical impact of DR-70 on monitoring treatment response[16].

The aim of the present study was to evaluate the clinical efficacy of novel biomarker DR-70 to predict treatment response in metastatic GI cancers. We also investigated the correlation between traditional tumor markers and DR-70 at the time of enrollment. Lastly, the association between baseline DR-70 level and DR-70 change following the treatment and survival outcomes were analyzed.

MATERIALS AND METHODS

Patient Selection

We prospectively enrolled patients with advanced GI cancer at the time of initiating any lines of systemic therapy, after obtaining an informed consent. The study group mainly included patients with colorectal, esophagogastric and pancreaticobiliary cancers. All patients were evaluated with chest and abdominal computed tomography (CT) or ^{18}F-fluorodeoxyglucose positron emission tomography/CT (PET/CT) at the time of admission. Blood samples were collected for both DR-70 examination and other tumor markers such as CEA and CA 19-9 at the same time. Patients then received the treatment of physician's choice for 3 months. At the end of this period, response evaluation was performed based on the Response Evaluation Criteria in Solid Tumors (RECIST) or PET Response Criteria in Solid Tumors (PERCIST) with the identical imaging method previously used. DR-70, CEA and CA 19-9 were also reanalyzed. The study was approved by the Marmara University Faculty of Medicine Clinical Research Ethics Committee (date of approval: 1 June 2018, protocol code: 09.2018.423).

DR-70 Immunoassay

A 5 ml of peripheral blood sample was drawn from each participant. After standing at room temperature for about half an hour, the blood was centrifuged at 1500 rpm for 10 minutes. All serum samples were then frozen and preserved at -80 ^{o}C until DR-70 level was analyzed. Serum concentration of DR-70 (µg/mL) was measured using AMDL DR-70 kits (AMDL, Inc., Tustion, CA, USA) according to the manufacturer's instructions. This is an enzyme-linked immunosorbent assay based serological test that was developed to quantify serum levels of FDPs.

Response Evaluation

We used RECIST (version 1.1) and PERCIST based on the imaging method to evaluate response to the therapy. We categorized patients into two groups, imaging responders and non-responders. Non-responders included patients whose disease progression was confirmed by imaging while responders included patients with complete response, partial response and stable disease. Regarding DR-70 response, we only analyzed patients with DR-70 level above 0.8 µg/mL at the time of admission, as this level was accepted as a threshold to

identify low risk patients for detecting cancer cell in previous studies[4,15]. Since there has been no established percentage change in DR-70 that is associated with disease progression in advanced GI cancer, we used the same threshold defined by Hung et al.[16] and divided patients into two groups, concerning DR-70 change, as follows: more than 20% elevation in DR-70 level defined non-responders, on the other hand remaining were considered as responders.

Statistical Analysis

Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as median (range) for continuous variables, and as percentages for categorical variables. After the normality of the distribution of continuous variables was tested by the Kolmogorov-Smirnov test, the Kruskal-Wallis test and Mann-Whitney U test were used to make inter-group comparisons for parameters that did not indicate a normal distribution. Correlation coefficient and its significance were calculated using the Spearman's rank correlation test. The Fisher's exact test was performed to highlight the relation between DR-70 response and imaging response. Survival analysis was performed with the Kaplan-Meier method and log-rank test. Confidence interval (CI) was selected as 95% and $p<0.05$ was accepted as the level of significance.

RESULTS

Clinical Characteristics of the Study Population

A total of 142 patients with advanced GI cancer were enrolled between July 2018 and January 2019. The median age was 60 (range 30-83) years. The majority of the patients were male (65.5%, 93 of 142 patients). More than half of the patients were evaluated with PET-CT (55%). In total, 3 groups of tumor types were represented among enrolled patients, most commonly colorectal cancer (52.1%), followed by esophagogastric (32.4%) and pancreaticobiliary cancers (15.5%). Most of the patients were enrolled just before first-line treatment (56.3%), the remaining had previously received one or more lines of treatment. Second blood sampling for DR-70 was performed in 81 of patients (57%), the remainder could not be evaluated due to loss of follow-up or death. The characteristics of participants are presented in Table 1.

The Relationship Between DR-70 and Clinical Characteristics

The median DR-70 levels of first and second blood sampling were 1.27 µg/mL (range 0.2-10) and 0.84 µg/mL (range 0.18-10), respectively. There were no significant differences between the median DR-70 levels in terms of tumor type ($p=0.37$), sex ($p=0.32$), and age ($p=0.42$). The number of previous lines of treatment also did not affect DR-70 level ($p=0.25$).

The Correlation Between Pre-treatment Tumor Markers and DR-70

For all study group, there was no correlation between pre-treatment DR-70 and CEA levels ($p=0.12$); however, a weak positive correlation was present between DR-70 and CA 19-9 levels ($p=0.001$, $r=0.287$). Considering tumor subtypes; there was a positive but weak correlation between DR-70 and CEA levels ($p=0.03$, $r=0.244$) in patients with colorectal cancer, while no correlation was seen between DR-70 and CA 19-9 levels ($p=0.16$). Concerning patients with both esophagogastric and pancreaticobiliary cancers, there was no correlation between DR-70 and CEA levels ($p=0.38$ and $p=0.70$, respectively). Nevertheless, a moderate positive correlation was present between DR-70 and CA 19-9 levels ($p=0.01$, $r=0.402$ and $p=0.04$, $r=0.515$, respectively).

Assessment of Treatment Response

Initially, we compared DR-70 response and imaging response after 3 months of therapy in all study group with a baseline DR-70 level higher than 0.8 µg/mL. Among 44 available patients, 25 were both DR-70 and imaging responders. On the contrary, 11 patients were non-responders for both DR-70 and imaging studies. We found a significant correlation between DR-70 response and imaging response based on the RECIST/PERCIST criteria by performing a Fisher's exact test ($p<0.001$). Then, we made the same comparison in subgroups regarding tumor type;

Table 1. Clinical characteristics of study population

	All patients (n=142)
Median age, years (range)	60 (30-83)
Sex, n (%)	
Male	93 (65.5)
Female	49 (34.5)
Tumor type, n (%)	
Colorectal	74 (52.1)
Esophagogastric	46 (32.4)
Pancreaticobiliary	22 (15.5)
Treatment line, n (%)	
1	80 (56.3)
2	35 (24.6)
≥3	27 (19.1)
Median pre-treatment DR-70 level (µg/mL), (min-max)	1.27 (0.20-10)
Colorectal	1.00 (0.20-10)
Esophagogastric	1.79 (0.24-10)
Pancreaticobiliary	1.28 (0.29-10)

min-maks: Minimum-maksimum

a significant correlation between DR-70 and clinical image response was only found in patients with esophagogastric cancer (p=0.007). Table 2 presents the detailed analysis of the correlation between DR-70 and imaging response.

The Association Between Baseline DR-70 and Survival Outcomes

We only analyzed the data of patients treated with first-line therapy for overall survival (OS) outcomes (80 patients). The median pre-treatment DR-70 level was used as cut-off for each tumor type. Only patients with esophagogastric cancer lived significantly longer in the low DR-70 group (14 months, 95% CI: 7.7-20.2) when compared to the high DR-70 group (4 months, 95% CI: 1.0-8.6) (p=0.02). No significant difference was observed in progression free survival (PFS) between low and high DR-70 levels in tumor subtypes. The Kaplan-Meier curves showing OS stratified by DR-70 level in each tumor type are presented in Figure 1.

The Association Between DR-70 Change (ΔDR-70) and Survival Outcomes

We set two different cut-off values for ΔDR-70 after the treatment: ΔDR70 ≥10% decrease and ΔDR70 ≥25% decrease. Among 81 patients with two blood samples, more than 25% reduction in DR-70 was related to significantly longer PFS (8.6 months vs. 5.8 months, p=0.01) irrespective of treatment line. Among 51 patients who received first-line therapy and had two samples of DR-70, more than 25% reduction in DR-70 was found to be associated with significantly longer OS (22.4 months vs. 15.3 months, p=0.03).

DISCUSSION

A close relationship between cancer and thrombosis has been recognized for more than a century. Four-to seven-fold increased risk of thromboembolism has been reported in cancer patients[17]. FDPs are over produced in cancer patients as a result of activation of tumor-induced degradation pathways. The novel tumor marker DR-70 is a polyclonal anti-FDP antibody-based immunoassay, which has been developed to detect the full complement of FDP[4]. This simple, rapid and non-invasive biomarker has been investigated in several trials as a screening and diagnostic tool, and was found to be promising in various malignant tumors such as colorectal, prostate, lung, gastric, tongue and liver[8-14]. However, there seemed to be insufficient data about monitoring role of this promising biomarker.

To the best of our knowledge, the current study is the first to evaluate the monitoring ability of DR-70 immunoassay in different types of advanced GI cancer treated with systemic therapy. Results of our study demonstrated a significant correlation between DR-70 and imaging response only in patients with esophagogastric cancer. This valuable finding is consistent with previous results of Hung et al.[16], which included a total of 51 patients with gastric cancer. Besides showing high sensitivity and specificity, an ideal tumor marker should have the potential to predict treatment response, which might actually save physicians from frequent and unnecessary imaging studies leading to financial toxicity as well as protect patients from waste of time and risk of radiation. DR-70 seems to be a promising marker to be used for treatment response evaluation in patients with esophagogastric cancer.

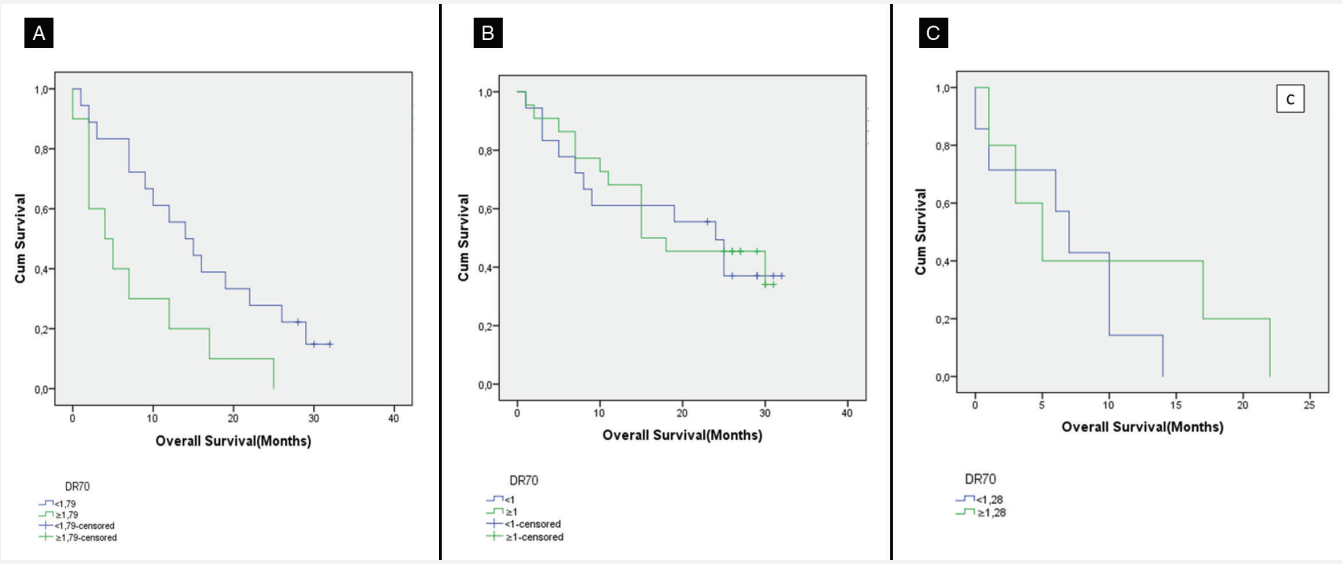

Figure 1. Kaplan-Meier curves showing overall survival stratified by DR-70 level in each tumor type: A) Esophagogastric cancer, B) Colorectal cancer, C) Pancreaticobiliary cancer

Table 2. The correlation between DR-70 and imaging response after treatment				
All group (n=44)	**Imaging responder (n)**	**Imaging non-responder (n)**	**Total**	**p value***
DR-70 responder	25	3	28	
DR-70 non-responder	5	11	16	<0.001
Total	30	14	44	
Colorectal (n=26)	**Imaging responder (n)**	**Imaging non-responder (n)**	**Total**	**p value***
DR-70 responder	14	2	16	
DR-70 non-responder	5	5	10	0.069
Total	19	7	26	
Esophagogastric (n=13)	**Imaging responder (n)**	**Imaging non-responder (n)**	**Total**	**p value***
DR-70 responder	8	1	9	
DR-70 non-responder	0	4	4	0.007
Total	8	5	13	
Pancreaticobiliary (n=5)	**Imaging responder (n)**	**Imaging non-responder (n)**	**Total**	**p value***
DR-70 responder	3	0	3	
DR-70 non-responder	0	2	2	0.10
Total	3	2	5	

*Fisher's exact test

We further investigated the correlation between pre-treatment tumor markers and DR-70. In this context, we examined conventional tumor markers, such as CEA and CA 19-9, which are commonly used in GI cancers. In our findings, there was a positive but weak correlation between DR-70 and CEA levels in patients with colorectal cancer, while a moderate positive correlation was present between DR-70 and CA 19-9 levels in both esophagogastric and pancreaticobiliary cancers. Previous studies suggested to use DR-70 in combination with CEA and CA 19-9 to increase the sensitivity in patients with gastric cancer[11,16].

The prognostic performance of DR-70 was discussed only in few studies. Lin et al.[14] showed a good correlation between DR-70 level and OS in patients with hepatocellular carcinoma. The concentration of DR-70 in serum was also found to be significantly associated with 3-year survival in patients with tongue carcinoma[13]. On the other hand, no significant difference in either OS or PFS was observed between high or low DR-70 in patients with gastric cancer[16]. We analyzed the data of 80 patients treated with first-line therapy for OS outcomes and our results significantly differed from the findings of Hung et al.[16]. We only found a significant difference between the low and high DR-70 groups in terms of OS in esophagogastric cancer. There was no difference between the groups regarding PFS.

We also analyzed the association between DR-70 change during treatment and survival outcomes. More than 25% reduction in DR-70 concentration was found to be associated with longer OS and PFS. The utility of ΔDR-70 was thus highlighted for the first time.

Finally, a number of potential limitations need to be considered. First of all, in the present study, approximately 1 out of 3 patients who were actually considered to have a life expectancy more than 3 months died within this period. Therefore, second blood sampling could not be obtained from this group of patients in addition to the patients who were lost to follow-up. This unexpected situation unfortunately led to decreased number of samples, which may negatively affect statistical analyses. Second, we included patients with advanced GI cancer in different treatment lines which caused actually a heterogeneous group; however, we only analyzed patients treated with first-line therapy for OS outcomes to overcome this bias. Third, since there have been no established thresholds for DR-70 in different types of cancers, we used median levels or previously defined cut-off values for detecting cancer cell in the literature.

CONCLUSION

We conducted this pilot study to provide preliminary evidence on the clinical efficacy of the DR-70 immunoassay in different types of advanced GI cancers. DR-70 seems to be a good candidate to be used as a tumor marker in advanced esophagogastric cancer. The immunoassay correlates well with treatment response and OS. However, further large-scale studies are needed to confirm our findings.

Ethics

Ethics Committee Approval: The study was approved by the Marmara University Faculty of Medicine Clinical Research Ethics Committee (date of approval: 1 June 2018, protocol code: 09.2018.423).

Informed Consent: Informed consent form was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.A.T., N.A.B., Ö.A., R.H., S.K., T.B., F.D., Concept: T.A.T., N.A.B., P.F.Y., Design: T.A.T., N.A.B., P.F.Y., Data Collection or Processing: T.A.T., S.H., E.T.Ş., Analysis or Interpretation: Ö.A., M.A.Ö., Literature Search: T.A.T., Ö.E., Writing: T.A.T.

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