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ORIGINAL ARTICLE

Pre-treatment lymphopenia as a prognostic biomarker in colorectal cancer patients receiving chemotherapy

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Abstract
Background Lymphopenia is a predictor of the efficacy and hematological toxicity of chemotherapy in various advanced cancers. There is little data about this relationship in colorectal cancer. In this retrospective study, the influence of pretreatment lymphopenia on hematological toxicity and the efficacy of chemotherapy was investigated in colorectal cancer patients.
Patients and methods In total, 260 patients were included in the study. Correlations between pre-treatment lymphopenia (lymphocyte count $< 1{,}000/\mu l$) and the occurrence of hematological toxicity and efficacy of first-line palliative chemotherapy were investigated.
Results Lymphopenia was found in 49/260 (19%) patients. Ten of these patients with lymphopenia (20.4%) experienced severe hematological toxicity compared with 17 of the remaining 211 (8%) patients ($P = 0.01$). Lymphopenia was identified as an independent factor for hematological toxicity. Among patients who received palliative chemotherapy, the objective response rate was significantly lower in lymphopenic patients than in the other patients (12.5% vs. 40.2%; $P = 0.004$). Lymphopenia was strongly associated with shorter progression-free survival (median 4 vs. 7 months; $P = 0.033$) and shorter overall survival (median 16 vs. 24 months, $P = 0.024$). Multivariate analysis revealed that lymphopenia had an independent effect on survival.
Conclusions Our findings show that lymphopenia is an independent predictive factor for both hematological toxicity and efficacy of chemotherapy in colorectal cancer. Pre-treatment lymphocyte count may represent a simple and new predictive biomarker of chemotherapy effects in colorectal cancer patients.

Keywords Colorectal cancer · Chemotherapy · Lymphopenia · Predictive factor

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Introduction

Colorectal cancer is one of the most common causes of cancer death in the Western world, ranking second in Europe and third in the United States of America [1]. In recent decades, substantial progress has been made in the treatment of colorectal cancer, and patients with metastatic disease are now living twice as long as they were a decade ago [2]. The use of adjuvant chemotherapy has increased the recovery rate by 30% in patients with stage III colon cancer [3]. Although palliative chemotherapy is able to increase survival and improve quality of life, the prognosis of patients with advanced colorectal cancer is still poor. Clinical trials in metastatic colorectal cancer have revealed

considerable heterogeneity in survival rates [4]. This can be accounted for not only by differences in treatment regimens but also by differences in patients' characteristics and prognostic factors [5–7]. New prognostic and predictive factors are sorely needed to improve patient management. With the exception of *KRAS* mutations, which are highly specific negative predictors of the response to anti-EGFR monoclonal antibodies, other factors predicting treatment failure following conventional therapies in advanced colorectal cancer are not well established [8, 9]. Identifying patients most likely or very unlikely to benefit from chemotherapy in colorectal cancer is crucial for physicians when considering different treatment options and for researchers and investigators who aim to perform new trials. Cancer development is partially due to the immune system's failure to control and eradicate transformed cancer cells [10]. This immune surveillance is mediated by both innate and adaptive components of cellular immunity [11, 12]. In colorectal cancer, the relevance of immune-mediated cancer control is supported by (1) the frequently observed positive correlation between tumor-infiltrating lymphocytes and prognosis, (2) the negative correlation between immunosuppressor cells, such as regulatory T cells, and prognosis and (3) the immune system's role in the early stages of metastatic invasion [13–16]. Determining immune parameters related to tumor progression might provide significant prognostic information. Previous studies have shown that lymphopenia is commonly observed in patients with advanced cancers and correlated to poor prognosis in terms of overall and progression-free survival in patients with different cancer types, including breast cancer, sarcoma, or lymphoma, treated by chemotherapy [17–20]. Lymphopenia is also a powerful predictor of chemotherapy-induced hematological toxicity, in addition to patient and disease characteristics, biological parameters, and previous treatments [21–25]. Although the predictive value of pre-treatment lymphocyte counts for efficacy and/or hematological toxicity of chemotherapy is now well established for several cancers, there is little data available for colorectal cancer [26].

The purpose of this retrospective study was to determine whether pre-treatment lymphopenia is a predictor of hematological toxicity, tumor response, and survival in a well-defined and closely monitored cohort of consecutive colorectal cancer patients receiving chemotherapy.

Patients and methods

Patient selection

This survey is a retrospective database analysis of a prospective cohort of colorectal cancer patients treated in a single center. Database procedure codes were used to identify consecutive patients who received first-line systemic chemotherapy for metastatic colorectal cancer or adjuvant chemotherapy for stages II or III colorectal cancer between October 1999 and October 2007. The hospital records of each patient were individually reviewed. The analysis was performed using anonymized patient data, and therefore informed patient consent was not required.

For patient inclusion, all of the following criteria had to be fulfilled: a histologically proven colorectal adenocarcinoma, first-line systemic palliative or adjuvant chemotherapy, no previous systemic chemotherapy, no previous malignancy other than colorectal cancer in the last 5 years, no documented human immunodeficiency virus infection, no suspected or documented bone marrow involvement, no concomitant radiotherapy or chemotherapy (previous neo-adjuvant radiotherapy for rectal cancer was authorized), and no primary prophylactic administration of granulocyte colony-stimulating factor (G-CSF) following chemotherapy. All patients were treated with conventional systemic chemotherapy regimens containing approved cytotoxic agents for colorectal cancer. In all cases, the treatment regimens were based on the recommended dosing range and schedule. The duration of adjuvant chemotherapy was 6 months. The same first-line systemic chemotherapy regimen for metastatic colorectal cancer was maintained until disease progression or severe toxicity, whichever occurred first. Physical examination and a complete blood count were performed prior to each chemotherapy cycle. Data were collected retrospectively from the patient data file. The absolute lymphocyte count prior to chemotherapy initiation was retrieved for all patients from the patient data file. Lymphopenia was defined as a lymphocyte count below 1,000/μl, as this threshold was found to be more discriminatory in predicting overall survival in a recent, large, prospective study [20].

Assessment of toxicity, response, and survival

Chemotherapy toxicity was recorded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 3.0. The occurrence of severe hematological toxicities (grades 3–4) was the primary end point. All patients who had received at least one course of chemotherapy were evaluated for hematological toxicity. Hematological toxicity was evaluated with a blood count performed in the 48 h before the next chemotherapy course. Hematological toxicity data were available for all patients. Tumor assessment was performed every 2–3 months using computed tomography scan, or earlier in cases of clinical suspicion of progression. Tumor assessment was made according to the response evaluation criteria in solid tumors [27]. For this analysis, patients with

complete or partial response were classified as responders, and those with stable or progressive disease were classified as non-responders. Progression-free survival (PFS) was defined as the interval from the first day of chemotherapy to the date of tumor progression, death from any cause, or last follow-up, at which time point data were censored. Overall survival (OS) was defined as the time from the first day of chemotherapy to death from any cause or last follow-up, at which time point data were censored. Survival data was available for all patients.

Statistical analysis

Prechemotherapy lymphopenia was considered a dichotomous variable (<1,000/μl vs. ≥1,000/μl). Different potential predictive variables for hematological toxicity were tested in univariate and multivariate analyses: World Health Organization performance status (WHO PS), age, sex, leukocyte count, previous radiotherapy, and chemotherapy regimen. The variables were considered as dichotomous variables. The correlation between a variable and the occurrence of chemotherapy-induced hematological toxicity was evaluated using chi-squared test or Fisher's exact test. A logistic regression analysis was performed using the parameters studied in the univariate analysis. The chi-squared test was used to calculate the P value for the association between lymphopenia and response to palliative chemotherapy. Both PFS and OS for patients who received first-line palliative chemotherapy were estimated using the Kaplan–Meier method [28]. The median time of survival was used to summarize the survival data. Potential predictive variables most commonly used in previous studies were evaluated in univariate and multivariate analyses, including age (<75 vs. ≥75 years), sex, WHO PS (0–1 vs. ≥2), primary tumor site (right colon vs. others locations), number of metastatic sites (1–2 vs. ≥3), leukocyte count (<10,000 vs. ≥10,000/μl), previous resection of primary tumor, and chemotherapy regimen (monotherapy vs. bi- or tritherapy). As alkaline phosphatase values (<300 vs. ≥300 IU/l) were available for 126 patients only, this parameter was excluded from the multivariate analysis. The first part of survival analysis consisted of the univariate comparison of factors, including baseline lymphopenia, which might potentially affect survival time using the log-rank test. Univariate analysis describes the survival with respect to the factor under investigation, while ignoring the other factors. A Cox proportional hazards regression model was, therefore, developed to estimate the effect of baseline lymphopenia on survival, in which each variable was adjusted for all others [29]. Calculations were performed using Stata statistical software (Stata Corporation, College Station, TX, USA). The level of significance was set at $P < 0.05$.

Results

Patient characteristics and treatments

A total of 282 patients were found in the database. Among these patients, 22 were excluded from the study for the following reasons: tumors that were endocrine rectal tumors rather than lieberkühnian adenocarcinomas ($n = 3$), missing lymphocyte count prior to initiating treatment ($n = 7$), patients presenting second primitive cancers (prostate $n = 4$; lung $n = 1$; larynx $n = 1$; bladder $n = 1$), lost or inaccessible medical files ($n = 4$), and a patient having congenital lymphopenia with Zinsser-–Engman–Cole syndrome ($n = 1$). Thus, 260 consecutive patients with colorectal cancer treated by first-line palliative or adjuvant chemotherapy between October 1999 and October 2007 were selected for this study.

Patient characteristics are summarized in Table 1. Of the 260 patients, 102 were women (39.2%) and 158 were men (60.8%); mean age was 64.8 years. Anatomic locations of primitive tumors were the colon in 195 cases (75%) and the rectum in 65 cases (25%). Among patients with rectal cancer, 63 (84%) received pre- or postoperative radiotherapy. The distribution of tumors according to the tumor-node metastasis (TNM) classification system was stages II or III in 121 (46.5%) patients and stage IV in 139 (53.5%) patients. The sites of metastasis at the palliative chemotherapy start date (139/260 patients) were the liver in 119 cases, the lungs in 44 cases, the peritoneum in 41 cases, and other sites in 26 cases.

The median follow-up time was 15 months (range 1–88 months) for patients with first-line palliative chemotherapy and 34 months (range 6–120 months) for patients with adjuvant chemotherapy. Overall, 45% of all patients received monochemotherapy, 50% received a two-drug combination, and 5% received a three-drug combination. In all, 121 patients received adjuvant chemotherapy. The adjuvant treatments were as follows: a monthly schedule of leucovorin and 5-FU bolus (Mayo Clinic regimen; $n = 10$), a semi-monthly regimen of 5-FU and leucovorin (LV5FU2 regimen; $n = 58$), capecitabine ($n = 2$), a semi-monthly regimen of 5-FU, leucovorin, and oxaliplatin (FOLFOX regimen, $n = 43$), a semi-monthly regimen of 5-FU, leucovorin, and irinotecan (FOLFIRI regimen, $n = 3$), a semi-monthly regimen of 5-FU, leucovorin, oxaliplatin, and bevacizumab (FOLFOX-bevacizumab regimen, $n = 2$), and a regimen of capecitabine, oxaliplatin, and bevacizumab every 3 weeks (XELOX-bevacizumab regimen, $n = 3$). With the exception of 10 patients who had to stop adjuvant chemotherapy for toxicity, the duration of adjuvant chemotherapy was 6 months. A total of 139 patients received first-line palliative chemotherapy. The palliative treatments were as follows: a LV5FU2

Table 1 Patient characteristics

Characteristics	
Number of patients	260
Sex m/f	158/102 (60.8%/39.2%)
Mean age (years) [range]	64.8 [34–85]
Mean pre-treatment lymphocyte count per µl (standard deviation)	1,624 (667.6)
First-line palliative chemotherapy	139 patients
Adjuvant chemotherapy	121 patients
Patients with pre-treatment lymphopenia	
First-line palliative chemotherapy	32/139 (23.0%)
Adjuvant chemotherapy	17/121 (14.0%)
Primary tumor location	
Right colon	75/260 (28.8%)
Transverse colon	9/260 (3.5%)
Left colon	23/260 (8.8%)
Sigmoid	88/260 (33.9%)
Rectum	65/260 (25.0%)
Total	260
Metastatic locations	
Liver	119/139 (85.6%)
Lung	44/139 (31.7%)
Peritoneum	41/139 (29.5%)
Intra abdominal nodes	13/139 (9.4%)
Bone	3/139 (2.2%)
Ovary	2/139 (1.4%)
Suprarenal glands	5/139 (3.6%)
Spleen	3/139 (2.2%)
WHO performance status (*all patients*)	
0–1	232/260 (89.2%)
>1	28/260 (10.8%)
WHO performance status (*metastatic patients*)	
0–1	113/139 (81.3%)
>1	26/139 (18.7%)

regimen ($n = 44$), a semi-monthly regimen of 5-FU, leucovorin, and oxaliplatin (FOLFOX regimen, $n = 53$), a semi-monthly regimen of 5-FU, leucovorin, and irinotecan (FOLFIRI regimen, $n = 27$), a semi-monthly regimen of 5-FU, leucovorin, irinotecan, and bevacizumab (FOLFIRI-bevacizumab regimen, n = 6), a regimen of capecitabine, oxaliplatin, and bevacizumab every 3 weeks (XELOX-bevacizumab regimen, $n = 2$), capecitabine monotherapy ($n = 2$), tegafur monotherapy ($n = 1$), raltitrexed monotherapy ($n = 1$), a regimen with raltitrexed and oxaliplatin every 3 weeks (TOMOX regimen, $n = 1$), a regimen with capecitabine and oxaliplatin every 3 weeks (XELOX regimen, $n = 1$) and a regimen with tegafur and oxaliplatin (TEGAFOX regimen, $n = 1$).

Among the 260 patients, the prevalence of lymphopenia, which was defined as a lymphocyte blood count less than 1,000/µl, was 19%. The lymphopenia prevalence tended to be lower in patients receiving adjuvant chemotherapy than in those receiving palliative chemotherapy (14% vs. 23%, respectively); this difference, however, did not reach statistical significance (chi-squared test, $P = 0.06$). In univariate analyses, number of metastatic sites, WHO performance status, age, sex and a palliative-aiming treatment were not significantly associated with lymphopenia (Table 2). The repartition of the chemotherapy regimen was not significantly different between patients with or without baseline lymphopenia (Table 3).

Hematological toxicity of adjuvant and palliative chemotherapies

Hematological toxicities are summarized in Table 4. The maximum hematological toxicity was grade 3 in 6.5% (17 patients) and grade 4 in 3.8% (10 patients) of patients. No toxic deaths occurred. The most prevalent hematological toxicity reported was neutropenia, with 8.4% (22 patients) of patients reporting grades 3 or 4 neutropenia. As expected, patients who received bi- or tri-chemotherapy experienced more frequent grades 3–4 hematological toxic events than those receiving monochemotherapy (16.3% vs. 3.4%, respectively; $P = 0.002$). The association between lymphopenia and hematological toxicity was assessed for 260 patients. Grades 3–4 hematological toxicity was observed in 10 of 49 patients with baseline lymphopenia (20.4%), and in 17 of 211 non-lymphopenic patients (8%), the difference being statistically significant ($P = 0.01$). Two others factors were significantly related to the risk of grades 3–4 hematological toxicity, notably female sex and bi- or tri-chemotherapy regimen (Table 5). Independent risk factors for grades 3–4 hematological toxicity were baseline lymphopenia (odds ratio [OR] 4.6 [95% CI 1.6–13.4]; $P = 0.005$), bi- or tri-chemotherapy regimen (OR 6.3 [95% CI 1.96–20.2]; $P = 0.002$), and female sex (OR 1.22 [95% CI 1.15–6.58]; $P = 0.023$).

First-line palliative chemotherapy efficacy

Response to treatment

All 139 patients were evaluated for response to first-line palliative chemotherapy. The overall response rate to chemotherapy was 33.8% (47 of 139; 95% CI 26.5–42.0%) and 20.1% (28 of 139; 95% CI 14.3–27.6%) in patients with tumor stabilization. The disease control rate was 54% (75 of 139; 95% CI 45.7–62.0%). As expected, patients with bi- or tritherapy regimen exhibited a significantly higher response rate than those with monotherapy consisting of 5-FU regimen alone (45% vs. 14%, respectively; $P < 0.0001$). Baseline lymphopenia was significantly

Table 2 Comparison of the characteristics of patients with or without pre-treatment lymphopenia

	Patients with baseline normal lymphocyte count	Patients with baseline lymphopenia	*P* value
Number of patients	211	49	N/A
WHO PS > 1	9.5% (20/211)	16.3% (8/49)	0.16
Age > 75 years	16.59% (35/211)	12.24% (6/49)	0.45
Number of metastatic sites > 1	62.6% (67/107)	50% (16/32)	0.2
Male sex	59.2% (125/211)	67.3% (33/49)	0.16
Palliative chemotherapy	50.7% (107/211)	65.3% (32/49)	0.065

N/A Non-applicable

Table 3 Repartition of the different chemotherapy regimen in patients

	All patients	Patients with baseline lymphopenia	Patients with normal pretherapeutic lymphocyte count
FOLFOX regimen	36.9% (96/260)	28.6% (14/49)	38.9% (82/211)
FOLFIRI regimen	11.5% (30/260)	12.2% (6/49)	11.4% (24/211)
5-FU monotherapy	43.8% (114/260)	51% (25/49)	42.2% (89/211)
Other regimen	7.7% (20/260)	8.2% (4/49)	7.6% (16/211)
Total	100% (260/260)	100% (49/49)	100% (211/211)

Chi-squared test $P = 0.76$

Table 4 Hematological toxicities among the 260 patients (NCI-CTC)

	1	2	3	4	Total
Neutropenia	52 (20.0%)	24 (9.2%)	13 (5.0%)	9 (3.4%)	98 (37.6%)
Febrile neutropenia	NA	NA	2 (0.8%)	1 (0.4%)	3 (1.2%)
Thrombocytopenia	42 (16.2%)	19 (7.3%)	2 (0.8%)	1 (0.4%)	64 (24.7%)
Anemia	80 (30.8%)	13 (5.0%)	4 (1.5%)	0	97 (37.3%)
Total	174 (67%)	56 (21.5%)	21 (8.1%)	11 (4.2%)	262

NA Not applicable

associated with lack of response to palliative chemotherapy. Only four of the 32 patients with baseline lymphopenia responded to chemotherapy versus 43 of the 107 patients without baseline lymphopenia (12.5% vs. 40.2%, respectively; $P = 0.004$).

Survival

Both PFS and OS were significantly lower in patients with lymphopenia at baseline than in those with normal baseline lymphocyte count. In univariate analyses, baseline lymphopenia, WHO PS $\geq$ 2, no resection of primary tumor, and leukocyte count of $\geq$ 10,000/μl were significantly linked to shorter PFS (Table 6; Fig. 1), and both baseline lymphopenia and leukocyte count $\geq$ 10,000/μl were significantly associated with shorter OS (Table 6; Fig. 2). In multivariate analyses, baseline lymphopenia, WHO PS $\geq$ 2, and no previous resection of primary tumor were significantly linked with shorter PFS, whereas only baseline lymphopenia was independently associated with poor OS (Table 7).

Adjuvant chemotherapy efficacy

All 121 patients were evaluated. Recurrence occurred in 43% (6/14) of lymphopenic patients, and in 34.7% (37/107) in non-lymphopenic patients. In an univariate analysis, pretherapeutic lymphopenia was associated neither with recurrence-free survival ($P = 0.73$) nor with global survival ($P = 0.59$). However, the weak effective of lymphopenic patients treated on an adjuvant basis (14 patients) do not allow to draw any conclusion of these results.

Discussion

In this study, we investigated whether pre-treatment lymphopenia may be linked to chemotherapy-induced toxicity as well as tumor response and survival in colorectal cancer patients receiving various chemotherapy regimens. Based on our study results, baseline lymphopenia appears to be

Table 5 Factors associated with grades 3–4 hematological toxicity

	N (%)	Grade 3 or grade 4 hematological toxicity	
		N (%)	*P* value
Lymphopenia			
Yes	49 (19.0%)	10 (20.4%)	**0.011**
No	211 (81.2%)	17 (8.0%)	
Age (years)			
<75	219 (84.2%)	24 (11.0%)	0.48
≥75	41 (15.8%)	3 (7.3%)	
Sex			
Male	158 (60.8%)	11 (7.0%)	**0.02**
Female	102 (39.2%)	16 (15.7%)	
WHO PS			
0–1	232 (89.2%)	22 (9.5%)	0.17
≥2	28 (10.8%)	5 (17.9%)	
Leukocyte count			
<10,000/μl	217 (83.5%)	23 (10.6%)	0.79
≥10,000/μl	43 (16.5%)	4 (9.3%)	
Previous radiotherapy			
Yes	59 (22.7%)	7 (11.9%)	0.23
No	201 (77.3%)	20 (10.0%)	
Chemotherapy regimen			
Monotherapy	119 (45.8%)	4 (3.4%)	**0.001**
bi- or tritherapy	141 (54.2%)	23 (16.3%)	
Palliative or adjuvant treatment			
Palliative	139 (53.5%)	18 (12.9%)	0.15
Adjuvant	121 (46.5%)	9 (7.4%)	

Bold values are statistically significant

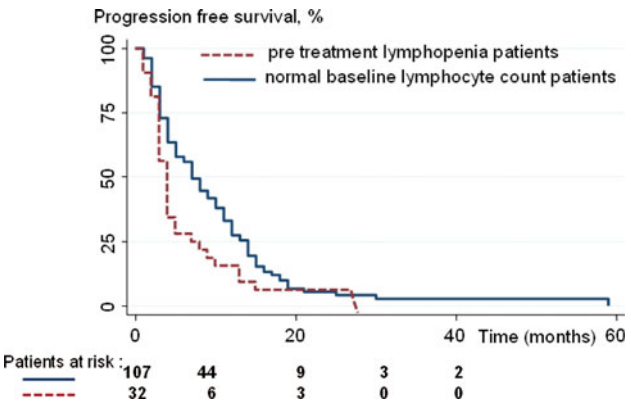

Fig. 1 Kaplan–Meier estimates of progression-free survival according to baseline lymphocyte count for patients who received chemotherapy for advanced colorectal cancer

predictive of low chemotherapy efficacy (PFS and OS) and severe chemotherapy-induced hematological toxicity in this patient population.

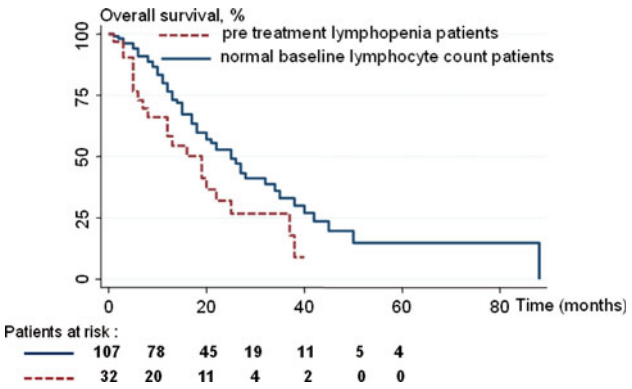

Fig. 2 Kaplan–Meier estimates of overall survival according to baseline lymphocyte count for patients who received chemotherapy for advanced colorectal cancer

Cancer prognosis depends on tumor aggressiveness and host immune response. Absolute lymphocyte count is assumed to reflect cancer patients' whole immune responsiveness [30]. Lymphopenia is a common biological finding in advanced solid tumors, reflecting cancer-related immunosuppression, and has been shown to be associated with an unfavorable prognosis in terms of survival time [31]. The threshold level of 1,000 lymphocytes per μl was selected, as a large study has recently revealed its predictive value in terms of prognosis, although its biological significance is still unclear [20]. In our series, the prevalence of patients presenting a lymphocyte count of < 1,000/μl was 19%, which was similar to that observed in previous reports for other advanced cancer types [20]. Only two previous studies have evaluated the prevalence of lymphopenia in advanced colorectal patients [21, 32]. In these series, with 31% (64/207) of patients presenting with a lymphocyte count of < 1,500/μl, the mean rate was lower than that observed in our series, notably 49% (68/139). Interestingly, the prevalence of lymphopenia was lower in patients who received adjuvant chemotherapy, although the difference was not statistically significant. This suggests some kind of relationship between lymphopenia and tumor mass cell, which needs to be defined further.

The cause of lymphopenia in chemo-naive cancer patients is still unclear but probably multifactorial. It may be due to increased tumor-related lymphocyte destruction or altered lymphocyte homeostasis [33–35] or reflect denutrition, which is commonly observed in cancer patients. Specifically, patients with cachexia syndrome usually exhibit lymphopenia, which seems to result from a direct effect of cytokines such as TNF-α [36, 37].

It has been reported that performance status, age, gender, nutritional status, pre-existing organ dysfunction, bilirubin and albumin levels, genetic polymorphism, and drug administration schedule could affect chemotherapeutic toxicities in colorectal cancer patients [39]. In our study,

Table 6 Factors associated with progression-free survival (PFS) and overall survival (OS) in the univariate analysis

	n	Median PFS (months)	*P* value	Median OS (months)	*P* value
Baseline lymphopenia					
Yes	32	4	**0.033**	19	**0.024**
No	107	7		25	
Age (years)					
≥75	20	5	0.8	15	0.18
<75	119	6		22	
Sex					
Male	88	8	0.15	22	0.74
Female	51	5		25	
WHO PS					
0–1	113	8	**<0.0001**	25	0.22
≥2	26	3		20	
Tumor site					
Right colon	38	5	0.6	21	0.83
Other localizations	101	6		28	
Number of metastatic sites					
1	56	7	0.19	22	0.23
≥2	83	5		21	
Leukocyte count					
<10,000/μl	103	7	**0.039**	25	**0.025**
≥10,000/μl	36	4		18	
Resection of primary tumor					
Yes	97	7	**0.0015**	25	0.067
No	42	3		20	
Chemotherapy regimen					
Monotherapy	50	5	0.08	22	0.62
bi- or tri-therapy	89	8		22	
Alkaline phosphatase value					
<300 IU/ml	99	6	0.22	22	0.44
≥300 IU/ml	27	5		18	

Bold values are statistically significant

Table 7 Factors associated with a shorter PFS and/or a shorter OS in multivariate analysis (Cox regression analyses)

Variable	Hazard ratio	95% CI	*P* value
PFS			
Baseline lymphopenia	1.56	1.00–2.43	**0.048**
WHO PS ≥ 2	2.93	1.81–4.74	**<0.001**
No previous resection of primary tumor	1.68	1.10–2.57	**0.015**
OS			
Baseline lymphopenia	2.35	1.34–4.14	**0.003**

Bold values are statistically significant

three independent risk factors for hematological grades 3–4 toxicity were identified, namely female sex, bi- or tritherapy chemotherapeutic regimen, and baseline lymphopenia. The hypothesis is raised that severe hematological toxicity may be dependent on concomitant immune suppression. Our results were obtained in a population of colorectal cancer patients treated with various chemotherapy regimens. This approach is justified for the identification of a predictive factor that would apply to any chemotherapy regimen. In other cancers, several studies have been conducted in order to identify patients that may be exposed to an increased risk of severe hematological toxicity. Baseline lymphopenia (lymphocyte count < 700/μl), lymphopenia assessed 5 days after treatment, and the type of chemotherapy regimen enabled the identification of patients at high-risk of febrile neutropenia, thrombocytopenia, anemia, and early death following chemotherapy [24, 25, 40]. One study report indicated that all lymphocyte subsets may be affected in lymphopenic cancer patients, although baseline CD4 lymphocyte count was identified as an independent risk factor for febrile neutropenia and early death in cancer patients receiving chemotherapy [38]. Additional studies are required to investigate different

lymphocyte subsets and evaluate the association between quantitative changes in lymphocyte subpopulations and chemotherapy-related toxicity in colorectal cancer patients.

In our series, baseline, pre-chemotherapy lymphopenia was also shown to be a predictor of decreased efficacy of first-line palliative chemotherapy, as baseline lymphopenia was found to be associated with reduced chemotherapy efficacy in terms of response and survival in the patient population studied. Two previous studies have used a threshold level of 1,500 lymphocytes per μl [21, 32]. In this study, we chose the threshold level of 1,000/μl as it was found to be more discriminatory in predicting overall survival in a large, recent and prospective study, which included three tumor types (NHL, metastatic breast cancer, advanced sarcoma) [20]. Although the mechanism of the association between low lymphocyte count and decreased chemotherapy efficacy in metastatic colorectal cancer patients is far from being understood, lymphopenia may reflect a state of immune depression. Of note is that immune suppression appears to play a role in tumor progression as reported in NHL, melanoma, and head and neck carcinoma as well [33, 34, 41]. Although total lymphocyte count is not specific enough to assess the host anticancer immunity, this parameter may be very useful in routine practice.

Determining whether lymphopenia is a cause or a consequence of colorectal cancer progression may be relevant, considering the perspective that lymphopenia may be corrected by means of lymphocyte growth factors such as IL-7 and IL-2 [44, 45]. In a study by Lissoni et al., IL-2 administration to metastatic colorectal cancer patients with pretreatment lymphopenia was shown to normalize lymphocyte counts in the majority of patients, while appearing to enhance chemotherapy efficacy [32].

In previous studies, PS, number of metastatic sites, white blood cell count, alkaline phosphatase, right-sided primary tumors, and chemotherapy regimens were shown to be the main clinical parameters associated with survival in metastatic colorectal cancer patients [5, 42, 43]. In our study, the prognostic value of lymphopenia was found to be independent from these factors in multivariate analysis using the Cox model. Large published studies evaluating prognostic indicators for metastatic colorectal cancer patients receiving palliative chemotherapy, such as the Kohne study, have not evaluated baseline blood lymphocyte counts. The decreased OS and response rates associated with baseline lymphopenia suggest that this factor may be considered to be prognostic, predictive or both. However, the new original observations arising from our study must be confirmed using independent clinical data sets that include both quantitative and qualitative assessments of peripheral blood lymphocytes.

Our study had some limitations, as we evaluated a relatively small number of patients, who received different chemotherapy regimens. Our findings must, therefore, be considered as preliminary results. A larger study with patients at the same stage and receiving an identical chemotherapy regimen is needed in order to confirm our results.

In conclusion, this study suggests that pretreatment lymphopenia is associated with a reduced chemotherapy efficacy in metastatic colorectal cancer patients. In addition, the pretreatment absolute blood lymphocyte count appears to be a significant predictor of chemotherapy-related toxicity in colorectal cancer patients. These findings could lead to individualized treatment for patients with colorectal cancer requiring chemotherapy. Moreover, in future randomized clinical trials, metastatic colorectal cancer patients may be stratified according to this simple biological parameter.

Conflict of interest The authors indicated no potential conflict of interest.

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