

## RESEARCH ARTICLE

# Treatment of Metastatic Colorectal Cancer With or Without Bevacizumab: Can the Neutrophil/Lymphocyte Ratio Predict the Efficiency of Bevacizumab?

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### Abstract

**Background:** The purpose of this study was to analyze the predictive value of neutrophil/lymphocyte ratio (NLR) to better clarify which patient groups will benefit the most from particular treatments like bevacizumab. **Materials and Methods:** A total of 245 treatment-naive metastatic colorectal cancer (mCRC) patients were retrospectively enrolled and divided into 2 groups: 145 group A patients were treated with chemotherapy in combination with bevacizumab, and 100 group B patients were treated as above without bevacizumab. **Results:** Group A patients had better median overall survival (OS) and progression-free survival (PFS) (24.0 and 9.0 months) than group B patients (20 and 6.0 months) ( $p=0.033$ ;  $p=0.015$ ). In patients with low NLR, OS and PFS were significantly longer in group A patients (27 vs 18 months,  $p=0.001$ ; 11 vs 7 months,  $p=0.017$ ). **Conclusions:** We conclude that NLR, a basal cancer related inflammation marker, is associated with the resistance to bevacizumab-based treatments in mCRC patients.

**Keywords:** Metastatic colorectal cancer - bevacizumab - neutrophil to lymphocyte ratio - response to therapy

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### Introduction

For several years, the standard treatment for metastatic colorectal cancer (mCRC) patients, has been the addition of irinotecan, oxaliplatin, or both to 5-fluorouracil (5-FU)-based chemotherapy (Ducreux et al., 2007). Recently, several phase III trials reported that the addition of targeted therapies to combination chemotherapy increased the treatment efficacy without increasing overall toxicity substantially. As such, the prognosis of mCRC has improved markedly with combination chemotherapy plus molecular agents (Saltz et al., 2008). Additionally, because of the heterogeneity of mCRC patients, personalized treatments came into prominence. For this reason, predictive markers became very popular as the response to treatments varies from patient to patient.

Bevacizumab is a humanized IgG1 monoclonal antibody that selectively binds to and neutralizes the biologic activity of human VEGF-A. For mCRC patients, bevacizumab is very active and improves outcomes when used with a variety of first-line and second-line regimens (Giantonio et al., 2007; Fuchs et al., 2008). Against this data, there are also studies which showed that bevacizumab

did not improve overall survival when added to standard chemotherapy (Stathopoulos et al., 2010; Price et al., 2012). In NO19666 study, only the subgroup analysis demonstrated an improvement in progression free survival of mCRC patients that were treated by the addition of bevacizumab to XELOX (capecitabine+oxaliplatin) (Saltz et al., 2009). There are no other randomized trials comparing the outcomes between chemotherapy and chemotherapy plus bevacizumab. Another problem with bevacizumab is the absence of the clinically available predictive marker. Despite nearly a decade of experience with bevacizumab, important questions remain regarding its optimal use, ideal patient population, and predictive biomarkers.

Owing to two major problems, there are doubts in the first line usage of bevacizumab in mCRC patients. Moreover, the morbidity and cost-effectiveness of this drug is also a problematic issue. The objective of this study was to evaluate the efficiency of bevacizumab in the first line treatment of mCRC patients when added to standard combination chemotherapy and to analyze the predictive role of clinicopathologic and biochemical parameters, including the neutrophil/lymphocyte ratio,

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shown to be relevant as a diagnostic or prognostic factors for many cancers (Unal et al., 2013; Zheng 2013; Cihan et al., 2014; Gunduz et al., 2014; Kacan et al., 2014; Kemal et al., 2014; Ozdemir et al., 2014).

## Materials and Methods

### Patients

The data of 245 patients diagnosed with metastatic colorectal adenocarcinoma and presenting at the Medical Oncology Outpatient Clinic of Izmir Katip Celebi University Atatürk Training and Research Hospital between January 2006 and January 2012 were evaluated retrospectively. All patients had a measurable metastatic lesion in at least one area. Eastern Cooperative Oncology Group (ECOG) performance status was between 0 and 2. Age, sex, date of diagnosis and metastasis, initial stage, localization of the tumor, histological data, site of metastasis and all subsequent treatments were registered from medical records. Pretreatment levels of neutrophil, lymphocyte and carcinoembryonic antigen (CEA) and their levels after the assessment of chemotherapy response were recorded. Patients with active infection (high fever, classical symptoms and signs of the infection in the systems like upper and lower respiratory system, urinary system etc, identification of the microorganisms in cultures of serous effusions and radiologic signs of the infection), active bleeding, blood transfusion within the last three months, chronic inflammatory or autoimmune disease and steroid treatment were excluded from the study.

### Neutrophil /lymphocyte ratio (NLR) and CEA

NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count. Cut-off levels of NLR and CEA was determined according to receiver operating characteristic (ROC) curve analysis. Additionally,  $\geq 50\%$  decrease in CEA level after the assessment of chemotherapy response with respect to the level at diagnosis was measured in every patient.

### Treatment plan

The study included 245 mCRC patients who had completed their first line treatments. 145 patients treated with chemotherapy in combination with bevacizumab (Group A) while 100 patients treated with chemotherapy only (Group B). Both group A and B patients received FOLFOX or XELOX or XELIRI or FOLFIRI regimens, together with or without bevacizumab regimens which were all standardized according to previous studies (de Gramont et al., 2000; Saltz et al., 2008; Pectasides et al., 2012; Uygun et al., 2013). Actual dosing of drugs given to patients evaluated in this study were Oxaliplatin 100-130 mg/m<sup>2</sup> or Irinotecan 180 mg/m<sup>2</sup>, Leucovorin 400 mg/m<sup>2</sup>, Capecitabine 1250 mg/m<sup>2</sup>, 5-FU 400 mg/m<sup>2</sup> iv bolus, 5-FU 2400 mg/m<sup>2</sup> 24 hour infusion with a chemotherapy infusion pump and bevacizumab 5 mg/kg every two weeks or 7.5 mg/kg every three weeks. In each arm, schedule of 5-FU (oral or infusional), irinotecan or oxaliplatin dose intensities with versus without bevacizumab were comparable.

### Response evaluation and toxicity

Tumor response was assessed according to the response evaluation criteria in solid tumors (RECIST 1.1), regarding complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). The investigated endpoint was TTP, which was defined as the period of time from the start date of chemotherapy to the first documentation of progression. First documentation of progressive disease (PD) was based on the definition of PD in the RECIST guidelines; the investigator's clinical judgment of PD or death as a result of any cause in the absence of previously documented PD (Eisenhauer et al., 2009). Patients were also evaluated for hematological and non-hematological toxicities and were graded according to the National Cancer Institute (NCI) Common Toxicity Criteria, version 3.0.

### Statistical analysis

All statistical analyses were performed using SPSS version 19.0 for Windows (Statistical Package for Social Sciences, Chicago, IL). P values  $< 0.05$  were considered statistically significant. Survival probability was calculated using the product limit method of Kaplan Meier. Differences in survival between groups were determined using the log-rank test. ROC curves were used to determine the cut-off values of hematological parameters and tumor marker. The effect of each significant predictor identified via univariate analysis was assessed via multivariate analysis using Cox's proportional hazards model.

## Results

### Response evaluation

There were 154 (62.9%) male and 91 (37.1%) female patients. The median age of all patients was 60 (range, 21-80) years. The median duration of therapy was 28 weeks in the group A and 26 weeks in the group B. In group A, 88 patients (60.7%) achieved an objective response (complete response, 9.7%; partial response, 51.0%), 27 patients (18.6%) had stable disease and 30 (20.7%) progressive disease. In group B, 43 patients (43.0%) responded (complete response, 5%; partial response, 38%), 21 patients (21.0%) had stable disease and 36 (36.0%) progressive disease. Objective response rate was statistically significant between the group A and group B ( $p=0.006$ ). The response rates of both groups are shown in Table 1.

### Efficacy

After a median follow-up of 32 months (range 2-72), 128 patients (88.3%) progressed and 82 (56.6%) died in group A, while 92 patients (92.0%) progressed and 72

**Table 1. Response Rates According to Groups**

	Group A n (%)	Group B n (%)	p
Complete Response	14 (9.7)	5 (5.0)	0.228*
Partial Response	74 (51.0)	38 (38.0)	<b>0.044**</b>
Stable Disease	27 (18.6)	21 (21.0)	0.744**
Progressive Disease	30 (20.7)	36 (36.0)	<b>0.005*</b>
Objective Response (Complete+Partial)	88 (60.7)	43 (43.0)	<b>0.006*</b>

\*Pearson Chi-Square \*\*Fisher Exact Test

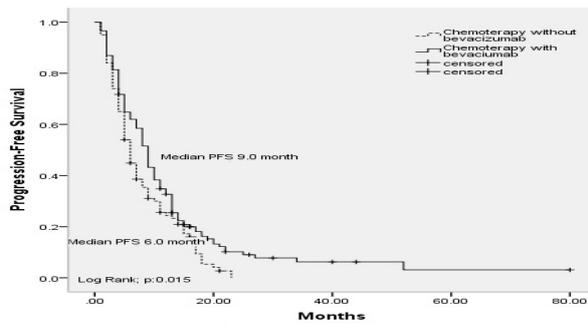


Figure 1. Kaplan-Meier Estimates for Progression-free Survival

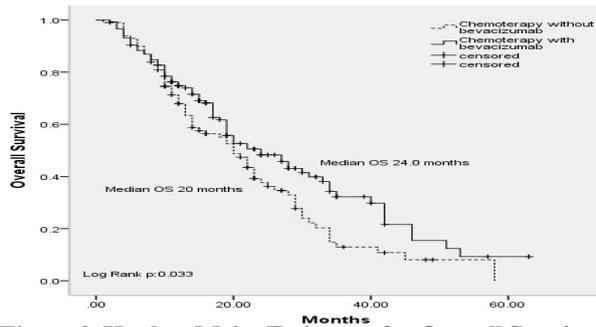


Figure 2. Kaplan-Meier Estimates for Overall Survival

Table 2. Efficacy of Treatment Subgroups

	Irinotecan-based		Oxaliplatin-based	
	CT plus Beva*	CT	CT plus Beva*	CT
No. of patients	112	57	33	43
Median PFS (months)	9	6	6	6
HR (95% CI)	0.68 (0.48-0.95)		0.84 (0.52-1.38)	
**p value	0.027		0.509	
Median OS (months)	24	20	19	19
HR (95% CI)	0.62 (0.41-0.93)		0.78 (0.43-1.41)	
**p value	0.021		0.415	

\*Beva; Bevacizumab; \*\*p values <0.05 were considered statistically significant; \*\*\*CT; Chemotherapy

Table 3. Clinicopathologic Characteristics Predictive for Progression Free Survival and Overall Survival According to Groups

Variable		PFS			OS		
		Group A Month (95%CI)	Group B Month (95%CI)	p*	Group A Month (95%CI)	Group B Month (95%CI)	p*
Age	≥ 65 (38/52)	11 (8.5-13.4)	7 (5.0-8.9)	0.02	31 (19.7-42.2)	15 (8.0-21.9)	0.002
	< 65 (104/48)	9 (7.3-10.6)	8 (4.2-11.7)	0.565	22 (15.6-28.3)	19 (12.6-25.3)	0.792
Gender	Female (53/38)	10 (8.4-11.5)	7 (5.0-8.9)	0.08	26 (12.9-39.0)	17 (11.9-22.0)	0.072
	Male (92/62)	9 (7.3-10.6)	7 (4.7-9.2)	0.572	21 (14.8-27.1)	21 (13.5-28.4)	0.137
Only Pulmonary Metastases (15/7)		15 (11.2-18.7)	7 (4.47-9.23)	0.377	34 (29.2-38.7)	21 (18.6-23.4)	0.126
Only Hepatic Metastases (31/17)		9 (2.98-7.0)	5 (6.4-11.5)	0.214	22 (13.6-30.3)	20 (2.6-37.3)	0.361
Only Hepatic and Lung Metastases (19/18)		12 (6.3-17.6)	8 (3.8-12.1)	0.137	23 (16.3-29.6)	19 (13.1-24.8)	0.237
Other Metastases (25/20)		11 (8.9-13.0)	10 (1.8-18.1)	0.648	21 (15.7-26.2)	16 (11.5-20.4)	0.257
	Metastases no.						
	≤2 (107/78)	10 (8.2-11.7)	7 (4.9-9.0)	0.148	30 (22.2-37.7)	21 (17.3-24.6)	0.015
	>2 (38/22)	9 (7.0-10.9)	7 (5.8-8.1)	0.75	19 (16.4-21.5)	12 (6.1-17.8)	0.514
Primary tumor localization	Rectum (50/41)	10 (7.3-12.6)	8 (6.0-9.9)	0.036	26 (11.7-33.2)	22 (14.2-29.7)	0.19
	Colon (83/51)	10 (8.7-11.2)	7 (5.6-8.3)	0.66	23 (16.0-29.9)	17 (10.3-23.6)	0.11
NLR	Junction (11/7)	9 (5.8-12.1)	11 (5.8-16)	0.84	17 (14.1-19.8)	21 (5.6-36.3)	0.63
	<3.5 (102/68)	11 (8.59-13.4)	7 (5.1-8.8)	0.017	27 (20.6-33.3)	18 (11.9-24.0)	0.001
	<1.5 (30/11)	9 (7.47-10.5)	8 (3.14-12.8)	0.661	28 (16.8-39.1)	15 (2.44-30.02)	0.193
	≥1.5 NLR <2.5 (47/30)	13 (10.9-15.06)	7 (6.14-7.8)	0.045	33 (23.4-42.5)	20 (14.0-25.9)	0.004
	≥2.5 NLR <3.5 (23/27)	10 (6.4-13.5)	6 (4.3-7.6)	0.048	20 (10.7-29.2)	19 (14.0-23.9)	0.251
	≥3.5 (39/30)	6 (4.48-7.5)	8 (5.4-10.5)	0.276	11 (5.6-16.3)	22 (13.2-30.7)	0.047
CEA	<18.4 (53/45)	9 (5.5-12.4)	7 (5.7-8.2)	0.156	30 (14.9-45.0)	22 (16.0-27.9)	0.806
	≥18.4 (62/41)	9 (6.8-11.1)	6 (2.9-11.0)	0.703	17 (14.1-19.8)	13 (10.6-15.3)	0.23
CEA yanıtı	≥50% (44/27)	11 (8.2-13.7)	9 (5.0-12.9)	0.41	27 (17.1-36.8)	22(18.6-25.3)	0.189
	<50% (39/39)	9 (4.1-13.8)	6 (4.3-7.6)	0.147	17 (4.1-29.8)	12 (5.0-18.9)	0.254

\*p: Long Rank analysis

(72.0%) died in group B. Median PFS was 9.0 months (95% confidence intervals [CI]: 7.94-10.05) in group A and 6.0 months (95%CI: 5.03-6.96) in group B (Log rank; p=0.015). Median OS was 24.0 months (95%CI: 17.39-30.60) in group A and 20.0 months (95%CI: 16.40-23.59) in group B (Log rank; p=0.033). Kaplan-Meier curves for OS and PFS according to group A-B are shown in Figure 1-2.

In subgroup analysis, the benefit of the addition of bevacizumab to standard chemotherapy in terms of PFS and OS was assessed for each chemotherapy regimen (oxaliplatin-based or irinotecan-based). Statistical superiority in terms of PFS in group A versus group B was evident in the irinotecan-based treatment subgroup (HR:0.68; % 95 CI, 0.48-0.95; p=0.027), but did not reach the significance level in the oxaliplatin-based treatment subgroup (HR:0.84; 95%CI, 0.52-1.38; p=0.509). Similarly, when bevacizumab was added to irinotecan-based treatment, median OS was statistically superior (HR:0.62, 95%CI 0.41-0.93, p=0.021) than oxaliplatin-based treatment subgroup. Use of second-line therapies in group A was 64.2% compared with 54.0% in the group B (Table 2).

#### Safety

The overall safety evaluation for patients in the group A and the concurrently enrolled patients in the group B is summarized. Hematologic toxicity (neutropenia and thrombocytopenia) was observed and was similar in patients of both arms. Hypertension were increased in the group A patients compared with the group B patients (for grade 1-2, p=0.003; for grade 3-4, p=0.001). However, no difference was seen in incidence of venous thrombosis and grade 3-4 bleeding. Arterial thrombotic events (myocardial infarction, stroke, or peripheral arterial thrombotic event) occurred in 2 patients in the group A; compared with

one patient in the group B. Bowel perforation was seen in two patients of group A. No patient was died due to these adverse effects. Vomiting, diarrhea, weakness, oral mucositis and neuropathy were similar in both groups.

#### *Clinicopathologic factors*

In both groups, predictive role of clinicopathologic factors in mCRC patients were analyzed (Table 3). We found that, treatment with bevacizumab in mCRC patients older than 65 had superior outcomes in terms of both PFS and OS ( $p=0.02$ ,  $p=0.002$ ). In group A patients, who had  $\leq 2$  sites of metastasis, treatment with bevacizumab provided an advantage only in the outcome of overall survival ( $p=0.015$ ). In group A patients, who had  $> 2$  sites of metastasis, there is no outcome difference between groups in terms of both OS and PFS. Patients with rectum localization had better PFS if they treated with bevacizumab-based treatments ( $p=0.036$ ), but no difference in OS. Interestingly, patients with rectosigmoid localization, although statistically not significant, had better PFS and OS if they treated with bevacizumab based treatments.

#### *NLR and CEA levels*

Pretreatment median NLR value of all patients was 2.51 (range: 0.62- 27.2). Patients were divided into 2 groups according to high NLR ( $\geq$  cut-off 3.5,  $n:75$ , 30.6%) or low NLR ( $<$  cut-off 3.5,  $n:170$ , 69.4%) values. When we compared the outcomes of the group A and B patients with low NLR, we found that group A patients had better PFS and OS (11 vs 7 months,  $p=0.017$ ; 27 vs 18 months,  $p=0.001$ ). However, when we compared the outcomes of group A and B patients with high NLR, we found that group A patients had significantly shorter OS (11 vs 22 months,  $p=0.047$ ) and shorter PFS (6 vs 8 months,  $p=0.276$ ) which was statistically non-significant.

Pretreatment cut-off value for CEA level was 18.4. Patients were divided into 2 groups according to high CEA ( $\geq 18.4$ ,  $n:103$ ) and low CEA ( $<18.4$ ,  $n:98$ ) levels. When we compared the outcomes of the group A and B patients with either low or high CEA levels, no difference was found in terms of PFS and OS. When we measured serum CEA levels after the response evaluation which was grouped as  $<50\%$  or  $\geq 50\%$ , there was also no difference between the treatment groups in terms of survival. Survival of the patients in both groups was not different in terms of gender and metastasis localization.

## **Discussion**

The integration of oxaliplatin and irinotecan chemotherapies in the treatment of patients with advanced colorectal cancer has improved median survival in a meaningful way. The recent administration of molecular agents such as bevacizumab in addition to chemotherapeutic agents provided better response rates and further significant improvements in survival (Algire et al., 1945; Ferrara et al., 2005). However, unlike EGFR-targeted therapies no clinical or biological factors clearly predictive of response or resistance to bevacizumab treatment have been identified.

The results from randomized phase III clinical trials conducted across the world showed that when bevacizumab was added to chemotherapies in the first line treatment, PFS and OS of mCRC patients was 8.6-10.6 months and 20.3-25.9 months respectively (Hurwitz et al., 2004; Fuchs et al., 2007; Saltz et al., 2008; Van Cutsem et al., 2009; Stathopoulos et al., 2010). In our study, median PFS and OS of group A patients were 9 months and 24 months which were compatible with the previous publications. There is only one trial which compared the efficiency of chemotherapy with or without bevacizumab in mCRC patients (Saltz et al., 2008). Only PFS improvement was observed when bevacizumab was added to oxaliplatin-based regimens (XELOX or FOLFOX) while no benefit was observed in OS. (FOLFOX/XELOX 8.0 months vs FOLFOX/XELOX+Bevacizumab 9.4 months,  $p=0.0023$ ). In subgroup analysis of this study, improvement in PFS was detected to be due to the improved PFS in patients who had XELOX regimen (XELOX 7.4 month vs XELOX+Bevacizumab 9.3 month,  $p=0.0026$ ). Overall survival differences did not reach statistical significance by the addition of bevacizumab. In our study, when we analyzed the both groups, we found that the addition of bevacizumab to both irinotecan and oxaliplatin-based chemotherapies significantly improved PFS and OS in this first-line treatment of patients with mCRC (Group A 9 months vs Group B 6 months; and  $p=0.015$ ; Group A 24 months vs Group B 20 months;  $p=0.033$  respectively). In subgroup analysis, the addition of bevacizumab to oxaliplatin-based chemotherapy did not improve PFS and OS while there is an improvement in PFS and OS in patients who were treated by irinotecan-based chemotherapy combined with bevacizumab ( $p=0.027$ ,  $p=0.021$ ). Mocado et al. (2012) made a wide search of randomized clinical trials using bevacizumab in first-line metastatic colorectal cancer and observed its effectiveness in limited subsets as bolus fluorouracil, capecitabine-regimens, and in combination with irinotecan. They indicated that, available data are insufficient to reach a conclusion about the optimum dosage of bevacizumab and its contribution to infusional regimens (FOLFOX and FOLFIRI) because of the high heterogeneity among studies. In E3200 study, they determined the effect of bevacizumab (at 10 mg/kg) in addition to oxaliplatin-based chemotherapy on survival duration for patients with previously treated mCRC (Giantonio et al., 2007). But, as E3200 study was a second-line study, we did not know if bevacizumab combined with chemotherapy was active and improved survival in previously untreated mCRC at dosage of 10 mg/kg.

In NO16966 trial, response rate was not improved by the addition of bevacizumab to oxaliplatin-based chemotherapy in this first-line trial in patients with mCRC (47% vs 29%) (Saltz et al., 2008). Stathopoulos et al. (2010) observed no statistically significant difference between the bevacizumab containing and not containing arms regarding the response rate (partial response; 36.8% and 35.2% respectively). In another trial evaluating bevacizumab combination with IFL regimen for mCRC patients, demonstrated a better overall response rate (ORR) which was not statistically significant (40% vs

37%;  $p=0.055$ ) (Hurwitz et al., 2005). In TREE-1 study, only chemotherapy was used and ORR was 20-41%. In TREE-2 study, bevacizumab was combined with these regimens and ORR was 39-52% (Hochster et al., 2008). In this study, partial response and ORR were statistically higher in bevacizumab containing group (PR 51% vs 38%,  $p=0.044$ ; objective response rate 60.7% vs 43%,  $p=0.006$ ). Fewer patients in bevacizumab containing group had progressive disease (20.7% vs 36.0%,  $p=0.005$ ) (Hochster et al., 2008).

Bevacizumab-associated adverse events were hypertension, bleeding, venous and arterial thromboembolic events and gastrointestinal (GI) perforation. Thromboembolic complications and perforations were life threatening adverse effects and required emergent intervention. Increased risk of bevacizumab-induced grade 3-4 hypertension was demonstrated by Hurwitz et al. (2004) and Kabbavar et al. (2005) [11% (control 2%) and 16% (control 3%)]. BEAT study which evaluated the safety and efficacy of bevacizumab plus first-line chemotherapy, grade 3-4 hypertension was reported as 5%. This result was not compared with the control group because of the absence of control group in BEAT study. In this study, grade 3-4 hypertension was significantly higher in group A patients (4.1% vs 0%,  $p=0.003$ ). In BRIT and BEAT trials; arterial thromboembolic events in 2% versus 1%, grade 3/4 bleeding in 3% versus 2% and GI perforation in 2% were reported respectively. These bevacizumab-associated adverse effects were prominently but non-significantly increased in group A patients (Group A vs Group B; bleeding 2.1% vs 1%, GI perforation 1.4% vs 0%, arterial thromboembolic events 1.4% vs 1%, venous thromboembolic events 2.8% vs 1%). Other grade 3-4 hematologic and non-hematologic adverse effects were similar between group A and group B patients.

Recently, there is an increasing data about NLR which was defined as a host inflammatory response. NLR seems to correlate with the prognosis of cancer patients (Wang et al., 2011; Cedrés et al., 2012; Ishizuka et al., 2012; Proctor et al., 2012). Botta et al firstly demonstrated the resistance to bevacizumab treatment in lung cancer patients with high NLR (Botta et al., 2013). They documented that high NLR was associated with shorter progression-free survival and overall survival in bevacizumab treated patients. (9.0 vs 7.0 months, HR:0.39,  $p=0.002$ ; and 20.0 vs 12.0 months, HR:0.29,  $p<0.001$  respectively). This result is exactly similar to our results. Because, we found out bevacizumab resistance in mCRC patients with high NLR and, longer PFS and OS in bevacizumab treated mCRC patients with low NLR. (11 vs 7 months,  $p=0.017$ ; and 27 vs 18 months,  $p=0.001$  respectively). Bevacizumab treated mCRC patients with high NLR had longer PFS and statistically non-significant OS (6 vs 8 months,  $p=0.276$ ; and 11 vs 22 months,  $p=0.0047$ ). There are other studies investigating the pretreatment inflammatory and proinflammatory serum markers and their association with the resistance to bevacizumab-containing regimens (Abajo et al., 2012; Gyanchandani et al., 2013). Besides, this is the first trial in literature evaluating the impact of NLR in mCRC patients treated with bevacizumab-based treatments. The

primary analysis of ASCENT trial will test the association between NLR and progression free survival in mCRC patients treated with bevacizumab. The results of this study will clarify the role of NLR in patients with previously untreated mCRC patients receiving bevacizumab-based first- and second-line treatment (Clarke et al., 2013).

In our study, we also investigated the other possible parameters that could predict bevacizumab efficiency. We could not demonstrate a significant predictive value of CEA levels either at diagnosis or at the response evaluation. Formica et al. found a similar result in mCRC patients when they evaluated the predictive value of CEA levels (Formica et al., 2009). We also evaluated the efficiency of bevacizumab in patients older than 65 and detected a superior outcome in terms of both PFS and OS (11 vs 7 months,  $p=0.02$ ; and 31 vs 15 months,  $p=0.002$  respectively). In patients who had  $\leq 2$  sites of metastasis, bevacizumab treatment provided an advantage only in the outcome of overall survival ( $p=0.015$ ). In patients who had  $> 2$  sites of metastasis, there is no difference between groups in terms of both OS and PFS. Patients with rectum localization had better PFS if they treated with bevacizumab-based treatments ( $p=0.036$ ), but no difference in OS. Survival of the patients in both groups was not different in terms of gender and metastasis localization.

In conclusion, we found a RR, PFS and OS benefit in favor of bevacizumab when it was added to chemotherapy in the first line treatment of mCRC. Subgroup analyses supported the survival advantage with bevacizumab restricted to irinotecan-based regimens. Grade 3-4 hypertension was more common with bevacizumab treatment, but there is no increase in the risk of other adverse effects. So, no new safety concerns were observed in this trial and the safety data were consistent with the known safety profile established in previous publications. The present study firstly demonstrated the correlation between NLR and the efficiency of bevacizumab-based treatment in mCRC patients. These results clearly emphasized that high NLR values predict resistance to bevacizumab treatment. However, as it is a retrospective analysis, more comprehensive and randomized trials are essential in order to determine a correct statement.

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