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Peri-tumoural CD3+ Inflammation and Neutrophil-to-Lymphocyte Ratio Predict Overall Survival in Patients Affected by Colorectal Liver Metastases Treated with Surgery

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Abstract

Background Systemic and local inflammation plays an important role in many cancers and colorectal liver metastases (CRLM). While the role of local immune response mediated by CD3+ tumour-infiltrating lymphocytes is well-established, new evidence on systemic inflammation and cancer, such as neutrophil-lymphocyte ratio (NLR), is emerging. The aim of this study is to seek an association between the CD3+ lymphocytes and NLR with patients' prognosis and possibly stratifying it accordingly.

Methods From January 2005 to January 2013, 128 consecutive patients affected by CRLM and treated with chemotherapy and surgery were included in the study. Different cutoff levels were calculated with ROC curves for each of the biomarkers, and their relative outcome in terms of overall survival (OS) and recurrence-free survival (RFS) was determined. Associating the two biomarkers, three risk groups were determined: low risk (two protective biomarkers), intermediate risk (one protective biomarker) and high risk (no protective biomarker).

Results After a median follow-up of 45 months, median OS and RFS were 44 and 9 months, respectively. For OS, 29 (22.66%), 59 (46.09%) and 40 (31.25%) patients were in the low, intermediate and high-risk groups, respectively. Adjusted Cox regression analysis showed an increased risk of death in the intermediate group (HR 2.67 $p = 0.007$ 95% CI 1.31–5.42) and high-risk group (HR 2.86 $p = 0.005$ 95% CI 1.37–5.99) compared to the low-risk group (reference).

Conclusion Systemic and local immune response index allows stratification of patients in different OS and RFS risk groups.

Keywords Colorectal liver metastases · Local inflammation · Sistemic inflammation · Neutrophil-to-lymphocyte ratio

Introduction

Colorectal cancer (CRC) is one of the most common human malignancies. Worldwide, approximately 1.2 million new cases are diagnosed, and over 600,000 deaths are estimated to occur annually [1]. About 40% of patients with CRC have developed liver metastases (CRLM) at the time of presentation, with approximately 20% presenting as synchronous (within 6 months from primary tumour resection) and the remaining 20% having metachronous metastases [2–4]. Despite therapeutic advancements, the prognosis for CRLM still remains dismal, with a 5-year survival rate of 25–47% and a median survival of 33–50 months after resection of liver metastases [5–8]. Furthermore, recurrences occur in half of patients after resection of liver metastases within 2 years [9]. Therefore, identification of reliable prognostic predictors for CRLM remains a priority [10, 11]. Indeed, cancer progression and outcome are dependent on the interactions between the host and the tumour. Such interactions depend on the host

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immune response against the tumour (the host inflammatory response). Several studies have analysed the relationship between intra-tumour and peri-tumour inflammatory infiltration and patient outcome, showing that robust local inflammation is associated with better prognosis [12, 13]. Tumour-infiltrating lymphocytes identified as CD3+ staining at immunohistochemistry in liver specimens seem to play a major role in local immune response [14, 15]. In contrast, the presence of systemic inflammation, as measured by biomarkers such as an elevated C-reactive protein or neutrophil-to-lymphocyte ratio (NLR) in peripheral blood, has been significantly associated with poor prognosis across many cancers including CRLM [16–21].

To date, the existing studies have focussed on local or systemic inflammatory response in isolation, so it is important to explore whether combining a marker for each process could further improve prognostication.

Here we examine the relationship between local and systemic inflammation, clinical and pathological data and outcomes in a consecutive series of patients affected by CRLM who underwent systemic therapy and surgery.

Methods

Study Design and Data Collection

This is a retrospective study that examined a cohort of patients undergoing curative hepatic resection for CRLM at our institution. Data were collected in a dedicated database. Each patient gave written consent for data storage and analysis. The study was approved by the local ethical committee.

Definitions

Based on the Brisbane classification [22], hepatic resection was considered major when at least three adjacent segments were removed. Postoperative morbidity was graded based on the Clavien–Dindo classification [23]. Postoperative mortality was recorded at 90 days after surgery. Metachronicity was defined as the diagnosis of CRLM after 6 months from the diagnosis of the primary tumour. Response to chemotherapy was assessed according to RECIST criteria [24].

Patient Selection

Patients who underwent hepatic resection for CRLM at the Division of Hepatobiliary and General Surgery at Humanitas Research Hospital between January 2005 and December 2013 were considered for this study. Inclusion criteria were histologically proven CRLM along with complete clinical, surgical, pathological and follow-up data. Patients with previous liver surgery in other institutions and/or missing data were excluded.

Patient Management

In all patients, hepatic magnetic resonance imaging (MRI) to assess the local disease extension and to evaluate chemotherapy response, thoraco-abdominal computed tomography (CT) and positron emission tomography (PET-CT) were systematically performed to evaluate the presence of extrahepatic disease.

The management of every patient was discussed by a multidisciplinary committee including surgeons, oncologists, radiologists, radiotherapists and nuclear medicine physicians. The indication for preoperative chemotherapy (CHT) was evaluated on the basis of disease extension and patients' performance status. Upfront surgery was considered in patients with metachronous or limited synchronous metastases, especially in the first period of the study. In chemotherapy-naïve patients, generally a short course of chemotherapy was administered to assess chemotherapy response. Only patients with stable disease or a partial response to chemotherapy were scheduled for surgery. In the case of disease progression, patients were usually scheduled for a second-line treatment.

The need for adjuvant chemotherapy was evaluated on a case-by-case basis. Follow-up of all patients was performed every 3 months (physical examination, CEA levels and abdominal ultrasonography, CT or MRI).

Pathological Data

All archival slides of CRLM were reviewed together at the multi-head microscope by two pathologists. They were blinded to any patient clinical data.

Assessment of Intra-tumoural and Peri-tumoural Inflammation

Regarding immunoreactivity to CD3+, two different areas for each case of CRLM were considered: (a) inside the tumour in the active part of the lesion excluding necrotic areas (intra-CD3+) and (b) inside the liver parenchyma at the invasive margin of the lesion (border-CD3+). For each of these areas (a and b), ten consecutive 10x microscopic fields were randomly selected from immunostained sections and optimized for the evaluation. Two pathologists scored the same areas of the analyses, and they were blinded to any patient clinical data. The number of CD3+ cells was evaluated as a percentage. In case of a different score, the mean value was taken into consideration.

As described in other solid tumours, the relationship between intra-tumoural and peri-tumoural CD3+ infiltration is

an important indication of the host immune response. According to these data, we have used intra-CD3+, border-CD3+ and ratio (CD3R) to evaluate host immune response [14, 15]. We used CD3R instead of intra-CD3+ and border-CD3+ because the AUC for OS values were 0.64, 0.62 and 0.53, respectively, meaning that CD3R would have been the most relevant factors in OS compared to intra-CD3+ and border-CD3+. The same observation was evident for AUC for RFS 0.70, 0.65 and 0.61, respectively. These data support the idea that the efficacy of local immune response is determined by the migration of the CD3+ lymphocytes inside the tumour more than the peri-tumoural concentration itself. For these reasons, we use CD3R—the higher the ratio, the better the OS and RFS.

Assessment of Systemic Inflammation

For each patient, perioperative neutrophil count and lymphocyte count were obtained from full blood count samples taken within 15 days prior to CRLM surgical resection. NLR was calculated as the absolute neutrophil count divided by the absolute lymphocyte count.

Follow-Up

The clinical follow-up was conducted at our institution and consisted of a physical examination, tumour marker evaluation and computed tomography or magnetic resonance imaging every 3 months after surgery. All the patients were followed up to 5 years.

Statistical Analysis

Descriptive statistics of the patient characteristics were reported using frequencies and percentages, medians and range, according to vital status and presence of relapses. Pearson's chi-squared test (or Fisher's exact test when appropriate) and the Kruskal–Wallis tests were used to compare the differences between frequencies and medians, respectively, among deceased and alive patients and among the relapsed and non-relapsed.

Overall survival (OS) was defined as time from surgery to death from any cause. Relapse-free survival (RFS) was defined as time to any relapse related to CRLM.

Different cutoffs for CD3R and NLR related to different outcomes (OS and RFS) were calculated with ROC curves. Figure 1 illustrates ROC curves for each factor related to OS and RFS.

Biomarkers' optimal cutoffs were calculated with the Liu method, which defines the optimal cutoff point as the point maximizing the product of sensitivity and specificity.

A Cox regression model was fitted to estimate hazard ratios (HRs) of OS and RFS. Proportions between hazards were tested with the Schoenfeld test. Two different models were fitted: (i) a crude model and (ii) a model with the additional covariates of age, sex, T, stage of the primitive lesion, N+ of the primitive lesion, neoadjuvant chemotherapy and number of lesions removed up to 4 (described in Table 1).

All analyses were performed using the software STATA version 14 (StataCorp LP, College Station, TX, USA).

Results

Patient Characteristics

A total of 141 patients that underwent hepatic resection for CRLM at our institution between January 2005 and January 2013 were retrospectively reviewed. Thirteen (10%) were excluded—10 did not have accessible slides for the CD3R assessment, and 3 were lost to follow-up. Table 1 details the demographics and clinicopathologic features of the patients stratified for OS (overall death vs alive) and RFS (overall, recurrence vs no recurrence). Analysing the overall population, 78 (61%) were male, and the median age was 64 (range 28–83). In 90 (70%) patients, the primary tumour was pathologically staged as T3–4, while in 72 (56%) patients, the regional lymph nodes were positive. Fifty-three (41%) patients had wild-type K-RAS, while 42 (33%) had the mutated form. Unfortunately, K-RAS type was unavailable in the remaining patients, and data on N-RAS and B-RAF mutations were not available since these mutations were systematically searched at our institution only from 2013. Globally, these features indicate relatively advanced stages of the tumour, a finding also confirmed by the size (median 3.3 cm; range 0.7–15.5) and number (median 4; range 1–49) of the CRLM, with 56 (37%) of the patients having more than 4 CRLM. Moreover, most of the patients (63%) had synchronous CRLM. Given such tumour burden, most patients (76%) were preoperatively treated with neoadjuvant chemotherapy (CHT). Based on the RECIST criteria, CHT was associated with a partial/complete response, stable disease and progressive disease in 65 (67%), 22 (23%) and 9 (10%) patients, respectively.

Overall Survival of Patients and Association with Selected Biomarkers

After a median follow-up of 45 months, 83 deaths occurred, and 45 patients were alive at the last clinical follow-up. Median OS was 44 months, and median RFS was 9 months. Figure 2 showed Kaplan–Meier curves of OS and RFS of the all series.

OS analysis was performed for each biomarker, categorized using the computed cutoffs (0.61 for CD3R, AUC = 0.65; 2.12 for NLR, AUC = 0.52). Table 2a shows crude and adjusted Cox analysis for OS according to the NLR and

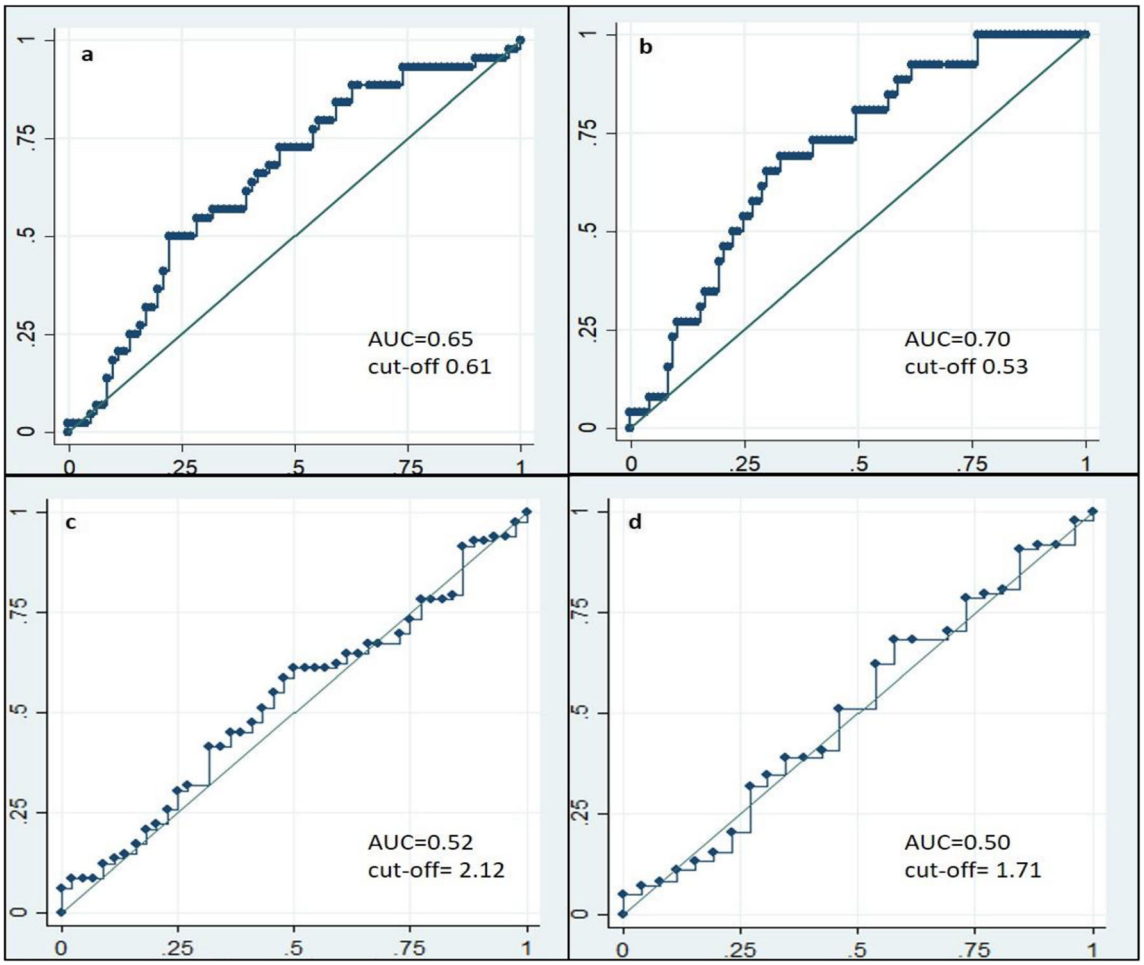

Fig. 1 AUC area under curve; (a) ROC curve CDR3 related to OS, (b) ROC curve CDR3 related to RFS, (c) NLR related to OS, (d) NLR related to RFS

CD3R cutoff. The Cox analysis was adjusted according to the following factors: sex, age (≤ 65 vs > 65), T of the primitive tumour (T1–2 vs T3–4), N of the primitive tumour (N0 vs N+), number of lesions removed (CRLM ≤ 4 vs CRLM > 4) and neoadjuvant chemotherapy (performed vs not performed). The mean number of CD3R cells was different not only for OS (0.92 in alive patients vs 0.70 in death patients $p = 0.028$) but also in RFS (0.80 in patients without recurrence vs 0.53 in patients with recurrence $p = 0.016$)

Combining the two biomarkers, we created 3 groups (low, intermediate and high risk). The low-risk group (29 patients, 23%) had both protective biomarkers (CD3R > 0.61 and NLR < 2.12); the intermediate-risk group (59 patients, 46%) had at least 1 protective biomarker (CDR3 < 0.61 or NLR > 2.12); and the high-risk group (40 patients, 31%) had no protective biomarkers (CDR3 < 0.61 and NLR > 2.12). Cox regression analysis for OS, comparing the three groups, was performed and then adjusted for the previously described variables. Table 2b describes the crude and adjusted Cox regression analysis comparing the three groups. The increase in the risk between the three groups was tested and resulted statistically significant ($p = 0.007$).

Recurrence-Free Survival of Patients and Association with Selected Biomarkers

After a median follow-up of 45 months, 98 (77%) patients had recurrence, and 30 (23%) were disease-free (median RFS = 9 months).

RFS analysis was performed for each biomarker, categorized using the computed cutoffs (0.53 for CD3R, AUC = 0.70; 1.71 for NLR, AUC = 0.50). Table 3a (see supplemental materials) shows the HRs of each biomarker calculated with crude and adjusted Cox regression analysis as shown with OS analysis. Proportions between hazards were calculated with Schoenfeld test.

Combining the two biomarkers, we defined 3 groups (low, intermediate and high risk): the low-risk group (29, 24%) had both protective biomarkers (CD3R > 0.53 and NLR < 1.71); the intermediate group (59, 44%) had at least 1 protective biomarker (CDR3 < 0.53 or NLR > 1.71); and the high-risk group (40, 32%) had no protective biomarkers (CDR3 < 0.53 and NLR > 1.71). Cox regression analysis for RFS comparing the three groups was performed and then was adjusted for the previously described variables and stratified for neoadjuvant

Table 1 Description of patients' characteristics stratified by vital status and presence of recurrence

	n (%)/median (range)						
	Overall series	Vital status			Presence of recurrences		
		Alive	Dead	P	No recurrence	Recurrence	P
Sex							
Male	78 (61)	28 (36)	50 (64)	0.897	20 (26)	58 (74)	0.505
Female	50 (39)	17 (34)	33 (66)		9 (18)	41 (82)	
Age	64 (28–83)	60 (28–80)	63 (30–83)	0.301	61 (28–80)	62 (32–83)	0.402
T status of the primary tumour							
T 1–2	16 (13)	9 (56)	7 (44)	0.041	6 (37)	10 (63)	0.107
T 3–4	90 (70)	27 (30)	63 (70)		18 (20)	72 (80)	
Missing	22 (17)	11 (50)	11 (50)		12 (55)	10 (45)	
N status of the primary tumour							
Negative	34 (27)	18 (53)	16 (47)	0.005	12 (37)	22 (63)	0.014
Positive	72 (56)	18 (25)	54 (75)		11 (11)	61 (89)	
Missing	22 (17)	11 (50)	11 (50)		14 (64)	8 (36)	
k-RAS status							
Wild type	53 (41)	15 (28)	38 (72)	0.527	3 (5)	50 (95)	0.083
Mutated	42 (33)	10 (23)	32 (77)		7 (17)	35 (83)	
Missing data	33 (26)	20 (61)	13 (39)		20 (60)	13 (40)	
Number of CRLM removed							
Median, range	4 (1–49)	6 (1–22)	6 (1–49)	0.087	3 (1–22)	7 (1–49)	0.006
> 4	56 (37)	15 (27)	41 (73)		6 (10)	50 (90)	
Preoperative CEA (ng/mL)	7 (1–2916)	54, 7 (1–97)	112, 8 (4–2916)	0.420	58 (5–450)	101(1–2916)	0.607
> 200	21 (16)	9 (43)	12 (57)	0.403	4 (15)	17 (85)	0.474
Timing of CRLM							
Synchronous	81 (63)	27 (33)	54 (67)	0.410	17 (21)	64 (79)	0.630
Metachronous	39 (30)	16 (41)	23 (59)		10 (25)	29 (75)	
Missing	8 (7)	4 (50)	4 (50)		4 (50)	4 (50)	
Neoadjuvant CHT	96 (76)	32 (33)	63 (67)	0.393	20 (21)	76 (79)	0.046
Type of neoadjuvant CHT							
5FU + oxaliplatin	53 (55)	17 (34)	36 (66)	0.835	11 (20)	42 (80)	0.886
5FU + irinotecan	35 (36)	12 (36)	23 (64)	0.859	5 (14)	30 (86)	0.216
5FU + oxaliplatin + irinotecan	8 (9)	5 (67)	3 (33)	0.243	4 (50)	4 (50)	0.366
Biological agents							
anti-VEGF	33 (34)	12 (36)	21 (64)	0.880	8 (22)	25 (78)	0.932
anti-EGFR	11 (11)	5 (45)	6 (55)	0.459	2 (18)	9 (89)	0.724
Number of CHT lines > 1	8	1 (12)	7 (88)	0.234	1 (12)	7 (88)	0.724
Number of CHT cycles	8 (3–17)	8 (3–17)	7 (3–12)		7 (3–17)	8 (3–12)	
Patients with > 6 cycles	86/96 (90)	29 (34)	57 (66)	0.679	19 (21)	67 (79)	0.885
Response neoadjuvant CHT							
Progression	9 (10)	1 (11)	8 (89)	0.120	0 (0)	9 (100)	0.089
Stable	22 (23)	7 (32)	15 (68)	0.185	4 (18)	18 (82)	0.079
Partial/complete response	65 (67)	23 (37)	42 (63)	0.191	15 (23)	50 (77)	0.049
CEA decrement after CHT	26 (20)	8 (31)	18(69)	0.810	3 (12)	23 (8)	0.389
Risk groups							
Low	29 (24)	16/44 (36)	13/84 (16)	0.033	11/28 (39)	18/100 (18)	0.053
Intermediate	59 (44)	16/44 (36)	43/84 (50)		9/28 (32)	50/100 (50)	
High	40 (32)	12/44 (28)	28/84 (34)		8/28 (29)	32/100 (32)	
Markers of local inflammation							
CD3+ border	8.5 (4.2–25)	11.40 (3.8–30)	10.97 (0–29)	0.716	8.5 (4.2–25)	9.2 (0–30)	0.244

Table 1 (continued)

	n (%)/median (range)						
	Overall series	Vital status			Presence of recurrences		
		Alive	Dead	P	No recurrence	Recurrence	P
CD3+ intra-tumoural	4.53 (1.8–25.5)	8.95 (1.8–24)	6.88 (1.8–25.5)	0.078	8.04 (2.8–24)	5.86 (1.8–25.5)	0.040
CD3R median, range	0.77 (0.14–5)	0.92 (0.14–5)	0.70 (0.15–3.87)	0.028	0.80 (0.40–5)	0.53 (0.15–3.87)	0.016
Markers of systemic inflammation							
NLR	2.60 (0.63–2.50)	2.34 (0.76–4.91)	2.74 (0.63–21.50)	0.343	2.16 (0.76–4.91)	2.2 (0.63–21.50)	0.596

CRLM colorectal liver metastases, *K-RAS* rat sarcoma virus, *CEA* carcinoembryonic acid, *CHT* chemotherapy, *5FU* fluorouracil, *VEGF* vascular endothelial growth factor, *EGFR* epidermal growth factor receptor, *CD3+ border* lymphocyte CD3+ in the liver tissue around the lesion, *CD3+ intra-tumoural* lymphocyte CD3+ inside the tumour, *CD3R* ratio between the absolute count of CD3 + intra-tumoural and CD3 + border, *NLR* neutrophil absolute count divided by the absolute lymphocyte count

chemotherapy. Table 3b (see supplemental materials) describes the Cox regression analysis comparing the three groups.

Discussion

This is the first study that describes the relationship between local and systemic inflammation in colorectal liver metastases in humans.

To individualize perioperative systemic therapy and to better select patients who may derive benefit from surgery, prognostic factors have been sought.

With the increasing evidence that the host immune system plays a crucial role in OS and RFS of patients affected by CRLM, several papers have reported that systemic and local immune responses are two faces of the same medal in the immune response against cancer [15–23].

The role of CD3+ intra-tumoural infiltration is now well-established, and several reports have shown that increased CD3+ intra-tumoural infiltration is a positive prognostic factor in several solid tumours and CRLM [14, 15]. CD3 cells infiltrate not only the tumour, as we pointed out in our recent paper [25], but also peri-tumoural tissue, being more abundant in this last compartment, suggesting that non-tumoural liver tissue elicits an inflammatory response against CRLM. For this reason, we use the CD3 tumoural/border ratio to describe the intensity of the local immune response.

By adjusted Cox analysis, we found that a CD3R greater than 0.61 is a protective factor for OS (HR 1.73, $p = 0.02$, 95% CI 1.05–2.84), whereas it has no role in RFS (HR 1.28, $p = 0.223$, 95% CI 0.86–1.91). The result for OS is a further confirmation of the role of CD3+ cells in the immune response

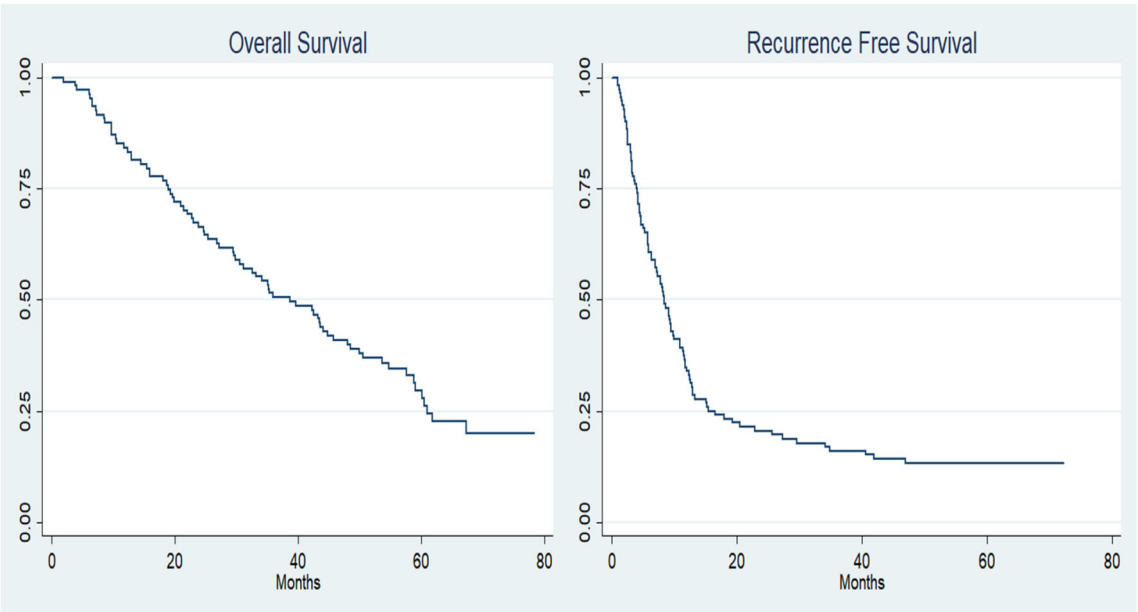

Fig. 2 Kaplan–Meier survival curves of the overall series: overall survival on the left recurrence-free survival on the right

Table 2 a OS analysis for CD3R and NLR. b OS analysis for the 3 different risk groups (low, intermediate, high)

OS analysis	HR	P	95% CI
Cox for CD3R	1.47	0.09	0.94–2.29
Adjusted Cox			
CD3R	1.73	0.02	1.05–2.84
Sex	1.07	0.78	0.65–1.79
Age (> 65 years)	1.01	0.34	0.99–1.03
T of the primitive tumour (T1–2 vs T3–4)	1.18	0.71	0.49–2.84
N of the primitive tumour (N0 vs N+)	1.74	0.09	0.93–3.28
Number of lesions removed (CRLM < 4 vs CRLM ≥ 4)	1.27	0.34	0.78–2.05
Neoadjuvant chemotherapy (yes/no)	1.23	0.53	0.64–2.38
Cox for NLR	1.34	0.18	0.87–2.09
Adjusted Cox			
NLR	1.58	0.07	0.96–2.61
Sex	1.06	0.80	0.64–1.79
Age (> 65 years)	1.01	0.45	0.99–1.03
T of the primitive tumour (T1–2 vs T3–4)	1.20	0.68	0.51–2.84
N of the primitive tumour (N0 vs N+)	1.73	0.09	0.93–3.25
Number of lesions removed (CRLM < 4 vs CRLM > 4)	1.28	0.31	0.79–2.07
Neoadjuvant chemotherapy (yes/no)	1.37	0.35	0.71–2.65
Cox for 3 risk groups			
-Low	1		
-Intermediate	1.94	0.038	1.04–3.63
-High	2.05	0.034	1.06–3.98
Adjusted Cox			
3 risk groups			
-Low	1		
-Intermediate	2.67	0.007	1.31–5.42
-High	2.86	0.005	1.37–5.99
Sex	1.24	0.394	0.74–2.12
Age (> 65 years)	1.01	0.327	0.98–1.04
T of the primitive tumour (T1–2 vs T3–4)	1.04	0.937	0.43–2.50
N of the primitive tumour (N0 vs N+)	1.93	0.042	1.03–3.66
Number of lesions removed (CRLM < 4 vs CRLM ≥ 4)	1.17	0.524	0.72–1.92
Neoadjuvant chemotherapy (yes/no)	1.42	0.328	0.73–2.72

CD3R ratio between the absolute count of CD3 + intra-tumoural and CD3 + border, *NLR* neutrophil absolute count divided by the absolute lymphocyte count, *T* tumour stage of the primitive lesion, *N* nodal status of the primitive lesion, *CRLM* colorectal liver metastases, *HR* hazard ratio, P *p* value, *CI* confidence interval

T tumour stage of the primitive lesion, *N* nodal status of the primitive lesion, *CRLM* colorectal liver metastases, *HR* hazard ratio, *P p* value, *CI* confidence interval

against tumours. Preoperative factors such as neoadjuvant chemotherapy, stage of the primitive lesion and intrahepatic tumour burden are deeply linked with the local immune response, as demonstrated by the adjusted Cox analysis.

The NLR is a promising biomarker that has been correlated with survival and response to chemotherapy. The correlation between NLR elevation and inferior survival in patients with CRLM has still to be elucidated. Persistent chronic inflammation is proved to trigger carcinogenesis of normal cells [13]. Previous reports have demonstrated that the inflammatory response, by promotion of angiogenesis and suppression of the immune response, will create a hospitable microenvironment in which the survival, expansion and epigenetic changes of premalignant cells can be supported and promoted [26]. As described in the published literature, CRLM patients who had the presence of an inflammatory response carried a more aggressive tumour in biological behaviour and a higher rate of tumour recurrence [27]. Neutrophils are regarded as the main source of vascular endothelial growth factor (VEGF), which acts as a proangiogenic mediator in tumour-related

angiogenesis and therefore accelerates the progression of malignancy [28]. Meanwhile, a heightened number of neutrophils lead to the up-regulation of cytokines and chemokines (interleukin-1, interleukin-6 (IL-6) or tumour necrosis factors), thus facilitating tumour proliferation [13]. In addition, in vitro assay found that increased neutrophils in peripheral blood inhibited the cytolytic activity of lymphocytes and natural killer cells towards tumour cells [29].

In our study, we aimed to analyse the role of local and systemic inflammation on the oncological outcome of patients affected by CRLM treated with surgery. NLR has been found to be the more relevant biomarker of systemic inflammation, whereas CD3+ infiltration has been selected as a relevant biomarker of local inflammation. Combining the two biomarkers, three different risk groups were determined. High levels of systemic inflammation and low levels of local inflammation are known as negative prognostic factors affecting survival (see Cox regression analysis, Table 2b). Similar findings on RFS were not statistically significant (see Table 3b in the supplemental materials).

As reported by Turner et al. in a series of patients affected by stage II colorectal cancer, systemic and local inflammation plays an important role in the prognostication of this disease compared to the standard classification [30], and the combinations of these two biomarkers can be helpful in the clinical practice to address different follow-up schedule and postoperative treatment. In our series of CRLM patients, this evidence is confirmed. In fact as shown in Fig. 3, low-risk patients and high-risk patients both treated with neoadjuvant preoperative chemotherapy have a statistically significant different OS at 3 and 5 years of 68% and 38% versus 50% and 28%, respectively, and a median survival of 58 versus 33 months, respectively (HR 2.25 CI 95% 1.05–4.83 $p = 0.036$).

In our paper we have found that associating systemic and local inflammation index, we could provide a stratification of

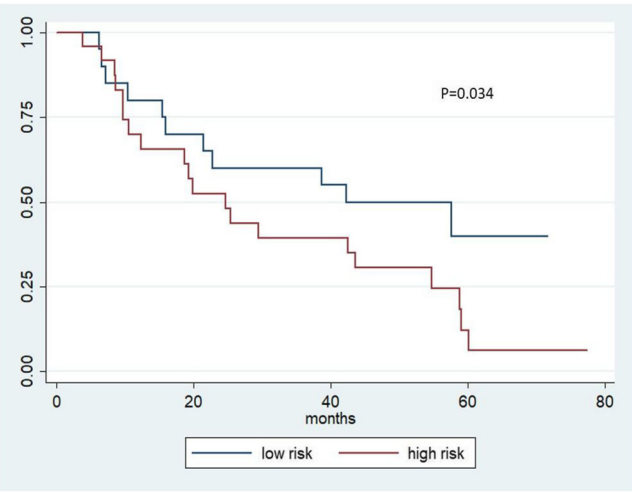

Fig. 3 Kaplan–Meier survival curves of low-risk group vs high-risk group in patients treated with preoperative chemotherapy

patients affected by CLRM independently from baseline characteristics and chemotherapy regimen (see Table 1) even if we didn't find a direct correlation. Nevertheless, several papers have found a possible link between local and systemic inflammation. In particular, the paper by Moles et al. [31] shows how neutrophils are recruited from the bloodstream to the liver in case of hepatic injury through the expression of chemokines. Neutrophils can leave the injured tissue once the healing process has been concluded and the inflammatory environment is turned off returning in the bloodstream with a process called inverse migration [32]. Translating these findings into clinical practice, we may speculate that high level of NLR may indicate an impaired ability of the peri-tumoural tissue to recruit neutrophils to elicit their anticancer activity or the development of immune-tolerance by the peri-tumoural tissue towards cancer cells that may escape from anti tumoural innate mechanism. Further analysis is needed to confirm these hypotheses.

A deeper analysis of the immune infiltration may help to discover new targeted therapies. Promising new drugs act against immunomodulators, and programmed cell death protein-1 (PD-1) is one of the most promising markers [33]. It has been demonstrated that tumours with a high grade of local inflammation (called “hot tumours”) are the ones that better respond to the anti-PD-1 therapies. PD-1 blockade has been tested in colorectal cancer (CRC) where a phase I study resulted in a complete response in one CRC patient [34], and recently, the FDA approved the combination of nivolumab and ipilimumab (2018) in metastatic CRC with genetic features such as mismatch repair deficiency or high microsatellite instability. It is now well-known that PD-1 is widely expressed on T cells and plays an important role in establishing local immunosurveillance. Local immune infiltration plays an important role on oncological outcome of patients affected by CRLM as reported by Mlecnik et al. [35] that demonstrated how local immunoscore defined as the local CD3/CD8 CRLM infiltration is related with a better oncological outcome and is correlated with the response to systemic treatment. These findings show that the immunological environment of CRLM must be one of the targets of new systemic therapies, but further efforts must be undertaken to better analyse the relationship between cancer cells and peri-tumoural environment to find new immune-checkpoint molecules (ICMs) such as PD-1.

This study has several weak points. First of all, retrospective analysis could introduce selection bias even if we analysed a consecutive series of patients. The cutoff that we calculated for NLR (2.12 for OS and 1.71 for RFS) is lower than the ones reported in the literature (2.5–5), probably due to the high number of patients (76%) treated with neoadjuvant chemotherapy that has an important immunosuppressive role. In particular, this element can be a confounder in case pegfilgrastim is administered to patients. Unfortunately, the use of stimulating growth factors is not available for all

patients, because half of our patients are submitted to chemotherapy in others centres. Another issue to consider is the time interval between the last cycle of chemotherapy and the day when a blood sample is taken. In all studies [17–21] analysing the importance of NLR in CRLM, the blood sample is taken in the perioperative workup that generally is within 2 weeks from surgery. As described in the Methods section, in our series, all blood samples were taken within the same period. Moreover, the study of Mao et al. [36] shows that NLR measured before chemotherapy is higher than after chemotherapy (2.4 ± 1.1 and 2.1 ± 1.6, respectively, $p < 0.001$), underlining the immunosuppressive role of neoadjuvant chemotherapy. In our series, we analysed patients with and without a history of chemotherapy, and this can be another confounder; but in order to build a universal immunoscore and considering that all patients were submitted to surgery after 4 weeks from the last chemotherapy regimen, we consider this effect negligible. Moreover, a recent internationally validated risk score did not consider chemotherapy as a possible variable in the immunoscore [37]. The lower median value of the NLR in the chemotherapy group can decrease its effect, but it still remains statistically significant in the adjusted Cox analysis. The improved OS of low-risk patients compared to high-risk patients in the subgroup of patients treated with neoadjuvant chemotherapy is evident in the Kaplan–Meier curves of Fig. 3. Undoubtedly, CD3R plays a more relevant role in the local immune response, but the association of the two tumour markers improves the level of stratification and is a further confirmation of the importance of the interaction between local and systemic inflammation on the tumour biology.

Even if the combination of the two biomarkers can be determined only after surgical resection, this immunoscore has not only a prognostic value but also may help clinicians to better select patients to address postoperative chemotherapy. In fact, data on colorectal cancers show that the degree of local inflammation can predict response to chemotherapy [14]. Similarly, a low level of NLR has been associated with increased response to chemotherapy in colorectal cancer [38]. Given these two findings, combined with the evidence that perioperative chemotherapy is important in selecting chemoresponsive patients that will benefit from surgery in terms of RFS but not OS [39], this new immunoscore will probably help clinicians to recommend postoperative chemotherapy only to high-risk patients.

The monocentric retrospective design of our study needs external validation in a prospective setting to confirm our findings.

Conclusion

NLR and CD3+ are both promising markers of systemic and local inflammation, respectively. The present analyses show that their interaction further demonstrates the tight relationship between systemic and local inflammation. Scoring this interaction improves prognostication and may help clinicians to plan different postoperative strategies such as adjuvant chemotherapy and different follow-up schedule.

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