

ANSWERING REVIEWERS

Dear Editors and Reviewers:

Thank you very much for your attention and reviewers' evaluation and comments concerning our case report as following:

Title: A Case: Monitoring disease progression and treatment efficacy with circulating tumor cells

Author: Yuan-Yuan Qiao, Kai-Xuan Lin, Ze Zhang, Da-Jin Zhang, Cheng-He Shi, Ming Xiong, Xiu-Hua Qu, Xiao-Hang Zhao*

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 15687

Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our further researches. We have studied comments carefully and have revised the manuscript according to your kind advices and detailed suggestions. We hope the revised case will meet with approval. Now I answer the questions one-by-one.

1 Format has been updated

I have modified the manuscript according to the format for case report of *World Journal of Gastroenterology*. Our report is a combination detection technology of laboratory and clinical utility. Because of its particularity, we have some difficulties to describe in the COMMENTS subsection. We expect editor to give more suggestions.

2 Revision has been made according to the suggestions of the reviewer

Reviewer *02441236*

Comment 1: Dear Sir Please explain more about the other examination comparing CTCs analysis in the table Regards.

Response: Thanks for the referee's kind suggestion. According to the advices, the 12 serum tumor markers results were added in addition to the imaging in the table 1. In the table, we can see that results of the serum markers, imaging and CTCs detection of the Changes in treatment and disease process. In the discussion section of the article, we also investigate CTCs as a useful complement detection can serve to monitor progress of tumor recurrence and metastasis (table 1; page 10, line 3-6; 24-27 et al.).

Reviewer *02570860*

Comment 1: The Introduction was lengthy, please re-write it.

Response: Thank you very much for this comment. We have re-written the Introduction section. The words of modified introduction are limited to 400 words. We hope that the meaning of the original text can be expressed clearly.

Comment 2: The author described that after traditional Chinese drug therapy, the patient exhibited a stable condition. Please specify what kind of traditional Chinese drug the patient used. What is the mechanism of that drug?

Response: Thank you very much for your comment. TCM for treating tumor mainly adjust the overall balance. As we know now because of the complex composition, their pharmacological effects showed multiple effects and multiple targets. TCM may promote the restoration and consolidation therapy; may comprehensively strengthen the body resistance. There are dozens of medications in prescription of this follow-up patient. Each component is not described in detail here; one of them is Astragalus which contains a lot of effective ingredients including polysaccharide, protein, amino acids, alkaloids, glycosides, flavonoids etc. Many studies showed that Astragalus membranaceus polysaccharide (AMP) can promote antitumor activity via improving immune responses of host organism and promote the synthesis of NO in macrophages, significantly enhance the cytotoxic effect of macrophages on tumor cells. (page 12, line 3-9).

Comment 3: What was the staging workup protocol? Why was neoadjuvant chemordiaion not performed?

Response: Neoadjuvant chemoradiotherapy of esophageal cancer is an important idea of comprehensive treatment, but there is no standard method to perform so far. Because of the difference in tumor site, progression and pathological, there are still many problems in neoadjuvant therapy for esophageal cancer and the effects are not clear. In theory, operation should be actively done for I / II A stage esophageal cancer; neoadjuvant chemotherapy should be used in preoperative for II B and III esophageal cancer to improve the resection rate and prolong the survival time of the patients.

But neoadjuvant chemoradiotherapy carried out in lung cancer treatment and not be used in the treatment of ESCC in our hospital. I have discussed the therapeutic regimen with the patient' doctor and he thought the patient was suitable for curatie surgery based on evaluation results for the patients, so the resection was performed to cut the tumor and metastasis lymph nodes.

Comment 4: Please explain why CTC elevated quickly after operation? Was it an incomplete resection? The clinical scenario is queer.

Response: The question is very important, and we have discussed about this. The CTC number was one before surgery, but increased to 14 after operation. It was considered about two reasons: firstly, the patient had lymph node metastases, and because the some lymph nodes around the gastric artery, they could not be removed completely, leaving predict tumor lesions; Secondly, researches showed that the operation procedure causes tumor cells to squeeze into the blood, significantly increased the CTC number in peripheral blood, and appearance of CTCs may associate with tumor metastasis. We will further summarize the relationship between the counts of circulating tumor cells before or after operations in peripheral blood in patients and prognosis in patients of ESCC. (page 11, line 6-15).

Comment 5: The author should discuss whether CTC can detect recurrence/metastasis earlier than image studies.

Response: Thanks. The CellSearch system approved by FDA for metastatic breast cancer, colorectal cancer and prostate cancer to evaluate the progression-free survival (PFS) and overall survival (OS). Our case report found that CTCs changes were consisted with imaging results. CTCs may be additional tests for metastatic cancer to supplement the current methods. But now we can't determine whether CTC can detect recurrence/metastasis earlier than image. In theory, the possibility that invasive but localized tumors may shed CTCs into the bloodstream before any bona fide metastases are established suggests that CTC detection may provide important information in individuals at risk. More researches are needed to prove it. Therefore, in addition to the evaluation of CTCs in prognostic value, in monitoring curative effect, recurrence and metastasis early diagnosis and individualized treatment guidelines, CTCs will continue to expand the scope of application in clinic. (page 9, line 21-28)

Reviewer02922262

We are very grateful for the editor's review my article twice and the kind suggestions for us. Thank you for your tolerance and patience.

Comment 1: In the presented form article does not qualify for publication in World Journal of Gastroenterology.

Respond: We fully agree with the reviewer and thank the suggestion. We have been re-modified our full text in accordance with the requirements of the magazine format.

Comment 2: Authors have corrected errors indicated in my previous review only partially. Legends in Fig.2 and Fig.3 were corrected. But the legend of Figure 1 is still incomplete. Authors should explain in this legend, what figures A-D in Fig.1 show.

Respond: Legends in Fig.2 and Fig.3 were corrected. We initially wanted to make figure 1 beautiful, I chose the four slices results with HE staining of the patients, while the pictures were repetitive. In this revision process, I have revised the figure 1 accordingly, added the legend of Figure and explained the implication of A-C in Figure 1.

Comment 3: Section of Results is still written incorrectly.

Respond: We thank you for your suggestion, and have made the advised changes in the Result Section. We hope the section should be able to fully describe the case report.

Comment 4: There is lack of corrected description of study results with quotation of appropriate figures in the text.

Respond: We thank you for your suggestion, and have made the advised

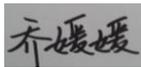
changes in the discussion section of the paper about the description of study results. Figure 1 is the results of histopathology characterization of the ESCC patient. We could conclude that the tumor cells were cytokeratin positive origin epithelial cells and active in proliferation and confirmed that we could obtain CTCs based on cytokeratin markers. Figure 2 showed the immunofluorescence analysis of the CTCs captured by negative selection at three months after operation. Negative selection method can effectively obtain the circulating tumor cells in peripheral blood, as auxiliary examination of tumors. The treatments, disease progression and all kinds of inspections were reflected in Figure 3, as a summary of a chart, can help the readers better understand complete information.

Special thanks to you for your good comments.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

A small square box containing a handwritten signature in Chinese characters, which reads '齐媛媛' (Qiao Yuan-yuan).

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