Peer Review File

Reviewer Comments

The paper titled "Circulating tumor cells and neutrophil-lymphocyte ratio are predictive markers for metastatic colorectal cancer patients" is interesting. The authors conclude that NLR value and CTCs counts could serve as robust prognostic predictors for patients with metastatic colorectal cancer. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 1 *Previous studies have tested the predictive value of the ratio of lymphocytes to the sum of monocytes and neutrophils for the prognosis of patients with metastatic colorectal cancer. What are the similarities and differences between the two articles? What are the advantages of this article?*

Reply 1: Thanks for your excellent comment. Firstly, cancer patients commonly present with local and systemic changes in the immune-inflammation index, including changes in the neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR). Many studies have shown that both NLR and PLR are powerful predictors of poor outcomes for CRC patients. The similarities between these articles were to further demonstrate the predictive value of immune-inflammation index in CRC patients.

Secondly, recent evidence has also shown that the interaction of inflammatory cells with CTCs in the peripheral blood may promote tumor metastasis to distant sites. The differences between these articles were that this study assessed the relationship between baseline CTC counts and immune-inflammation index, namely, NLR and PLR; and further determined if baseline CTC counts in combination with NLR and PLR were associated with clinical outcomes in CRC patients.

The advantages of this article were that this study indicated that combining the immune-inflammation index and CTC detection as predictive tools allowed for more accurate predictions of CRC patient survival.

We have cited these similar papers and elaborated introduction in red color and italics

(Please see Page 3, Line 19-27).

Comment 2: *What are the prognostic factors for patients with colorectal cancer?*

Reply 2: Thank you for your question. The prognostic factors for CRC patients include gender, old age, tumor localization, TNM stage and metastatic sites. We have compared CTCs counts, immune-inflammation index and clinical features. The results were presented in Table 1 and Table 2.

Comment 3: *Is it too limited to only detect circulating tumor cells? The detection of other inflammatory cells should be increased.*

Reply 3: Excellent comment. Theoretically, CTCs that leave the primary tumor site will be more vulnerable to attack by immune cells; the survival of CTCs in vessels might be the "Achilles' heel" of metastasis. Recent publications have shown that some CTCs closely interact with blood cells, such as neutrophils, platelets, and monocytes, to survive in the bloodstream. Therefore, in this present study, we combined the detection of other inflammatory cells and CTCs as predictive tools to allow for more accurate predictions of CRC patient survival. We demonstrated that CTC counts were positively associated with the immune-inflammation index (as measured by NLR and PLR) in mCRC patients, and that baseline CTC counts, together with NLR, were independent prognostic factors for mCRC patients.

Comment 4: *It is recommended to increase the comparison of traditional predictive markers.*

Reply 4: Thank you for your helpful suggestion. The traditional predictive markers for CRC patients include gender, old age, tumor localization, TNM stage and metastatic sites. We have compared CTCs counts, immune-inflammation index and clinical features. The results were presented in Table 1 and Table 2.

Comment 5: *The samples were too small. How to handle with the limitation. Such limitations should be addressed in the discussion.*

Reply 5: Excellent comment. We have addressed the limitations in the discussion part (Please see Page 9, Line 22-26).

Comment 6: *Will the patient's clinical classification, complications, underlying diseases and treatment measures affect the results?*

Reply 6: We appreciate your comment. To elucidate whether the patient's clinical classification, complications, underlying disease and treatment measures affect the results or not, we reanalyzed the correlations between baseline CTCs counts and clinical parameters and the results showed in Table 1-3.

Comment 7: *It is recommended to increase the detection indicators of circulating tumor cells.*

Reply 7: We appreciate your suggestions. As depicted in methods, fluorescently labeled monoclonal antibodies specific for leukocytes (CD45) and epithelial cells (cytokeratin 8, 18, 19) are used to distinguish epithelial cells from leukocytes. Circulating tumor cells were defined as nucleated cells lacking CD45 and expressing cytokeratin and EpCAM. We have added the detection indicators of CTCs in methods part as advised (see Page5, Line 6-13)