

112256_Auto_Edited_011254.docx

WORD COUNT

5587

TIME SUBMITTED

18-SEP-2025 03:32PM

PAPER ID

118234486

Name of Journal: *World Journal of Gastrointestinal Surgery*

Manuscript NO: 112256

Manuscript Type: META-ANALYSIS

Effect of perioperative glutamine-enriched nutritional support on ⁹patients with colorectal cancer

A systematic review and meta-analysis of randomized controlled trials

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Abstract

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. Although a large number of previous studies have reported that perioperative Gln supplementation helps improve short-term clinical outcomes in patients with CRC, however, some studies have not found a benefit, it need to be further explored.

AIM

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.

METHODS

A comprehensive literature search was conducted from inception until June 2025. The search covered PubMed, Embase, Web of science, Cochrane Library, China Biomedical Database (CBM), China National Knowledge Infrastructure (CNKI),VIP Medical Information System (VIP), and Wanfang Electronic Database. 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. A random-effects model was used to pool relative risks(RR) and mean differences(MD) with 95% confidence intervals (CIs).

RESULTS

For the primary endpoint, the pooled analysis showed that Gln intervention reduced the morbidity of postoperative infectious complications (RR = 0.36; 95%CI: 0.24-0.54)

and non-infectious complications (RR = 0.32; 95%CI: 0.19–0.55) and shortened the length of hospital stay(LOS) 2.31 days(MD = -2.31; 95%CI: -3.21 to -1.41) in CRC patients. For secondary endpoints, Gln-supplementation increased serum albumin(ALB), prealbumin(PA) , peripheral blood lymphocyte, and nitrogen balance(NB) levels, improved humoral and cellular immune function. Meanwhile, we found that postoperative tumor necrosis factor α (TNF- α) and C-reactive protein(CRP) levels were lower in Gln-supplemented patients. However, Gln-supplementation did not improve CD8⁺ and CD4⁺/CD8⁺ levels.

CONCLUSION

In conclusion, our study concluded that Gln supplementation is effective in improving short-term clinical outcomes in CRC patients.

Key Words: Colorectal cancer; Immune function; Inflammation levels; Length of stay; Nitrogen balance; Nutritional status; Postoperative complications

Huang Y, Yang XZ, Qin SH, Zhang T, Xie M, Wang JW. Effect of perioperative glutamine-enriched nutritional support on patients with colorectal cancer. *World J Gastrointest Surg* 2025; In press

Core Tip: Our study found that glutamine(Gln) supplementation could not only effectively improve the postoperative nutritional status, reduce the incidence of postoperative complications and shorten the length of hospital stay(LOS), but also, importantly, maintain nitrogen balance(NB), enhance immune function and reduce inflammation levels of colorectal cancer(CRC) patients, without the interference of comorbidities and other immune nutrients. On the basis of previous studies, this further confirmed that perioperative application of Gln can bring positive prognosis to CRC patients, and provided an evidence-based basis for the widespread clinical application of Gln.

INTRODUCTION

Colorectal cancer(CRC) originates from malignant transformation of the epithelial cells of the colorectal mucosa, including colon cancer and rectal cancer. The latest statistics of global cancer show that there are 1,926 million new cases and 904,000 deaths of worldwide in 2022, which are the third and second causes of cancer incidence and death, respectively[1]. Currently, according to the European Society for Medical Oncology (ESMO) guidelines[2] and National Comprehensive Cancer Network (NCCN)[3,4] assessment, surgery-based comprehensive treatment is the main treatment for resectable CRC. However, preoperative anxiety and depression[5], surgery-induced tract mucosal injury, ischemia-reperfusion injury[6], postoperative pain, nausea and vomiting, intestinal dysfunction[7] and anastomotic edema often result in delayed oral feeding, leading to impaired nutrient intake and absorption of nutrients[8]. In addition, the use of perioperative antibiotics may cause an imbalance in intestinal flora and damage the intestinal barrier. And previous studies have reported malnutrition rates as high as 45-60% in patients with CRC, which are higher in patients undergoing surgery[9]. Most postoperative complications are caused by malnutrition, and the presence of complications worsens short-term clinical outcomes and severely impairs patient prognosis[10,11]. Previously, prevention and reduction of postoperative complications were mainly achieved by eradicating pathogens and improving medical techniques [12,13]. Currently, there is a growing trend to reduce complications through adequate nutritional support to enhance host immune function and, consequently, decrease inflammation levels[14]. Accordingly, perioperative nutritional support for patients with CRC has attracted much attention.

Since the 1990s, immunonutritional therapy supported by specific nutrients, including Gln, omega-3 polyunsaturated fatty acids, arginine, and nucleotides, has been gradually found to have many advantages over traditional formulations of nutritional support, as well as to result in nitrogen levels and protein synthesis[15], regulate host immunity[16] and inflammatory responses[17], and help maintain the mucosal barrier

function to reduce the entry of bacteria and toxins into the bloodstream[18,19]. This reduces the occurrence of postoperative infectious complications and shortens the LOS[20]. The 2021 European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines[16] strongly recommend immunonutrition during the perioperative period for gastrointestinal malignancies. Parenteral Gln supplementation is recommended in patients who cannot be adequately fed enterally. Although authoritative guidelines recommend immunonutrition, its use remains controversial. Gln has long been the most commonly used immunonutritional therapy for gastrointestinal malignancies in the perioperative period of gastric cancer; however, as the overall morbidity and mortality of CRC are showing a significant upward trend, the incidence of perioperative nutritional risk or malnutrition in patients with CRC is increasing, and immunonutritional therapy represented by Gln has received increasing attention.

Previous studies have indicated that Gln plays a positive role in the short-term clinical efficacy of CRC surgery[21]. It may influence the gut microbiota through several mechanisms. These include reducing the Firmicutes-to-Bacteroidetes ratio, activating the nuclear factor kappa B and phosphoinositide-3-kinase-Akt pathways, and increasing the production of intestinal secretory immunoglobulin A (SIgA). Moreover, it is speculated that SIgA promotes bacterial aggregation in the intestinal lumen and avoids their adhesion to the epithelial surface, thereby reducing bacterial and endotoxin translocation into the blood to reduce infectious complications[22]. However, one study reported that patients undergoing gastrointestinal surgery did not show significant benefits in terms of the morbidity of complications and LOS after Gln administration at a dose of 0.5 g/kg/d the day before and three days after surgery[23]. Marta Sandini *et al.*[24] and Rotovnik Kozjek N *et al.*[25] also found that Gln supplementation did not improve the prognosis of CRC patients. Even Lee SY *et al.*[26] raised the question that **the routine use of immunonutrition in the perioperative period of colon cancer is unreasonable**. Moreover, ESPEN guidelines[16] did not clearly indicate the specific use and dosage of Gln in CRC patients during the perioperative period. Because of the small sample size of most trials, the design of each study varied, resulting in

inconsistent findings. Therefore, to further study the effects of perioperative glutamine-enriched nutritional support on postoperative nutritional status, immune function, inflammatory level, incidence of postoperative complications and LOS in CRC patients by conducting a meta-analysis of prospective clinical trials on the effect of perioperative glutamine-enriched nutritional support on short-term clinical outcomes of CRC patients after surgery. In addition, the best way, maintenance time and dosage of glutamine are explored to provide new clinical evidence for immunonutrition in CRC patients.

MATERIALS AND METHODS

Data sources and search strategy

We have registered this protocol previously in PROSPERO in March 2023 (registration no. CRD42023412285; <https://www.crd.york.ac.uk/PROSPERO>). This meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines[27]. The relevant Chinese and English databases were comprehensive searched for articles meeting the inclusion and exclusion criteria from the default date to June 2025, and the search strategy is described in **Table 1**.

Study selection

Inclusion criteria: (1) Adult patients who were diagnosed with colon or rectal cancer and treated surgically, regardless of race and gender[28];(2) The following intervention measures are present: The experimental group was given Gln for nutritional intervention during the perioperative period, regardless of pre-operative, postoperative, enteral, or parenteral administration; the control group underwent the same period of isonitrogenous and isocaloric conventional nutritional therapy; for studies with more than two randomized groups, only the Gln intervention and standard control groups were included;(3) The study design was a randomized controlled trial (RCT), regardless of whether it was blinded[29];(4) At least one of the following outcome indicators was recorded: Indicators related to nutritional status (eg. ALB, PA, peripheral blood

lymphocytes), immune function indicators (CD3⁺, CD4⁺, CD8⁺, and CD4⁺/CD8⁺ ratio, IgA, IgG, and IgM), inflammation level (TNF- α and CRP), NB, complications, the detailed classification is shown in **Table 2**. And LOS. Exclusion criteria: (1) Study subjects with comorbidities (*e.g.* intestinal obstruction, chronic obstructive pulmonary disease, and so on); (2) animal experiments; (3) interventions involving radiotherapy, chemotherapy, or a combination with other immunonutrients (*e.g.* probiotics, arginine, nucleotides, omega-3 fatty acids, and so on); (4) narrative literature review, meta-analysis, or unavailability of full-text literature; (5) cross-over studies; (6) retrospective studies; (7) inconsistent reported outcome indicators.

Data extraction

Two researchers(Huang Y and Yang XZ) independently read ⁵ the titles, abstracts, and full texts of the obtained literature, screened them according to the inclusion and exclusion criteria, and extracted the data using a pre-designed spreadsheet. After screening, cross-checking, discussing, or soliciting opinions from a third researcher(Wang JW) to resolve suspicious data or inconsistent opinions, the included studies were finally selected.¹² The following information was extracted from studies that met the inclusion criteria: Study name, year, tumor type, sample size, age, sex, glutamine dose, route of supplementation, dose, time, and outcome indicators. The primary objective of this meta-analysis was to assess whether perioperative Glu-enhanced nutritional support affects the short-term clinical outcomes of patients with CRC.

Quality assessment

Literature quality was independently assessed by two researchers(Qin SH and Zhang T) according to the Cochrane Risk of Bias Assessment Tool, including (1) the method of random allocation; (2) allocation concealment; (3) whether blinding was used; (4) ⁴ completeness of outcome data; (5) whether there was selective reporting of study results, and (6) whether there were other sources of bias (*e.g.*, early trial closure and

baseline inconsistency).¹ Different colors (green, red, and yellow) and symbols ("+", "-", and "?") are used to indicate "low risk of bias," "high risk of bias," and "unclear," respectively.

Outcomes

As the primary study outcomes, we assessed the infectious and non-infectious complications morbidity, length of hospital stay; as secondary outcomes, we assessed the indicators related to nutritional status (including levels of ALB, PA, and peripheral blood lymphocytes), immune function indicators (including levels of CD3⁺, CD4⁺, CD8⁺ and CD4⁺/CD8⁺ ratios, IgA, IgG, and IgM), indicators of inflammation levels (TNF- α and CRP), NB and so on.

Data syntheses and analyses

Meta-analyses were performed using RevMan 5.0, and forest plots were generated to measure the combined effects of all meet the inclusion and exclusion criteria studies on the outcome variables. Infectious and non-infectious complications were deemed a dichotomous variable, and the relative risk(RR) was used as the effect size. The remaining¹¹ indicators were continuous variables, with the mean difference (MD) as the effect size, and standardized mean differences (SMDs) were used when there was a difference in magnitude between the studies. The results were presented as pooled effect sizes⁴ and 95% confidence intervals (CI). $P < 0.05$ was considered to indicate statistical significance. The meta-analysis process was analyzed⁸ using the DerSimonian-Laird random-effects model, heterogeneity was assessed using the χ^2 test, and quantified using I^2 (low, < 50%; moderate, 50-75%; and high, > 75%), when $I^2 < 50\%$, we consider heterogeneity to be acceptable; for $I^2 > 75\%$, We concluded that significant heterogeneity existed and that sensitivity analyses(one-by-one elimination method) and subgroup analyses were needed to explore sources of heterogeneity. We hypothesized that supplementation time, timing, route, tumor type, and sample size might have different effects on the results. Therefore, subgroup analysis was performed based on

these hypotheses: Tumor type (CC/RC/CRC), Gln supplementation route (parenteral nutrition [PN]/enteral nutrition [EN]), Gln supplementation timing ($> 7/ \leq 7$ d or $> 5/ \leq 5$ d), Gln supplementation timing (postoperative/preoperative+postoperative), and sample size ($> 30/ \leq 30$). Finally, publication bias was tested by Egger's test for outcome indicators with more than ten included pooled studies; $P < 0.05$ indicated a high likelihood of publication bias.

RESULTS

Search results

The initial review yielded 540 articles (PubMed, $n = 27$; Embase, $n = 44$; Web of science, $n = 52$; Cochrane Library, $n = 56$; CNKI, $n = 47$; Wanfang database, $n = 85$; VIP, $n = 155$; CBM, $n = 61$; and 13 from other sources), and 211 duplicates were removed. The remaining 137 potential articles were read in full, 110 were excluded (9 with comorbidities, 14 with other immunonutrients, 47 with radiotherapy or chemotherapy, 18 with other diseases, 5 with incompatible study design, 3 with incompatible outcome, 3 with inconsistent indicators, 7 with unavailable full texts, 1 with duplicate literature, and 6 with irrelevant contents), and 27 articles were finally included in this meta-analysis. Of the studies included, there were 3 English and 24 Chinese literatures. And complete selection protocol detailed in **Figure 1**. All studies performed baseline comparisons prior to study entry and were comparabl.

Characteristics of the included trials

The meta-analysis ultimately comprised 27 single-center randomized controlled trials, encompassing a total of 1643 patients, 827 (50.3%) of which received glutamine perioperatively (experimental group), and 816 (49.7%) re-ceived conventional nutritional support (control group). The mean number of patients per study was 60.8. In 66.7% (18/27) of the trials[30-47], patients received PN, and in 33.3% (9/27) of trials, patients received EN[48-56]. The maintenance doses of Gln were mainly 0.3–0.5 g/kg.d; the timing of initiation was divided into preoperative and postoperative, with 37.0%

(10/27) of trials[30,31,33,40-42,44,45,47,53] initiating Gln intervention pre-operatively and 63.0% (17/27) of trials[32,34-39,43,46,48-52,54-56] initiating Gln intervention postoperatively, mainly between 1 and 7 days postoperatively; two studies[35,51] did not specify the duration of maintenance. The details of all trials incorporated in the meta-analysis were presented in **Table 3**. As for primary outcomes, infectious complications were reported in 13 studies[30,31,34-37,38,41,42,45,49,52,54,56], noninfectious complications in 10 studies[30,33,35,41,42,44,45,52,54,56], and length of stay in 10 studies[34,35,38,45,46,48-50,52,55]. As a secondary outcomes, in terms of nutritional status, 21 studies[30,33-39,41-44,48-56] reported postoperative albumin levels, 10 studies[30,36,41,42,44,50,51,53,54,56] reported pre-ALB levels, and 5 studies[33,35,37,44,52] reported peripheral blood lymphocyte numbers. In terms of cellular immune function, 6 studies[32,33,37,44,51,56] reported postoperative CD3⁺ levels, 12 studies[32,33,37,39,41,42,44,48,51,53,55,56] reported CD4⁺ levels, 11 studies[32,37,39,41,42,44,48,51,53,55,58] reported CD8⁺ levels, and 5 studies[29,38,41,45,50] reported CD4⁺ /CD8⁺ levels; in terms of humoral immune function, 5 studies[32,37,48,55,56] reported postoperative IgA levels, 6 studies[32,36,37,39,48,56] reported IgG levels, and 7 studies[32,36,37,39,48,55,56] reported IgM levels. In terms of inflammatory levels, 6 studies[31,40,47,49,53,54] reported TNF- α levels, and 4 studies[31,53,54,56] reported CRP levels. Finally, 3 studies[33,36,38] reported NB levels.

Evaluation of the methodological quality of included trials

In terms of selection bias, 7 of the 27 included studies used the random number table method[30,40,41,49,53-55], one used the unsealed envelope method[37], one randomized by surgical order[33], and one used 1: 1 paired randomization[56], while the remaining studies[31,33-36,38,39,42-48,50-52] only mentioned "randomization" without specifying the randomization method and were assessed as "low risk." All included studies did not state whether the allocation scheme was concealed and were rated as "unclear." Only 1 study blinded its investigators and participants[46] using a

clinical pharmacist who was not involved in the study to prepare the infusion solution and was rated as “low risk”; the remaining studies were not blinded and considered “high risk.” The risk of bias from incomplete or selective reporting of study results was minimal, as most included RCTs adequately reported all outcomes of interest and were considered “low risk.” Of the other biases, one study[47] with a small sample size ($n = 11$) was found to be at “high risk,” and the remaining studies were found to be at “low risk” with no other biases. The quality evaluations of the RCT articles are shown in **Figure 2**.

Primary outcomes

Infectious complication

Thirteen RCTs[30,31,34-36,38,41,42,45,49,51,54,56](943 patients) provided information on morbidity due to infectious³ complications. The morbidity rate was 6.58% in patients receiving Gln and 18.57% in controls. The effect of Gln was significant (RR = 0.36, 95%CI: 0.24-0.54, **Figure 3**).

Non-infectious complication

Ten trials[30,33,35,41,42,44,45,52,54,56] comprising 724 patients described non-infectious³ complications, The rate was 4.12% in patients receiving Gln vs 14.72% in controls. A significant effect of glutamine supplementation was observed (RR = 0.32, 95%CI: 0.19- 0.55, **Figure 4**).

LOS

Ten studies[34,35,38,45,46,48-50,52,55](592 patients) reported LOS, the heterogeneity of which was significant ($I^2 = 77\%$). We concluded that patients who received Gln dipeptides had a significantly shorter hospital stay of 2.31 days (MD = -2.31, 95%CI: -3.21 to -1.41, **Figure 5**). However, this result should be interpreted with caution owing to higher heterogeneity.

Secondary outcomes

Nutrition-related outcomes

A total of 1301 participants from 21 studies[30,33-39,41-44,48-56] were enrolled in the serum ALB analysis, 764 participants from 10 studies[30,36,41,42,44,50,51,53,54,56] were enrolled in the serum PA analysis, and 233 participants from 5 studies[33,35,37,44,52] were enrolled in the peripheral lymphocyte analysis. Pooled analysis showed that there was a statistically significant difference between the experimental and control groups for ALB (MD = 2.62 g/L, 95%CI: 1.78-3.46, **Figure 6A**), PA (MD = 25.37mg/L, 95%CI: 16.07-34.66, **Figure 6B**), and peripheral lymphocyte (MD = 0.21×10^9 /L, 95%CI: 0.10-0.31, **Figure 6C**).

NB levels

Three RCTs[33,36,38](160 participants) reported NB levels, and the statistical heterogeneity was mild ($I^2 = 28\%$). The addition of Gln significantly enhanced the postoperative nitrogen balance in patients(MD = 1.44 g/d, 95%CI: 0.40- 2.49, **Figure 7**).

Immune function-related indicators

Cellular immunity

Six RCTs[32,33,37,44,51,56](396 participants) reported CD3⁺ levels, 12 RCTs[29,30,34,36,38,39,41,45,48,50,52,53](746 participants) reported CD4⁺ levels, and 11 RCTs[32,33,37,39,41,42,44,48,51,53,55,56](686 participants) reported CD8⁺ levels. Moreover, 352 participants from 5 studies[32,41,44,48,53] were enrolled in the CD4⁺/CD8⁺ levels analysis. All variables demonstrated a statistically significant difference between the experimental and control groups for CD3⁺ levels(MD = 2.99%, 95%CI: 1.29-4.69, **Figure 8A**), CD4⁺ levels(MD = 3.82%, 95%CI: 2.80-4.84, **Figure 8B**), CD8⁺ levels(MD = -1.75%, 95%CI: -4.02 - 0.52, **Figure 8C**), and CD4⁺/CD8⁺ levels (MD = 0.11, 95%CI: -0.24-0.46, **Figure 8D**).

Humoral immunity

Five RCTs[32,37,48,55,56](330 participants) reported IgA levels, 6 RCTs[32,36,37,39,48,56](376 participants) reported IgG levels, and 7 RCTs[32,36,37,39,48,55,56](420 participants) reported IgM levels. Gln supplementation was associated with a significant difference in IgA levels (MD = 0.30 g/L, 95%CI: 0.21-0.39, **Figure 9A**), IgG levels(MD = 1.45 g/L, 95%CI: 0.35-2.56, **Figure 9B**) , and IgM levels (MD = 0.33 g/L, 95%CI: 0.13-0.53, **Figure 9C**). These results suggest that Gln supplementation effectively improves the immune function in patients with CRC.

Inflammation-related indicators

A total of 385 participants from 6 studies[31,40,47,49,53,54] were enrolled in the TNF- α levels analysis, and 366 participants from 4 studies[31,53,54,56] were enrolled in the CRP levels analysis. There was a significant difference between the experimental and control groups for TNF- α (MD = -13.10pg/mL, 95%CI: -20.72 to -5.48, **Figure 10A**) and CRP levels (MD = -4.45mg/L, 95%CI: -7.03 to -1.86, **Figure 10B**), suggesting that Gln downregulates inflammatory cytokines in patients with CRC after radical surgery. However, this result should be interpreted with caution owing to higher heterogeneity.

Sensitivity Analyses

In this study, sensitivity analysis (removing one by one) was performed on the pooled analysis results with high heterogeneity ($I^2 > 75\%$) to verify the robustness of the pooled analysis (LOS , ALB, PA, CD8⁺, CD4⁺/CD8⁺, IgG, IgM, TNF- α , CRP, **Figure 11A-11I**). Sensitivity analysis of IgM results showed that the combined results fluctuated greatly after excluding the studies by Chen AH[55], the pooled results changed from 0.33 g/L [MD = 0.33; 95%CI: 0.13-0.53] to 0.20 g/L [SMD = 0.20; 95%CI: 0.15-0.24]. Reviewing the study of Chen, it was found that on the premise of ensuring research methods and quality control, We found no reason to affect robustness and cannot explain this change for the time being. However, sensitivity analyses of the other measures did not find that the removal of one of the studies had a significant effect on the original pooled results, which suggests that the results of the meta-analysis are more robust and reliable.

Subgroup analysis

This meta-analysis included a subgroup analysis of studies with an $I^2 > 75\%$ to explore the sources of heterogeneity (**Table 4**). The criteria for subgroup analysis were tumor type (CC/RC/CRC), Gln supplementation route (PN/EN), time ($> 7/ \leq 7$ d or $> 5d/ \leq 5$ d), timing (postoperative/preoperative+postoperative), and sample size ($> 30/ \leq 30$). From the results of the subgroup analysis, we found that for the primary end point of LOS and the secondary end points of ALB and PA, glutamine supplementation was shown to shorten patients' LOS and improve postoperative ALB and PA levels regardless of the subgroup analysis under any criteria. However, for CD8⁺ levels, the results showed that glutamine supplementation did not improve CD8⁺ levels in patients by any criteria. In addition, for CD4⁺/CD8⁺ levels, ¹the results showed that there was no statistically significant difference in glutamine supplementation under the stratification criteria of tumor type, time and sample size, while under the stratification criteria of supplementation route and timing, preoperative to postoperative enteral glutamine supplementation could improve the levels of CD4⁺/CD8⁺; whereas, for IgG and IgM, it appeared that parenteral glutamine supplementation improved the levels, and enteral supplementation was not statistically significant. For TNF- α levels, the results showed that enteral supplementation, with a duration of > 7 days and a sample size of > 30 in a single group, significantly reduced patients' postoperative TNF- α levels, regardless of the timing of initiation; similarly, supplementation for > 7 days also reduced postoperative CRP levels.

Publication bias

Publication bias was tested *via* Egger's test for outcome measures by combining over ten studies(**Figure 12A-12G**). By this test, we found no statistically significant publication bias for the morbidity rate of infectious complications ($P = 0.631$) and non-infectious complications($P = 0.287$), LOS($P = 0.954$), ALB levels ($P = 0.695$), PA levels ($P = 0.054$), CD8⁺ levels ($P = 0.850$). However, publication bias was found for CD4⁺ levels($P = 0.014$),

this suggests that the effect of small studies is smaller than that of large studies, therefore, the pooled result of CD4⁺ levels should be interpreted conservatively.

DISCUSSION

Gln is an α -amino acid that is abundant in plasma and skeletal muscle[57]; it is the energetic substrate of intestinal mucosal cells, lymphocytes, and neutrophils and can promote lymphocyte proliferation, macrophage phagocytosis[58,59], and stimulate crypt cell proliferation in the crypt basal region[60]; moreover, Gln is the precursor of glutathione[61]; it can resist free radical-induced cell damage[62,63], induce intestinal epithelial cell regeneration, and repair damaged intestinal mucosa, thus reducing the entry of bacteria and endotoxins into the bloodstream[64,65]. Our analysis showed that, compared with conventional nutritional support, perioperative Gln intervention reduced the morbidity of postoperative infectious complications and non-infectious complications, and shortened LOS 2.31 days in CRC patients. This finding is consistent with the results of recent studies. A study reported by Yangt *et al.*[29] in 2021, which included 31 studies with 2201 patients after radical colorectal cancer, showed that SSI (RR = 0.48; 95%CI: 0.30–0.75; $P = 0.001$), anastomotic leak (RR = 0.23; 95%CI: 0.09–0.61; $P = 0.003$), and length of stay (SMD = -1.13; 95%CI: -1.68 to -0.58; $P = 0.000$) were significantly lower in the Gln supplementation group. However, some patients in the study had concomitant diseases such as intestinal obstruction and chronic obstructive pulmonary disease; moreover, some received Gln treatment along with other immunonutrients, including arginine, probiotics, and so on. We believe that there are some confounding factors in including conditions such as concomitant comorbidities with combined immunonutrition in this meta-analysis when exploring the risk of postoperative complications among patients with CRC undergoing Gln therapy; therefore, our study excluded these situations and updated the recently published RCTs. Marta Sandini *et al.*[24] showed that Gln supplementation reduces 2.67 days of hospitalization (95%CI = -3.83 to -1.50; $P < 0.0001$) in patients undergoing elective major abdominal surgery but did not have an impact on the morbidity of complications (RR =

0.64; 95%CI = 0.38-1.07; $P = 0.087$), which is somewhat different from our findings. The reason for this discrepancy may be that the study included other gastrointestinal tract tumors in addition to CRC. Moreover, this study was limited to patients who received PN, and compared with our studies on both EN and PN, the positive effect of PN alone on postoperative complications may not be significant. All aforementioned analyses suggest that Gln supplementation has a protective role pertaining to the LOS, but this conclusion should be interpreted with caution, as length of stay is only plausible in the context of uniform discharge criteria; otherwise, it is highly subjective and relevant. ¹³ In recent years, with the rapid development of enhanced recovery after surgery (ERAS), the reduction in LOS has been affected to some extent. According to the research of Greco *et al.*[66], effective ERAS protocols can reduce LOS by almost 2 days without increasing readmission rates, as well as disease severity. Changes in clinical and surgical practice, patients' economic status, hospital technology, and regional medical policy changes will have an impact on LOS; in the future, we perhaps focus on complications rather than LOS.

Patients with tumors suffer from uncontrolled growth and division of tumor cells due to metabolic disorders and immunosuppression[67], which leads to enhanced catabolism and increased nutritional requirements in the body, ultimately resulting in malnutrition[68]. Nutritional risk has been shown to be an independent predictor of postoperative complications, patients with higher nutritional risk having a higher complication morbidity, increased postoperative mortality, and reduced quality of life postoperatively compared with well-nourished[68]. The nutritional indicators of surgical patients will have a downward trend after surgery, and then gradually recover, but the changes are different. Usually, the half-life of prealbumin is shorter and the sensitivity is higher than that of albumin, and it is more likely to show an early downward trend after surgery. However, albumin showed a lagging trend. Our analysis found that glutamine supplementation improved postoperative levels of ALB and PA, as well as counts of peripheral lymphocyte. This is similar to the findings of Beltrán Chaidez YL *et al.*[69], who investigated the effects of parenteral Gln

supplementation on patients undergoing surgery for gastrointestinal tumors and found that, after 7 days of postoperative intravenous Gln infusion (0.4 g/kg/d), the nutritional status of patients improved significantly; the concentration of lymphocytes ($P < 0.014$) and PA levels increased ($P < 0.012$). Therefore, Gln may increase serum ALB levels by improving liver function, regulating protein synthesis, and reducing protein decomposition, which is mediated by the p38 MAPK pathway[70], in addition to stimulating protein synthesis in skeletal muscle and intestinal epithelial cells, and inhibiting related degradation signaling pathways[71,72]. It is worth noting that PA is sensitive in judging acute changes in protein because of its short half-life and low plasma content, whereas ALB has a longer half-life (about 15–19 days), and the intervention time of Gln in most RCTs included did not last more than two weeks. The short-term monitoring of nutritional indicators may not accurately reflect its real changes; therefore, the above mentioned results should be interpreted with caution. Moreover, Gln provides a nitrogen source for the body and plays an important role in NB levels. Few previous studies have explored the effect of glutamine on NB in patients with CRC; therefore, we added an analysis of NB, our pooled analysis suggests that perioperative Gln supplementation cannot reverse the hypercatabolism caused by surgery but can help improve negative NB levels(MD = 1.44 g/d; 95%CI: 0.40– 2.49). This was found in the first clinical study using Ala-Gln, in which the postoperative NB improved more significantly after 5 days of dipeptide infusion in patients undergoing colon or rectal resection compared with conventional TPN and isonitrogenous and isocaloric intake[73]. In the animal experiment[74], parenteral nutrition containing Gln was injected into rats before and after gastrectomy. The NB of the rats improved within 1d postoperatively, and the phagocytic activity of peritoneal macrophages was higher in the Gln group ($P < 0.05$). Perioperative Gln intervention can improve the negative NB; however, more clinical studies are needed to confirm this.

Surgery induces strong local and systemic inflammatory responses characterized by changes in the plasma concentrations of various acute-phase proteins and pro-inflammatory factors. Gln can induce CD4⁺ T cells to release the anti-inflammatory

factor IL-10 by enhancing the expression of heat shock protein 70[75,76] and regulating the expression of p38 MAPK, thus inhibiting the expression of TNF- α and IL-6[13]. Moreover, Gln increases the number of Pan cells and goblet cells in the intestinal mucosa, and increases IL-4, IL-10, and IL-13 Levels[64]. Our pooled results showed that postoperative TNF- α (MD = -13.10pg/mL; 95%CI: -20.72 to -5.48) and CRP levels (MD = -4.45mg/L; 95%CI: -7.03 to -1.86) were lower among Gln-supplemented patients compared with controls, and one of the possible mechanisms is that it inhibits the synthesis of TNF- α through the negative regulation of NF- κ B phosphorylation[77]. Leclaire S *et al.* found that Gln supplementation decreased the levels of IL-8 and IL-6 by regulating NF- κ B expression, and when combined with Arg, p38 MAPK expression was observed, leading to a decrease in TNF- α production[13]. Moreover, animal experiments showed that Gln supplementation could reduce the production of TNF- α , IL-1, and IL-6 and intestinal mucosal injury by inhibiting the expression of TLR4, MyD88, and TRAF6, respectively, in the intestinal mucosa of endotoxemia rats[78]. Patients with tumors are prone to immunosuppression due to protein-energy malnutrition[79], and the consumption of Gln increases in inflammatory states, which aggravates immunosuppression[80] and finally increases the postoperative infection rate[81]. Our analysis showed that Gln intervention improved cellular and humoral immune function. This suggests that Gln can exert its immune effects by acting on immune cells[82], it is noteworthy that our analysis showed that glutamine intervention did not improve CD8⁺ levels and CD4⁺/CD8⁺ ratio. The reason for this may be that the sample size included in this study was small and no statistically significant results were observed, of course, this is worthy of further investigation in subsequent studies. Parry-Billings *et al.*[83] found that the proliferation of human immune cells was reduced when Gln levels were ⁷ below physiologically normal concentrations, despite the presence of other amino acids and fuels (e.g. glucose). The following are possible reasons for this phenomenon: 1. Gln promotes the proliferation of immune cells by activating the transcription of genes related to cell proliferation, including ERK and JNK kinases, and further activating JNK, AP-1, and other transcription factors[84] and 2. the expression of

ASCT2 and GLS promotes the production of IgG and IgM during the decomposition of Gln[85]. Gln improves innate immune function by maintaining the number of pancreatic and goblet cells, normalizing Th2 cytokines, and increasing resistance to bacterial mucosal invasion[86]. In summary, we found that perioperative Gln supplementation not only reduced postoperative complications, but also shortened LOS, which was consistent with most previous studies. More importantly, we found that it could also reduce the inflammatory response and improve the immune function in patients with CRC.

However, we should not ignore the following limitations: 1. The exploration of the timing of glutamine intervention has been the focus of research in recent years, but our analysis could not yield the ideal timing and dose of Gln application. However, most of the current clinical data support that patients with gastrointestinal tumors receive preoperative immunonutrition for at least 5-7 days; it would be more helpful in modulating the impaired immune function and inflammatory response of the body in the early postoperative period[87-89]. 2. Most of the 27 RCTs in our study did not specifically elaborate on the randomized method. Moreover, none of the trials explained whether the allocation regimen was hidden, indicating that there are some methodological limitations. 3. The RCTs included in this study comprised most RCTs in China, and whether the results obtained are applicable to other national populations needs to be studied. 4. Although the RCTs we included underwent rigorous literature screening and inclusion and exclusion criteria and were somewhat homogeneous, we noted a large number of inter- and intra-trial differences, including surgery types, Gln supplementation route, dose, and duration of maintenance, and some of the available trial reports did not allow us to definitively determine the extent to which enrolled patients received treatment. Moreover, some studies had small sample sizes and lacked intention-to-treat(ITT) analysis; all these potential biases could have interfered with the results and affected the true effect of glutamine intervention. In summary, our findings indicate that the clinical application of glutamine-enhanced nutritional support for CRC patients is conducive to ¹reducing the incidence of postoperative complications,

shortening LOS, and improving postoperative short-term prognosis of patients, which has certain reference value for guiding clinical immune nutritional support.

CONCLUSION

In conclusion, our study found that Gln supplementation could not only effectively improve the postoperative nutritional status, reduce the incidence of postoperative complications and shorten LOS, but also, importantly, maintain NB, enhance immune function and reduce inflammation levels of CRC patients, without the interference of comorbidities and other immune nutrients. Compared with previous studies, the endpoints of our research are more comprehensive and can more convincingly demonstrate that the application of Gln during the perioperative period can bring positive prognoses to CRC patients. However, while accepting this conclusion, it is important to avoid ignoring its limitations, and the results should be interpreted cautiously. Future RCTs with large sample sizes and higher methodological quality to further evaluate the efficacy of Gln and, most importantly, explore the ideal timing of Gln application are warranted. Second, because there are few studies on intestinal barrier function, blood glucose level, and insulin resistance in patients with CRC after Gln intervention in current RCTs, more clinical trials are needed to fill the gap in this area in the future to comprehensively evaluate the clinical benefits of Gln among this patient population.

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