Dear Editor,

We strongly appreciate you have considered our manuscript as acceptable for publication in your Journal. We also thank you and the reviewers for your constructive comments to improving the quality of our work.

Following the latter, mainly, we have improved the language quality of the manuscript by contracting the services of a specialised biomedical editing company (MOROTE TRADUCCIONES S.L.), and we have developed two clinician-friendly nomograms in order to better conceptualise the use of the predictors we describe in our work.

Please find below our point-by-point responses to all issues raised.

Reviewer #1:

This prospective study reporting value of combining circulating leukocytes ratios and tissues infiltrated leukocyte ratios on predicting OS and RFS of both RCRC and LCRC patients. The study is well conducted and presented.

We thank this reviewer for her/his valuable commentaries.

Major point:

1. As a prospective cohort study, whether this clinical trial has been registered on clinicaltrials.gov or similar. The corresponding website and registration number must be showed in the manuscript.

Even though we consider this study as a prospective one, as it well fits to your definition in the guidelines ("…*Prospective Study articles are submitted by any author and describe an observational study of a population for a sufficient number of persons over a sufficient number of years to generate incidence and/or mortality rates subsequent to the selection of the study group.*"), the study was not conceived as a clinical trial since neither drugs nor supplements would be under assessment to establishing usage, dosage, etc., but it will be only an observational characterisation of patients that had undergone surgical treatment for colorectal cancer condition. Hence, we cannot provide any document to justify this as a clinical trial, as it has never been registered as such nowhere.

2. The heterogeneity of the tumor is relatively strong, and the degree of tumor infiltrating cells in different lesion may be different. In the study, is there any corresponding material standard when selecting the tumor tissues of the enrolled patients for immunohistochemistry? Is the middle part or the tumor edge to be selected? Whether these parts can represent the overall infiltration situation, the author needs further explanation.

We agree that high heterogeneity of the tumour, with respect to the degree of immune infiltrating cells within its different parts, arises a serious methodological problem in order to compare by immunohistochemistry the immune composition of tumours from different patients or even, from different sections of a specific tumour. But it is precisely because of this heterogeneity that a corresponding material standardisation would hardly fit to all actual situations. What we standardised instead was the sample collection protocol, for all samples were excised by specialised pathologists, from the middle part of the tumour, avoiding both the epicentre (due to likely necrosis presence) and the edge (because of the risk to include healthy tissue) thereof; always prioritising sufficient tumour integrity to warrant further histopathologic diagnosis. With these criteria, albeit the selected sections might not be representative of the overall infiltration of the tumour,

a comparison amongst different pieces is reliable. In this revisited version of the manuscript, we have made explicitly clear these recommendations (*Materials and Methods, Tissue preparation section*).

3. The author found that all six indicators are related to the patient's prognosis. How to evaluate the prognosis when these 6 indicators conflict in patients? The author should build a model for integrated evaluation such as nomogram.

We recognise that such a tool like nomogram, to facilitate surgeon and physician's decision-making from all variables described, is missing in our work and we thank the reviewer for the suggestion. Thus, we have developed two clinician-friendly nomograms for both OS and RFS prognosis, based on the 6 and the 3 predictors respectively, we describe for RCRC patients' outcome.

Reviewer #2:

We really thank this reviewer for her/his evaluation of our manuscript.

1. Will postoperative chemotherapy affect overall survival time and RFS?

Though oncologists logically hope that adjuvant chemotherapy (CT) will improve both OS and RFS, when administered to CRC patients in the first-line therapy, multiple factors influence a poor or even none response (1). As can be seen in the tables below, in our cohort neither RCRC nor LCRC patients who received adjuvant CT showed differences regarding the OS with respect to those who did not (Non-CT). In the case of RFS, only RCRC patients showed significant differences with Non-CT. After all, since our main goal was to assess the composition of leukocytes in the different compartments as numerical indexes of survival, we decided not to go into their association with highly diverse CT effects.

Univariate analysis for OS.

	Total patients		Univariate analysis			
	n (%)	HR	95% CI		*P*	
Variables			Low	High		
Non-CT						
Right	34 (63,0)	1,201	0,230	6,270	0,828	
Left	20 (37,0)	0,706	0,121	4,125	0,699	

Univariate analysis for RFS.

	Total patients		Univariate analysis			
	n (%)	HR	95% CI		*P*	
Variables			Low	High		
Non-CT						
Right	34 (63,0)	4,658	1,371	15,826	0,014	*
Left	20 (37,0)	3,106	0,817	11,812	0,096	

2. The case collection is from 2017 to 2019. Not every patient has a 4-year follow-up period, so the survival chart should indicate the number of cases

We agree with this observation, thus we have added to Figure 1 a table with the number of patients at risk at different times, for both OS and RFS rates.

3. CD14 includes mCD14 and sCD14. How to distinguish between mCD14 and sCD14 when detecting CD14 by immunohistochemistry? Does CD14+ alone represent monocytes well?

When human monocytes/macrophages are aimed to be detected by following their CD14 expression, it is very important to consider the tissue to be assessed. In bloodstream, monocyte-CD14 can be found associated to monocyte membrane (mCD14) but also in its soluble form (sCD14) after excised from the membranes. For the former, flow cytometry (FACS) is sufficient enough to get accurately monocyte composition since only a minor proportion of circulating monocytes exhibit a $CD16^{+}CD14^{dim}$ phenotype (2) then escaping to CD14-binding FACS analysis; for the latter, proper ELISA kit will work. Nonetheless, in this study blood leukocyte counts (monocytes, lymphocytes, neutrophils and platelets) were obtained from a laboratory-routine haemogram, then soluble epitopes are not considered for the calculus of blood cell ratios.

On the other hand, albeit soluble epitopes (such as sCD14) are found in living solid tissues, FFPE samples do not contain them in detectable amounts following IHC protocols, mainly due to the dehydration-rehydration processes inherent to the paraffin-embedded tissue. Thence, what we have detected by IHC is only mCD14, which in turn represents 100% of the macrophages contained in the colon tissue (3).

Reviewer #3:

The manuscript is interesting. "Intertwined leukocytes balances in tumours and peripheral blood as robust predictors of right and left colorectal cancer survival." A prospective study of patients with colorectal cancer found differences in prognosis after surgical intervention between patients with RCRC and LCRC colorectal cancer. However, it requires some answers to comments particularly related of the prognosis.

We really thank also this reviewer for her/his commentaries and suggestions.

#Core tip: This was a prospective study involving 65 patients with colorectal cancer, seeking to find robust predictors of survival after surgical intervention, amongst the leukocytes balances in peripheral blood and tumour tissues. A number of these variables are shown to predict OS and RFS, in both RCRC and LCRC patients, thus allowing to improve pre- and postoperative patient's treatments. Method: Paraffin-fixed samples of tumour and peritumour tissues were assessed for leukocytes concentrations by immunohistochemical detection of CD4, CD8 and CD14 markers expression.

Q: How to improve pre-operative patient's treatments?

With this expression, what we want is to emphasise our hope that the implementation of these indexes and nomograms, may make surgeons and clinicians think about the possibility of delaying the scheduled surgical intervention until better indexes predict a better patient outcome. This is, for instance, after a routine preoperative colonoscopy showing a poor index-based outcome, physicians can change the patient's regime in order to improve leukocyte ratios and then achieve better predictive values on a second colonoscopy.

#CONCLUSION: Easily obtainable variables at preoperative consultation, defining the status of leukocytes balances between peripheral blood and tumour tissues, are reported as robust predictors for OS and RFS of both RCRC and LCRC patients.

Q: The leukocytes of tumour tissues were easily obtainable variables at preoperative consultation?

We thank the reviewer for this observation. In fact, we were referring to the peritumoural tissue, which is the one involved in all indexes described and is easy to obtain in a routine preoperative colonoscopy, but we were wrong to use the generic term "tumour" to describe it. We have made the appropriate changes throughout the text.

#Patient selection: They were surgically treated according to each patient's condition for right (caecum, ascending or transverse colon) or left (descending or sigmoid colon) hemicolectomies followed by anastomosis, with partial hepatectomy if 8 synchronous metastasis was presented.

Q: What was the outcome of these 8 patients? Are they considered as influencing factors?

"Eight" is not the actual figure: this is a typo, inserting the page number 8 into the rest of the phrase. In fact, they were 13 patients with synchronous (liver) metastasis in the studied cohort (6 with RCRC and 7 with LCRC).

As shown in tables below, this condition did not affect the OS in a significant manner with respect to those patients without it, neither for RCRC nor LCRC patients. On the contrary, as extensively established elsewhere (1), synchronous metastasis significantly influenced the RFS in our cohort, since it is present in patients at advanced stage II and above, then in a poorer condition at the time of surgery. Despite that, we did not delve into the influence of this variable in our model since hepatic lesions were resected as part of the surgical procedure itself (*Materials and Methods, Patient selection section*).

#RESULTS: They ranged from stages 0 to IV, based on tumour-nodes-metastasis (TNM) classification, 28 (43.1%) of them presenting metastasis (either synchronous or metachronous at the time of surgery); and approximately a half, 30 (46.1%), received adjuvant therapy after surgery.

Q: 28 (43.1%) of them presenting metastasis (either synchronous or metachronous at the time of surgery); 30 (46.1%), received adjuvant therapy after surgery. What effect did they have on the outcome of the follow-up?

This question can be divided into three parts. Its first part, the one regarding the effect of synchronous metastases, has been already responded with the previous question above.

A similar response deserves its second part about the metachronous cases, for the emergence of metastatic lesions after the resection of the primary tumour is part of the outcome variable RFS that it is being analysed. Then, despite metachronous incidence showed to be statistically influencing RFS in both RCRC and LCRC patients (please see tables below) we did not sum up this variable neither to our model, as it constitutes the proper RFS manifestation.

With respect to the third part of the question, regarding the effect of chemotherapy on the outcome, although oncologists logically hope that adjuvant chemotherapy (CT) will improve both OS and RFS, when administered to CRC patients in the first-line therapy, multiple factors influence a poor or even none response (1). As can be seen in the tables below, in our cohort neither RCRC nor LCRC patients who received adjuvant CT showed differences regarding the OS with respect to those who did not (Non-CT). In the case of RFS, only RCRC patients showed significant differences with Non-CT. Despite that, since our main goal was to assess the composition of leukocytes in the different compartments as numerical indexes of survival, we decided not to delve into their association with highly diverse CT effects.

Univariate analysis for OS.

	Total patients n (%)	HR	Univariate analysis 95% CI		*P*	
Variables			Low	High		
Synchronous						
Right	34 (63,0)	0,313	0,061	1,618	0,166	
Left	20 (37,0)	1,060	0,194	5,803	0,946	
Metachronus						
Right	34 (63,0)	0,285	0,055	1,481	0,135	
Left	20 (37,0)	0,777	0,137	4,411	0,776	
Non-CT						
Right	34 (63,0)	1,201	0,230	6,270	0,828	
Left	20 (37,0)	0,706	0,121	4,125	0,699	

Univariate analysis for RFS.

	Total patients n (%)	HR	Univariate analysis 95% CI		*P*	
Variables			Low	High		
Synchronous						
Right	34 (63,0)	0,097	0,024	0,395	0,001	**
Left	20 (37,0)	0,219	0,065	0,737	0,014	*
Metachronus						
Right	34 (63,0)	0,141	0,041	0,490	0,002	**
Left	20 (37,0)	0,226	0,053	0,966	0,045	*
Non-CT						
Right	34 (63,0)	4,658	1,371	15,826	0,014	*
Left	20 (37,0)	3,106	0,817	11,812	0,096	

#Patients' progression follow-up: The survival analysis, with a median follow-up of 26 months,... Patient selection .....were recruited .....from January 2017 to September 2019.

Q: What was the end date of the follow-up? How to get "mean follow-up of 26 months"?

The follow-up ended on March 31, 2021. Since patients were progressively recruited from January 2017 to September 2019, this means that patients could be followed from 50 months (for the first recruits) to only 18 months (for those latest recruited), although some would die during the follow-up. Eventually, the calculated median for the duration of follow-up of the 65 patients enrolled in the study was 26 months.

METHODS : **Ratios of leukocytes concentration in blood and tissues were calculated, and evaluated for their predictor values for OS and RFS with Spearman correlations, Cox univariate and multivariate proportional hazards regression, followed by the calculation of the Receiver Operating Characteristic (ROC) and Area Under the Curve (AUC), and thedetermination of Youden s optimal cutoff values for those variables which significantly correlated with either RCRC or LCRC patient s outcome.**

Q : **C-index was calculated?**

Yes, it was.

We performed the Receiver-Operating Characteristic (ROC) analysis and the Area under the Curve (AUC) quantifying marker, to determine the potential accuracy of our variables to predict OS and RFS. Then, the discrimination value of AUC was corroborated with C-

index (please see table below) for those significant variables, although this information is not included in the manuscript.

Surv.	Predictor	AUC	C-index
OS	CD8ptR	0.585	0.75
OS	CD4CD8ptR	0.550	0.58
OS	LMRptR	0.807	0.54
OS	CD8MRptR	0.757	0.63
OS	LMRb/LMRptR	0.672	0.531
OS	LMRb/CD8MRptR	0.601	0.64
OS	LMRb/CD4MRptL	0.786	0.8
RFS	LMRptR	0.737	0.5
RFS	LMRb/LMRptR	0.678	0.48
RFS	LMRb/CD8MRpt	0.601	0.7
OS	CD4CD8tL	0.524	0.55
OS	PLRbL	0.619	0.65
OS	CD4CD8ptL	0.726	0.75
OS	CD8MRtL	0.571	0.75
RFS	CD8MRptR	0.672	0.606
RFS	CD8ptR	0.528	0.55
RFS	CD4CD8ptR	0.506	0.56
RFS	CD4CD8L	0.656	0.65
RFS	PLRbL	0.635	0.7
RFS	CD4CD8ptL	0.760	0.75
RFS	CD8MRtL	0.781	0.75
RFS	LMRb/CD4MRptL	0.563	0.65

Editorial Office's comments and suggestions:

(1) Science editor: 1 Scientific quality: The manuscript describes a prospective study of the leukocyte balances and colorectal cancer survival. The topic is within the scope of the WJGO. (1) Classification: Grade B, Grade C and Grade D; (2) Summary of the Peer-Review Report: This prospective study reporting value of combining circulating leukocytes ratios and tissues infiltrated leukocyte ratios on predicting OS and RFS of both RCRC and LCRC patients. The study is well conducted and presented. The author should build a model for integrated evaluation such as nomogram. The questions raised by the reviewers should be answered; (3) Format: There are 4 tables and 6 figures; (4) References: A total of 39 references are cited, including 16 references published in the last 3 years; (5) Self-cited references: There are no self-citations; and (6) References recommendations: The authors have the right to refuse to cite improper references recommended by the peer reviewer(s), especially references published by the peer reviewer(s) him/herself (themselves). If the authors find the peer reviewer(s) request for the authors to cite improper references published by him/herself (themselves), please send the peer reviewer's ID number to editorialoffice@wjgnet.com. The Editorial Office will close and remove the peer reviewer from the F6Publishing system immediately.

All these questions have been developed above as part of the responses to each three reviewers; just to highlight that following suggestions of both Reviewer#1 and Editorial Office, we have developed two nomograms for OS and RFS respectively, which are included and discussed in the revisited version of our manuscript, along with their calibration and validation curves.

2 Language evaluation: Classification: Grade A and Grade B and Grade C. One of the authors (Karla Marina Montalbán-Hernández) is an English native speaker. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the CONSORT 2010 Statement, the Institutional Review Board Approval Form, and the Signed Informed Consent. The authors should provide the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement, and Clinical Trial Registration Statement. No academic misconduct was found in the Bing search.

All documents requested have been provided, with the exception of the Clinical Trial Registration Statement. This is due to, as above explained to Reviewer#1 with the same criterion, the fact that even though we consider this study a prospective one, as it well fits to your definition in the guidelines ("…*Prospective Study articles are submitted by any author and describe an observational study of a population for a sufficient number of persons over a sufficient number of years to generate incidence and/or mortality rates subsequent to the selection of the study group.*"), the study was not conceived as a clinical trial since neither drugs nor supplements would be under consideration to establishing usage, dosage, etc., but it will be only an observational characterisation of patients that had undergone surgical treatment for colorectal cancer condition. Hence, we cannot provide any document to justify this as a clinical trial, as it has never been registered as such nowhere.

4 Supplementary comments: This is an invited manuscript. The study was supported by Foundation for the Hospital La Paz Institute for Health Research and the European Union's Horizon 2020 research and innovation program under the Marie Sklodowaska-Curie-'laCaixa'. The topic has not previously been published in the WJGO. 5 Issues raised: (1) I found the language classification was grade C. Please visit the following website for the professional English language editing companies we recommend: https://www.wjgnet.com/bpg/gerinfo/240;

The revisited version of the manuscript has been edited by English-native editors (MOROTE TRADUCIONES, S.L.), as availed by the certificate we have uploaded.

(2) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);

Requested documents have been uploaded.

(3) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;

Original figures have been uploaded in a ready-to-edit file (ppt).

(4) PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout;

All DOI numbers and PMID (if exist) have been added and references are listed with all authors.

and (5) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text.

"Article Highlights" section has been added in the revisited version of the manuscript.

6 Recommendation: Conditionally accepted.

Thank you for conditionally accepting our work. We hope now the revisited manuscript is enough improved as to attain all acceptance requirements.

REFERENCES

1. Modest DP, Pant S, Sartore-Bianchi A. Treatment sequencing in metastatic colorectal cancer. Eur J Cancer. 2019;109:70-83. DOI: 10.1016/j.ejca.2018.12.019.
2. Kapellos TS, Bonaguro L, Gemund I, Reusch N, Saglam A, Hinkley ER, Schultze JL. Human Monocyte Subsets and Phenotypes in Major Chronic Inflammatory Diseases. Front Immunol. 2019;10:2035. DOI: 10.3389/fimmu.2019.02035. PMCID: PMC6728754.
3. Isidro RA, Appleyard CB. Colonic macrophage polarization in homeostasis, inflammation, and cancer. Am J Physiol Gastrointest Liver Physiol. 2016;311(1):G59-73. DOI: 10.1152/ajpgi.00123.2016. PMCID: PMC4967174.