

Dear reviewer,

Thank you for the constructive comments on our manuscript entitled“Effect of perioperative glutamine-enriched nutritional support on patients with colorectal cancer: A systematic review and meta-analysis of randomized controlled trials” (Manuscript ID: 112256). We appreciate the time and effort that you have dedicated to providing insightful feedback on our work.

We have carefully considered all the comments and have made revisions to the manuscript accordingly. We believe that the manuscript has been significantly improved as a result of these changes.

Below is our point-by-point response to the comments. All changes in the manuscript have been highlighted in yellow for easy identification. We look forward to hearing from you regarding our submission.

We thank you again for your comments on the manuscript. If further modifications are needed in the future, please let us know and we will do our best to cooperate with you to complete the relevant work. Thank you again.

Sincerely,

Jiwei Wang

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Reviewer #1:

Abstract Section : Section #1 :The rationale is well presented; however, for clarity across disciplines, key terms such as glutamine and colorectal cancer should be scientifically justified in the background. Strengthening these definitions will enhance reader understanding and support the well-structured rationale.

**Reply:**

We have accepted this suggestion and revised it. We further emphasize the two keywords of colorectal cancer and glutamine in the background section, and emphasize the connection between them. Add in the background "Colorectal cancer (CRC) is one of the common malignant tumors of the digestive system, seriously threatening human health. Patients with CRC during the perioperative period are prone to nutritional risk or malnutrition. Compared with traditional nutritional support, immune nutrients represented by glutamine(Gln) have attracted increasing attention.”

Section #2 : While the study topic is clear, the objective in the abstract appears fragmented and lacks clarity. The sentence is overly long and presents an unfinished idea. For example, the statement: “To evaluate the clinical significance of perioperative glutamine (Gln)-enriched nutritional support on postoperative nutritional status, nitrogen balance, immune function, inflammation levels, morbidity of postoperative complications, and length of hospital stay in patients with CRC” ends without clearly stating what is being measured or concluded regarding these variables. I recommend restructuring the objective for coherence, conciseness, and completeness. Abstract Methods & Results Section: The Methods section of abstract contains an overly long sentence that may hinder clarity and readability. It is recommended to break it into shorter, more concise statements to ensure the methodological approach is clearly communicated to readers from diverse backgrounds. The Methods and Results sections in the abstract appear to be mixed. For instance, the sentence “The meta-analysis ultimately included 27 studies, with a total of 1643 patients; 827 patients received perioperative Gln treatment, and 816 received conventional nutritional therapy” reflects methodological detail and should be placed in the Methods section, not under Results. Additionally, the Results section should focus on presenting the key findings rather than overloading it with multiple statistical values. While statistical outputs are essential to validate observations, they should support rather than overshadow the main conclusions NB: Please check that the keywords are listed in alphabetical order, as per journal guidelines.

**Reply:**

1. We accepted the suggestion and revised the sentence and paragraph to make it clearer and more concise. We have revised our previous objective to "To study the influence of perioperative glutamine-enhanced nutritional support on postoperative outcomes. Such as nutritional status, immune and inflammation levels, morbidity of complications, and length of hospital stay."
2. We divide the excessively long sentences in the method section of the abstract into shorter, more concise statements for readers to understand;
3. "The meta-analysis ultimately included 27 studies, with a total of 1643 patients; 827 patients received perioperative Gln treatment, and 816 received conventional nutritional therapy "moved to the methods section;
4. The Results section focuses on presenting key findings. Some statistical data for secondary research results have been removed to make the expression more concise and highlight the main research results.
5. According to the journal guidelines modified the keywords order.

#### Introduction section

#1 : The concept of colorectal cancer should be scientifically justified in the background to enhance clarity for a broader readership. Additionally, regarding citation [1], the authors refer to data from Freddie B et al., but the referenced figure in Table 1 indicates a global incidence of 9.6%, ranking colorectal cancer third not second. Please revise this statement to accurately reflect the original source.

#### Reply:

In the introduction section, we emphasize the concept of colorectal cancer and add "Colorectal cancer originates from malignant transformation of the epithelial cells of the colorectal mucosa, including colon cancer and rectal cancer." In addition, after reading reference 1 again, we found that Table 1 in the article showed that CRC was the third and second leading cause of cancer incidence and mortality (see the figure below). Our statement in the original article is accurate, please review.

234 | GLOBAL CANCER STATISTICS 2022

**TABLE 1** New cases and deaths for 36 cancers and all cancers combined in 2022.

| Cancer site   | Incidence |           |                | Mortality |           |                |
|---------------|-----------|-----------|----------------|-----------|-----------|----------------|
|               | Rank      | New cases | % of all sites | Rank      | Deaths    | % of all sites |
| Lung          | 1         | 2,480,301 | 12.4           | 1         | 1,817,172 | 18.7           |
| Female breast | 2         | 2,308,897 | 11.6           | 4         | 665,684   | 6.9            |
| Colorectum    | 3         | 1,926,118 | 9.6            | 2         | 903,859   | 9.3            |
| Prostate      | 4         | 1,466,680 | 7.3            | 8         | 396,792   | 4.1            |

#2 The statement “Previous clinical studies have indicated that Gln plays a positive role in the short-term clinical efficacy of CRC surgery” lacks specific citations. Please clarify which studies are being referred to by providing appropriate references to support this claim. in addition this section has long sentence which hinder clarity

**Reply:**

We added specific citations to this mention in the article to support this claim; In addition, we modified the long sentences in this part to make them more concise and clear

#3 [21]. However, one study reported which study is it ?

**Reply:**

The study referred to here is that of Yao et al. In Reference 23, the citation mark is placed at the end of this sentence.

#4 Data Sources and Search Strategy: For improved clarity and readability, it is recommended to present the data sources and search strategy in a table format, supported by a brief narrative explanation. This will help readers easily follow the databases used, search terms, and inclusion criteria.

**Reply:**

We provide the data sources and search strategies in tabular form, making it easy for readers to understand the databases used, search terms and inclusion criteria.

| Data sources  | search strategy   |
|---|---|
| PubMed, Embase, Web of Science, Cochrane Library, Chinese Biomedical Database (CBM), China National Knowledge Infrastructure (CNKI), VIP Medical Information System (VIP), and Wanfang Electronic Database. | "Rectal Neoplasms," "Colorectal Neoplasms," "Colonic Neoplasms," "Glutamine," and "randomized controlled trial" as subject terms combined with their corresponding free words for all possible combinations. Search terms were combined using the Boolean operators "AND" and "OR". |
| Others  | Additional articles were manually searched in the reference lists of articles selected for the full-text search. For overlapping authors, institutions, or patients, the most recent or highest-quality articles were selected.   |

# 5 Study Selection: The inclusion criteria, such as limiting to adult patients diagnosed with colon or rectal cancer and treated surgically regardless of race or sex, should be supported with justified reasoning or relevant citations. Similarly, the selection of randomized controlled trials (RCTs) as the study design should be backed by appropriate references that align with the study's objective and methodological rigor. Providing this context will strengthen the transparency and scientific foundation of the study selection process.

**Reply:**

Regarding this modification suggestion, we have added corresponding references, which are relevant to the background of our study, to illustrate that when including patients in our study, we should not consider gender or race, and should preferentially choose randomized controlled studies; in order to enhance the transparency and scientific foundation of the selection process.

# 6 Data Extraction The statement that “two researchers” conducted the data extraction lacks clarity. Please specify who these researchers are (e.g., by initials) and describe their roles in the process, including how discrepancies were resolved. Additionally, this section would benefit from a summarized presentation of the data sources and search strategy in a table format, supported by a brief narrative explanation with citation . This will enhance transparency and reproducibility of the methodology.

#### Reply

We added the names of two researchers(Huang Y and Yang XZ), described their roles in the research process, and decided whether to include controversial literature after discussion with a third researcher(Wang JW).

Data Syntheses and Analyses: The phrase “the combined effects of all studies on the outcome variables” is vague please clarify what “all studies” refers to and ensure it aligns with your inclusion criteria. Additionally, the statement “The results were presented as pooled effect sizes and 95% confidence intervals (CI)” is more appropriate for the Results section rather than the Data Analysis section. Moreover, the Data Syntheses and Analyses section should include or reference the specific statistical models or equations used (e.g., fixed/random effects models, heterogeneity tests), to ensure methodological transparency and reproducibility.

#### Reply

1. We further clarified that the "all studies" in this section refer to those that meet the inclusion and exclusion criteria and are ultimately subject to data analysis.

2.Regarding The reviewers' opinion that "the results were presented as pooled effect sizes and 95% confidence intervals (CI)" is more applicable to the results section rather than the data analysis section. Based on the previous writing structure of the Meta-analysis (see Examples 1 and 2), we want readers to clearly understand the presentation of our results, which is part of the data analysis. We believe it is acceptable to include it in the data analysis section. If the reviewer consider this inappropriate, we are willing to actively make changes in subsequent modifications.

eg.1

### **Statistical analysis**

To conduct the meta-analysis, we utilized RevMan 5.3.5 and Stata 14.0 software (Cochrane Collaboration, Oxford, United Kingdom). For continuous variables, we calculated the weighted mean differences along with their corresponding 95% confidence intervals (CIs). Similarly, for categorical variables, we computed odds ratios (ORs) with their respective 95% CIs. To assess heterogeneity, we performed a chi-square test, considering  $P > 0.05$  as non-significant. Statistical heterogeneity was assessed using  $I^2$  values, with a threshold of 50% or higher indicating the presence of heterogeneity. When the studies exhibited homogeneity ( $I^2 < 50\%$ ), we adopted the fixed-effects model. Conversely, if studies presented heterogeneity ( $I^2 \geq 50\%$ ), we utilized the random-effects model.

eg.2

### **Quality assessment, data extraction, and analysis**

The Newcastle-Ottawa Scale (NOS) was employed for study quality assessment<sup>[15]</sup>, and two independent reviewers used a standardized data extraction form to systematically extract relevant data such as author's name, publication year, study location, number and characteristics of the participants, duration of follow-up, the operational definition of "frailty" employed, and the outcomes of interest. All differences were resolved by consensus or a consultation with the third author.

All statistical analyses were performed with STATA version 15.0. Data are reported as relative risk (RR) when the outcome is categorical and as weighted mean difference (WMD) when it is continuous. Survival outcomes after a long-term follow-up are expressed as hazards ratio (HR). All estimates included 95% confidence interval (CI). A random-effects model was used for all analyses. To evaluate potential publication bias, we utilized both Egger's test and funnel plots<sup>[16]</sup>.  $P < 0.05$  was considered significant.

3. In response to the reviewers' opinion that the section on data synthesis and analysis should include or mention the specific statistical models or equations used (e.g., fixed/random effects models, heterogeneity tests), we have elaborated on them in detail in the section. Please see the markings.

