At the following, the points mentioned by the reviewers will be discussed and the changes can be identified by highlighted printing in the revised manuscript.

Point by point reply to editor's and reviewers' comments:

Reviewer 1

The wide confidence intervals for sensitivity and specificity raise questions about the precision of the estimates.

Response:

Thank you for the comment.

After the pooled analysis, the sensitivity of methylated BCAT1/IKZF1 in plasma for diagnosis of CRC was 60% (95%CI 53-67), and the specificity was 92% (95%CI 90-94). Only the confidence interval of sensitivity analysis was slightly wide, which was considered to be caused by the heterogeneity. In the subgroup analysis, it was found that the heterogeneity was likely to come from the purpose of testing. In CRC screening subgroup, the I2 of sensitivity and specificity was 48.58% and 26.75%, respectively. The sensitivity was 64% (95%CI 59-69), and the specificity was 92% (95%CI 91-93), and the confidence interval was narrowed to a certain extent. Therefore, to be more accurate, the diagnostic efficacy of this method for postoperative follow-up may need to be confirmed by further studies.

The authors should discuss the potential sources of heterogeneity among the included studies and consider conducting subgroup analyses to explore the reasons behind the variability.

Response:

Thank you for the comment.

In the subgroup analysis, the heterogeneity of CRC screening was significantly reduced when we grouped studies according to the purpose of testing. However, there was still some heterogeneity in subgroup of postoperative follow-up and assessment of disease recurrence. It was considered that the source of heterogeneity within this subgroup may be caused by the variables of the detection time point and the disease stage, but these variables were not clearly presented in the included studies, so further analysis cannot be conducted to reduce heterogeneity.

We added this in paragraph 2 of the Discussion.

The conclusion states that the detection of methylated BCAT1/IKZF1 in plasma is "safe, convenient, fast, and economical." However, no evidence or rationale is provided to support this

claim. The authors should either present relevant literature supporting their statement or modify the conclusion accordingly.

Response:

Thank you for the comment.

Yes, our previous conclusion was indeed not rigorous, and the conclusion was changed to: The detection of methylated BCAT1/IKZF1 in plasma, as a non-invasive detection method of circulating tumor DNA, has potential CRC diagnosis, but the clinical application prospect needs to be further explored.

The discussion of the results could be improved by comparing the findings of this study with other existing biomarkers for CRC screening and follow-up. This would provide a broader context for the significance of methylated BCAT1/IKZF1 in the field of CRC diagnosis.

Response:

Thank you for the suggestion.

In the discussion, we introduced the area of CRC screening and follow-up. The research and application of serum CEA, Faecal occult blood test, Faecal immunochemical test, gut microbes, FIT-DNA test and Multi-Target Stool DNA, SEPT9 methylation, CanerSEEK and GRAIL were introduced, respectively. All of the above items are considered to be important or cutting-edge detection methods for current CRC screening and follow-up. Based on your comments, we have also added relevant contents for postoperative follow-up of disease recurrence.

We added the content in paragraph 2 of the Discussion.

Overall, the article presents important findings on the diagnostic accuracy of methylated BCAT1/IKZF1 in CRC. However, addressing the mentioned concerns and incorporating the suggested improvements would further strengthen the validity and impact of the study. I recommend acceptance of the article pending the revisions mentioned above. The study has the potential to contribute significantly to the field of CRC diagnosis and would be of great interest to the readership of the journal. The authors conducted a systematic review of twelve eligible studies, involving a total of 6561 participants, to analyze the sensitivity, specificity, and diagnostic test accuracy of methylated BCAT1/IKZF1. The data analysis presented in the abstract indicates that the sensitivity of methylated BCAT1/IKZF1 for diagnosing CRC is 60%, with a specificity of 92%. The diagnostic odds ratio of 19 and area under the curve of 0.88 suggest good diagnostic accuracy for CRC detection. The study provides valuable insights into the potential of methylated BCAT1/IKZF1 as a biomarker for CRC diagnosis. The inclusion of multiple databases in the search strategy enhances the robustness of the results. The analysis of sensitivity and specificity for CRC screening and recurrence detection in the follow-up provides important information for clinical application.

Response:

Thank you for Thank you for your wonderful and careful review.

Reviewer 2

1 / 1 ASSESSING THE DIAGNOSTIC VALUE OF METHYLATED BCAT1 AND IKZF1 IN PLASMA FOR COLORECTAL CANCER: A META-ANALYSIS The aim of this meta-analysis was to analyze and evaluate the diagnostic accuracy of DNA methylation markers, in particular BCAT1/IKZF1, in plasma for screening and postoperative follow-up of colorectal cancer (CRC). Colorectal cancer (CRC) is the most common malignancy and it's third for his percentage of death and recurrence; even in patients who have recived radical treatment the recurrence in about 25-40%. Due to this scenario the necessity of early diagnosis and treatment are the main content of secondary cancer prevention. Both the initial diagnosis and the diagnosis of relapse after radical treatment have a significant impact on the overall survival of patients. At present, although the diagnostic accuracy of CRC has greatly improved through the wide application of CEA testing, colonoscopy, and imaging examination, it's still necessary to explore more safe, convenient, economical, and accurate diagnostic methods. In order to that, in recent years liquid biopsy technology's value has increased rapidly in disease diagnosis and treatment. This method is becoming an important pathway to follow because of the process of tumor development, to its aggressiveness and the biological phenomenon of cell necrosis and apoptosis with the production of circulating tumor DNA (ctDNA). This latter, infact, may enter the circulation in the early stage of the disease, suggesting that tumor markers based on ctDNA may play an important role in the early diagnosis of tumors. Among the DNA methylation markers, this meta-analysis chose to explore the value of BCAT1/IKZF1 for CRC. To do so twelve eligible studies were included with a search period from May 31, 2003 to June 1, 2023 for a total of 6561 partecipants. This study is very important for the improvement of the screening and post-treatment follow-up methods for CRC. It shows that methylated BCAT1/IKZF1 in plasma had a sensitivity of 64% (95%CI 59-69) and a specificity of 92% (95%CI 91-93) for CRC screening and a sensitivity of 54% (95%CI 42-67), and a specificity of 93% (95%CI 88-96) for CRC postoperative follow-up. Moreover, for his use as diagnostic value, some reports suggest that changes of methylated BCAT1/IKZF1 in plasma occur before imaging changes and, for the follow-up, researchers have found that methylated BCAT1/IKZF1 in plasma may also be valuable in the prognostic prediction of CRC, suggesting that methylated BCAT1/IKZF1 in plasma may be more likely to be found in patients with postoperative incisal margin deficiency, lymph node invasion or distant metastasis. In the end the role of this meta-analysis allows to make the difference in the diagnosis research field for CRC, showing how the detection of methylated BCAT1/IKZF1 in plasma, as a non-invasive detection method of circulating tumor DNA, has good diagnostic and prognostic accuracy, and is safe, convenient, fast, and economical, which is easy to be popularized. There were some limitations in this meta-analysis: the studies included were all conducted in Australia or the United States so the capability of methylated BCAT1/IKZF1 testing to diagnose CRC in other ethnic groups and regions needs to be further investigated. In addition, not all the studies clearly record the

diagnostic sensitivity for patients with different stages. Neverthless, the value of this study is still high and define the necessity to continue the research in this field.

Response:

Thank you for your wonderful comment.

You have introduced all the contents of this paper in a general way, and the shortcomings you raised were also mentioned in the Discussion of this research. Due to the limitations of the data extracted from the included research, further analysis cannot be made. These problems need to be further explored by prospective studies in the future.

Reviewer 3

There is quite some literature about using methylated BCAT1/IKZF1 as diagnostic marker in colorectal cancer, therefore this meta-analysis is of interest to the field. I do have some remarks/suggestions before this manuscript could be suited for publication:

- The conclusion might be somewhat overstated as a general sensitivity of 60% is still low for clinical use, especially since diagnostic sensitivity seems to decrease substantially in the early stages of CRC, as has been shown with other methylation markers as well. It is not clear whether all 6561 patients were included in the analysis of sensitivity at the different stages and what the clinical implication of this finding would be as early diagnosis seems not to be that effective. An additional paragraph about CRC stages could be added in the discussion.

Response:

Thank you for the comment.

At present, methylated BCAT1/IKZF1 in plasma has a diagnostic sensitivity of 64% (95%CI 59-69) for CRC, which is indeed limited in scope of application. However, its sensitivity is better than some FDA-approved diagnostic methods, such as faecal occult blood tests and serum CEA, and similar to the sensitivity of faecal immunochemical test and SEPT9 methylation detection. It still has certain value of exploration and application.

As you mentioned, the previous conclusion was indeed not rigorous, and the conclusion was changed to: The detection of methylated BCAT1/IKZF1 in plasma, as a non-invasive detection method of circulating tumor DNA, has certain efficacy in the diagnosis of colorectal cancer, but the clinical application value still needs to be further explored.

There is usually a decrease in diagnostic sensitivity when the diagnostic methods are applied to patients with early-stage CRC. When we investigated the diagnostic sensitivity of stage I, II, III, and IV patients in 6561 patients, the diagnostic sensitivity

in the stage I was only 32% (95%CI 22-43). The diagnostic sensitivity of stage II, III, and IV was 66% (95%CI 59-73), 71% (95%CI 63-78), and 91% (95%CI 81-96), respectively. Therefore, the diagnostic efficiency of the methylated BCAT1/IKZF1 in plasma was indeed limited in patients with early stage. It needs to be combined with other diagnostic methods to improve the diagnostic efficiency. According to your requirements, a discussion of the differences in the diagnostic effectiveness of its use in patients with different stages was added in the 2nd paragraph of Discussion.

The content added was: In terms of diagnostic sensitivity for patients with different stages of CRC, the detection of the methylated BCAT1/IKZF1 in plasma, similar to other detection methods, has a poor diagnostic sensitivity for patients with early-stage CRC, which may limit its clinical application in CRC screening. In the future, the clinical application value of methylated BCAT1/IKZF1 in plasma can be enhanced by combining it with other tests.

- This manuscript is written in a very statistical manner. Additional biological information in the introduction would welcome. For example
o why did you choose to perform this meta-analysis specific on methylation of BCAT1/IKZF1, as there are a multitude of other markers available.

Response:

Thank you for the comment.

Firstly, the studies of methylated BCAT1/IKZF1 in plasma as diagnostic method for CRC has only recently published the results, and this topic is novel. In pooled analysis, plasma methylated BCAT1/IKZF1 had a diagnostic sensitivity of 60% (95%CI 53-67) for CRC, which was indeed limited in diagnostic sensitivity. However, it was better than some FD-approved diagnostic methods, such as faecal occult blood tests and serum CEA, and similar to the sensitivity of faecal immunochemical test and SEPT9 methylation detection. It still has certain value of exploration and application.

Secondly, this method is a liquid biopsy method, detecting ctDNA in plasma as the object, which has the characteristics of less trauma, convenience, fast and economy.

Finally, DNA methylation is an epigenetic phenomenon and its change begins thought to be years before the onset of disease. Mechanistically, DNA methylation markers may have earlier diagnostic implications.

According to your requirements, the above content was added in paragraphs 2 and 3 of the Introduction.

o you mention that this has advantages over SEPT9, while a similar sensitivity. What is the proven sensitivity of SEPT9, as you state in the discussion that it is less sensitive than other current tests.

Response:

Thank you for the comment.

According to a recent meta-analysis on the diagnosis of SEPT9 methylation in CRC, its diagnostic sensitivity was 0.679 (95%CI 0.622-0.732), specificity was 0.904 (95%CI 0.881-0.923), and AUC of 0.883. From the descriptive statistical results, the diagnostic efficacy of methylated BCAT1/IKZF1 was similar to SEPT9 methylation in CRC. We only considered that methylated BCAT1/IKZF1 had a similar diagnostic efficacy to SEPT9 methylation, but not superior.

According to your requirements, the diagnostic efficacy of SEPT9 methylation in CRC was added in the paragraph 1 of the Discussion.

o Does mutational status of tumors have an influence on methylation of BCAT1/IKZF1?

Response:

Thank you for the comment.

After a comprehensive review of the literature, there is no relevant research to answer this question. From my personal understanding, DNA methylation is not directly related to DNA mutation. DNA methylation is an epigenetic phenomenon, which is more common and has complex causes, and is affected by factors such as age, growth and development, living habits, environment, etc. From the perspective of research, DNA methylation belongs to different omics studies with DNA mutation, and whether there is interaction between them needs to be further explored in future studies.

According to your requirements, this issue has been added in in paragraph 4 of the Discussion.

o It is not clear for an unexperienced reader that you look into ctDNA

Response:

Thank you for the comment.

In the first draft, we did not focus on ctDNA. In the revised vision, we add this part in paragraph 2 of the Introduction.

o Could there be anything done in the future to increase the sensitivity of this test?

Response:

Thank you for the comment.

We considered that the most effective way to improve the sensitivity of this method was to combine it with other assays, including making panel detection with other methylation markers, or combining with other types of assays, such as serum CEA, faecal occult blood tests, faecal immunochemical tests and so on.

This point has been added in paragraph 2 of the Discussion.

- The meaning of some sentences is not clear/Minor mistakes.
o First sentence of paragraph 3.5

Response:
Thank you for the comment.
We have invited native spreaker to make language editing to this article. We have corrected the error.

o Paragraph 2.4: “if there is any significance, …” I suppose significance is not the correct term here.

Response:
Thank you for the comment. We have corrected the error.

o 7th sentence discussion. Sensitivity is written with a capital.

Response:
Thank you for the comment. We have corrected the error.

o Discussion: “Another one new method” is not a correct sentence

Response:
Thank you for the comment. We have corrected the error.

- Quality of figure 3 and 4 needs to be improved. It currently is impossible to read and therefore could not be assessed.

Response:
Thank you for the comment. We have improved the image quality.