

Mueller et al.
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To the Company Editor-in-Chief and Science editor

January 2021

*Re: Colorectal Cancer of the Young Displays Distinct Features of Aggressive Tumor Biology:
A Single-Center Cohort Study*

Dear Professor Lian-Sheng Ma,

We would like to thank you for the opportunity to submit a revised version of our manuscript. We have made every effort to address all comments and questions from the reviewers in a point-by-point manner. If you see a need for additional clarification, please let us know. Please convey our thanks to the reviewers for their interest in our manuscript and their constructive criticism, which have enabled us to prepare a better manuscript.

We hope you find the revised version of our manuscript to be suitable for publication in *World Journal of Gastrointestinal Surgery*.

With best regards,

Matthias Turina

POINT-BY-POINT REPLY

Reviewer 1:

Remark #1: The authors have chosen a shorter period of time from 2013 to 18 and state that the trend is increasing based on the percentage of patients below 50 years, which may not be true unless either one compares this data with data of a previously defined period (eg. 2008 to 2013) or a joinpoint regression analysis of data of a longer time frame analyzed to arrive at this conclusion. That would have been a more authentic data to incur that the trend is increasing. This is especially true since Fanny ER Vuik et al report that there was no change in the trend of CRC incidence in young in Switzerland in their article (Increasing incidence of colorectal cancer in young adults in Europe over the last 25 years Fanny Er Vuik , Stella Av Nieuwenburg , et al . Gut - 2019 Oct; 68(10):1820-1826)

Our reply: As the reviewer correctly points out, our retrospective study covers the time from 2013-2018 and shows a relatively high incidence of CRC among the young. The conclusion that early-onset CRC seems to be increasing worldwide is drawn from the existing literature and not from our cohort data. A comparison with previous cohorts from the same region would have been mandatory to be able to demonstrate such an increase. However, as Fanny Vuik and colleagues state in their study, a lack of statistical power comparing regions with low population numbers hinders such analyses. We have clarified this claim in the discussion of the paper, which now reads: "Although our population does not provide the power to conclusively show an increase of CRC among the young of the same region, a rising incidence of CRC in young patients has been described by others" on page 8, 2nd paragraph.

Remark #2: While analyzing the colorectal cancer trend, the authors could also have provided the trend for colon and rectal cancers separately too. In those younger than 50 years, the authors could have provided data about those with family history of colon cancer, with IBD, with hereditary colon cancer syndromes, who already have established guidelines for screening. Only data from those with sporadic colorectal cancer in young may help to decide about initiating screening at a lower age than agreed upon.

Our reply: This is a very important point. Data on the frequency of IBD and hereditary colon cancer syndromes is indeed provided in Table 1 and does not reveal major differences between the age groups, which may however be due to the low incidence of such cancers. An analysis of the patients below 50 years of age with sporadic CRC depicted the same trends of significantly higher lymph node metastases and distant metastases compared to patients over 50 as did the cohort including patients with IBD and hereditary CRC syndromes. Part of the result section and discussion was edited accordingly on page 7, 1st paragraph and on page 9, 1st paragraph respectively.

Remark #3: Again to imitate screening program or to advice preventive measures in those below 50 years, no risk stratification has been attempted by the authors, based on the clinical data. though they state that "parameters of interest included clinical as well as histopathological characteristics". Also the authors have not provided the regional incidence (within Switzerland) based on data from more than 400 patients

seen in their institution, to direct the preventive measures to those regions with significantly higher incidence.

Our reply: Indeed, large-scale multi-centric studies or national datasets are required to rewrite cancer screening recommendations. Switzerland has started such initiatives over the past few years, however, comprehensive and complete data sets are still lacking. Due to the lack of power, a risk stratification within our cohort may be misleading. Regional incidence of hospitalized CRC patients below 50 in Switzerland is approximately 7% according to the nation-wide hospitalization database (MSK) of the Federal Statistical Office (BFS). While being of interest to Swiss political authorities, we have decided against including data on regional disparities within a small country such as Switzerland as we believe this not to be of interest to the international readership of the "World Journal of Gastrointestinal Surgery".

Remark #4: Having brought out obesity as risk factor the authors could have indicated the temporal relationship of CRC in young and obesity in this population.

Our reply: Having performed a sub-group analysis of obese patients (BMI>30kgm²) we found no difference between obese vs. non-obese patients in regard of age, tumor differentiation (e.g. signet cell cancer) or tumor stadium (TNM). However, the distribution of obesity among age groups was added to Table 1 to make the readership aware of this important aspect.

Remark#5: They state that "young patients showed a similar stage-dependent overall survival and recurrence-free survival" and attribute this to more aggressive surgical treatment, though there cannot be different oncological procedures for colorectal cancer management in young and older.

Our reply: Indeed, the comparable oncologic outcome between young vs. old and pictures of aggressive tumors among the young are conflicting. As we cannot analyze this issue with our data, we rely on possible explanations by others, as stated in the discussion: "Possible explanations are the more aggressive surgical treatment and higher rates of (neo-) adjuvant chemotherapy offered in younger patients [18, 27, 35]" or "The fact that lymph node dissection was reported to be far more extensive in young patients [27], ..., suggests a more aggressive surgical approach in younger patients. Obviously, younger patients have fewer comorbidities and a better physical/nutritional status, which also make them more suitable to more aggressive operations associated with increased perioperative morbidity." Appropriate comments were made in the text page 9, last paragraph.

Remark#6: It is quoted that higher Percentage of poor tumor grade and signet ring cell type histology was seen in those below 50 years. However, the table show that only 17 out of 411 had signet ring cell type histology. It is also noted that out of 411 patients, histological grade was not available in 120 patients.

Our reply: 4% of all CRC patients show a signet cell differentiation, with 8.8% of CRC showing these features among the young. This number is consistent with the mentioned and discussed literature. However, we acknowledge that due to the retrospective nature of this study many histological or mutational details are missing. Nevertheless, we decided to show the complete data set as it reflects the daily clinical experience. However, to make the reader

aware of this methodologic weakness, we have added appropriate comments on page 11, 2nd paragraph.

Reviewer 2:

This manuscript is an interesting paper. The study shows that the patients with CRC of young-onset have more frequently with locally advanced tumors, lymphatic invasion and with more frequent lymphatic metastases. It suggests the importance of early diagnosis and treatment in young patients with colorectal cancer, so it is important for clinical practice.

Our reply: We would like to thank the reviewer for the kind review of our manuscript.

Reviewer 3:

The manuscript is well written and meets most criteria on the check list, however, there is no new message from the manuscript about colorectal cancer in the young, except that the incidence of colorectal in the young is highest in Switzerland. In addition, to draw a more cogent conclusion on such a topical issue, I think a multi-centre study with larger population size would have been more appropriate. However, I have few comments for the authors.

Our reply: We agree with this comment and included this very message in our discussion section on page 11, 2nd and 3rd paragraph.

Remark#1:

I would have expected a comparison on the 5 years survival in the <50 and >50 we if we were discussing aggressive biology of CRC, which is highly predicated on prognosis. There is no data on CRC recurrence, or why was it not discussed.

Our reply: We thank the reviewer for underlining this point. As stated as limitation in the discussion ("Regarding oncological outcome, long-term survival and recurrence data may by definition not be drawn from the recent time period studied"). Further studies (in collaboration with other centers) may show evidence of cancer aggressiveness among the young with long-term outcome data, including data on tumor recurrences Appropriate note is given to this important aspect on page 11, 2nd paragraph.

Remark#2:

In discussing colorectal cancer, the author made allusion to the fact the rectal cancer may have a different biology as a result of the different embryological origin, which I agree its true. However, nothing was mentioned about the use of MRI in rectal cancer to further describe the radiomic features such as the EMVI, perineural invasion and Circumference resection margin involvement which would have helped to distinctly characterise rectal cancer in both age groups and give us some clue on the need for neo or adjuvant chemotherapy which is another surrogate maker for aggressiveness of the rectal in question.

Our reply: We agree that MRI findings such as those mentioned by the reviewer are crucial for determination of neoadjuvant therapy. Based on our data, we know that among patients with rectal cancer the rate of neo-adjuvant treatments did not differ between the groups

(36%<50yrs vs. 44%>50yrs, $p=0.53$, added on page 6, 2nd paragraph). Our data however do not allow to answer the reviewer's question as to which MRI findings were used as rationale to decide on neoadjuvant therapy in individual patients. As this was not the aim or focus of this study, we have decided against including available incomplete data on this topic.

Remark#3:

Why didn't the author use Prealbumin and ferritin level which are more reliable and reflective of nutritional status.

Our reply: Indeed, there is a lively discussion on the best marker for nutritional status and on flaws of albumin as marker for malnutrition. Since serum albumin is reported to be a solid marker for i.e. mortality, length of stay and infections after CRC surgery (Truong et al., WJGIS 2016, DOI: 10.4240) our center has now routinely measured albumin before surgery over the past decade. Due to its availability (and not superiority), we have chosen albumin as marker for nutritional status.

Remark#4:

Could the high nodal yield in young patient be a function of the immunocompetency in the that age group, knowing that younger patients a more likely to show higher lymphoproliferative ability. This a statement from line 210 " The fact that lymph node dissection was reported to be far more extensive in young patients [27], a result that is concordant with operations performed at our institution, suggests a more aggressive surgical approach in younger patients.

Our reply: This is a very interesting point. In fact, lymph nodes undergo extensive alterations during the aging process. Lymph nodes show degenerative features like fibrosis and lipomatosis over time, which can make a correct staging difficult (Hadamitzki et al. DOI: 10.1111/j.1469-7580.2010.01213.x). The lymphoproliferative ability and cellular activity of lymph nodes may be significantly reduced with advanced age, however, this may not be reflected in the total number of lymph nodes found in a specimen. As we found only very limited evidence for higher numbers of lymph nodes in tumor specimens of young patients in the literature (e.g. Shen et al., Arch Pathol Lab Med, 2009, DOI: 10.1043/1543-2165-133.5.781), we have decided against adding a specific paragraph to discuss this aspect in our manuscript. Hopefully future histopathological studies will shed light into this matter.

Remark#5:

Obviously, younger patients have fewer comorbidities and a better physical/nutritional status, which also make them more suitable to more aggressive operations associated with increased perioperative morbidity.- Is the author suggesting that age plays a factor in the degree of radicality of the surgery offered". Isn't this a bias already against the over 50yrs

Our reply: With the mentioned statement we want to underline, that not age per se, but comorbidities and physical state are decisive for therapy selection. Not only surgery, but also radicality of the (neo-) adjuvant regimen may differ in respect to side effects experienced. However, we did note that apparently, operations were carried out in a more "aggressive" manner in young patients, presumable as this cohort may benefit more and suffer less from potential complications of more aggressive lymph node dissection. We believe this to reflect the individual physician's attitude to cancer treatment in young versus older patients, which

is why we believe this to be an important finding of this study. It remains disputable however, if this attitude is morally correct of medically justified, and we have added a section on this crucial aspect in the discussion chapter on page 9, last paragraph.

Remark#6:

In line 226 "A better understanding of the molecular make-up of young-onset cancers is being achieved by recent advances in decoding the genome with next-generation sequencing, which I agree that is correct. However, the other correlation that would have been more interesting is to see if the molecular mutation has any correlation with CRC metastasis in either group.

Our reply: We thank the reviewer for bringing up this constructive suggestion. We performed a sub-group analysis in either group and depicted significant differences. The results section (page 7, 2nd paragraph) was edited and reads now as follows: "However, a subgroup analysis of young patients with a molecular mutation (incl. MSI, K/RAS, BRAF, HER2) vs. young patients without mutations, revealed a significantly higher incidence of distant metastases in those with a mutation (60% vs. 30% M+ respectively, $p=0.03$). Additionally, individuals >50 years with a molecular mutation showed a significantly higher incidence of N+ (57% vs. 37% respectively, $p<0.01$) and M+ status (45% vs. 22% respectively, $p<0.001$) compared to non-mutated CRC patients in the same age group."

EDITORIAL OFFICE'S COMMENTS

Science editor:

1 Scientific quality: The manuscript describes a retrospective cohort study of the colorectal cancer of the young displays distinct features of aggressive tumor biology. The topic is within the scope of the WJGS. (1) Classification: Grade B and two Grades C; (2) Summary of the Peer-Review Report: The manuscript is well written and meets most criteria on the check list. The questions raised by the reviewers should be answered; and (3) Format: There are 3 tables and 1 figure. A total of 47 references are cited, including 12 references published in the last 3 years. There are no self-citations. 2 Language evaluation: Classification: Two Grades A and Grade B. A language editing certificate issued by a native English speaker was provided. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the Institutional Review Board Approval Form, and the written informed consent. No academic misconduct was found in the CrossCheck detection and Bing search. 4 Supplementary comments: This is an unsolicited manuscript. No financial support was obtained for the study. The topic has not previously been published in the WJGS. 5 Issues raised: (1) The "Author Contributions" section is missing. Please provide the author contributions; (2) 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; (3) 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; and (4) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text. 6 Recommendation: Conditional acceptance.

Our reply: We have added the requested information (Author contributions, original pictures, PMID/DOI and Article Highlights) to the manuscript and thank the science editor for this kind review of our manuscript. The manuscript was revised according to the "Guidelines and Requirements for Manuscript Revision: Retrospective Cohort Study".