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# Perioperative management and cancer outcome

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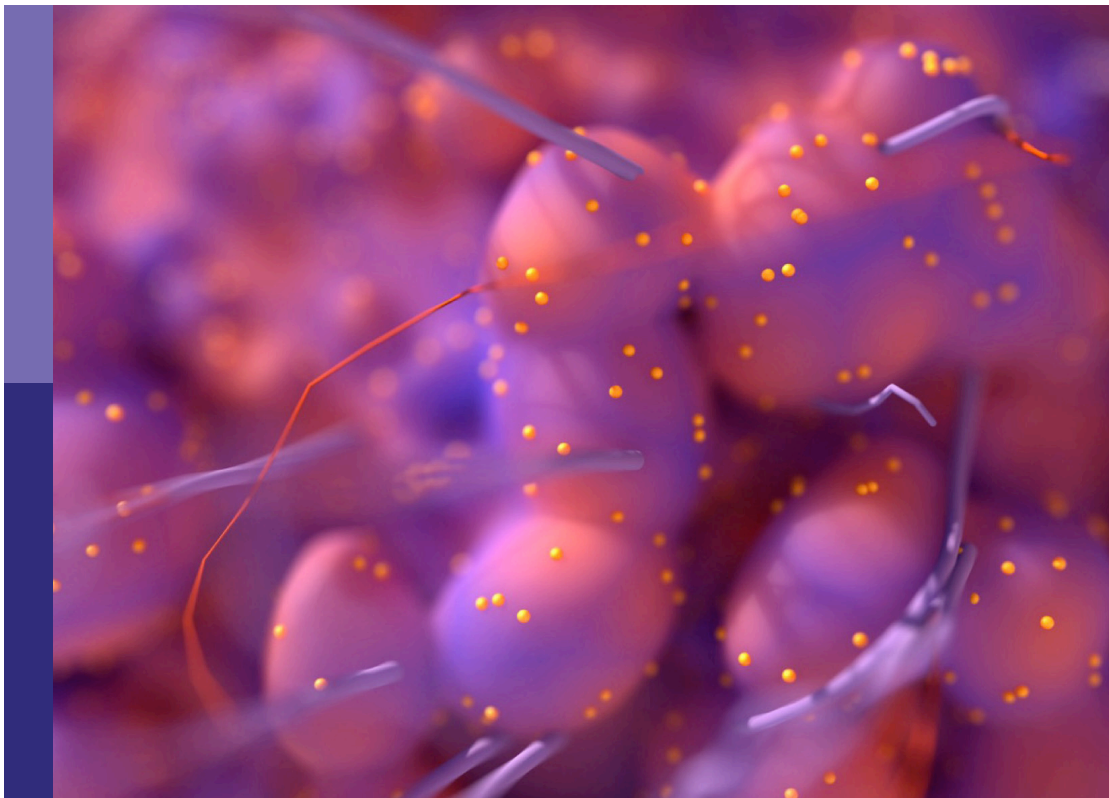

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# Perioperative management and cancer outcome

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# Editorial: Perioperative management and cancer outcome

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KEYWORDS

perioperative management, cancer outcome, prognosis, postoperative complication, prehabilitation

Editorial on the Research Topic
Perioperative management and cancer outcome

Perioperative management plays a critical role in determining the outcomes of patients with cancer undergoing surgery. It encompasses a comprehensive approach that involves careful planning, meticulous execution, and attentive postoperative care. The perioperative period, which includes the preoperative, intraoperative, and postoperative phases, presents numerous challenges and opportunities to optimize patient care and improve treatment outcomes. The Research Topic aimed to present some of the more recent evidence integrating clinical observations and experimental findings linking perioperative management and cancer-related outcomes.

Several studies focused on the potential long-term effects of perioperative and intraoperative pharmacological management on tumors. The influence of anesthetic approaches on cancer patients is complex. Abundant evidence from animal studies has suggested that different types of anesthetics can influence tumor progression and survival outcomes in patients with malignancies (1, 2). The impact of intraoperative low-dose dopamine administration in hepatic surgery emerges as another intriguing topic within this Research Topic. The propensity score matching analysis examining its association with survival rates in hepatocellular carcinoma patients conducted by Wang et al. highlight the potential implications of such intervention on long-term outcomes. Dexmedetomidine is a frequently used sedative during surgery. Xu et al. conducted a meta-analysis and showed the impact of dexmedetomidine in reducing systemic inflammation and postoperative cognitive dysfunction and improving recovery in patients undergoing digestive tract cancer surgery.

Importantly, the Research Topic also addresses the value of non-surgical interventions in perioperative care. Prehabilitation is a proposed modality for optimizing preoperative conditions to improve postoperative outcomes. Studies reported potential advantages for various surgical procedures (3, 4). However, according to Zhang X. et al.'s systematic reviews and meta-analyses, rehabilitation did not significantly enhance postoperative

outcomes for colorectal surgery patients when postoperative complications, length of hospital stay and functional capacity were considered.

Other studies focused on the association between specific biomarkers and long-term survival rates in patients with cancer. According to Zhang H. et al., mu-opioid receptor (MOR) expression in ovarian cancer patients undergoing surgery is not an independent predictor of worse survival but is related with high rates of perineural invasion. Furthermore, one team conducted an intensive study on perioperative management and biochemical markers of colorectal cancer (CRC) patients. The articles shed light on the significance of factors such as anemia tolerance, blood transfusions, neutrophil and white blood cell (WBC) count levels in CRC. Weng et al. demonstrated that preoperative anemia and blood transfusion increased the risk of colorectal cancer surgery recurrence, thus promoting the idea of anemia tolerability and limiting the use of blood transfusion. Considering the characteristics of CRC, which is an inflammation-related tumor characterized by the infiltration of heterogeneous immune cells into the tumor microenvironment and peripheral hematological disorders (5), they included neutrophil and WBC as variables of interest in the study. Weng et al. indicated that elevated myeloperoxidase (MPO) levels in CRC patients were substantially linked with high preoperative neutrophil counts, implying that neutrophils may be crucial participants in the mechanism connecting MPO levels with poor CRC outcomes. Also, they have found that a high preoperative WBC count was a poor prognostic indicator and was associated with an immunosuppressive microenvironment in CRC patients (Weng et al.). By dissecting the relationships between these variables and patient outcomes, researchers provide a deeper understanding of the disease's progression and offer valuable insights for tailored perioperative management strategies.

Additionally, the articles emphasize the significance of prevention and care of postoperative complications and hospital volume-patient outcome relationships. Dai et al. identified risk factors associated with an increased incidence of postoperative pulmonary complications (PPCs) in patients aged over 60 years who underwent elective colorectal surgery. The retrospective analysis revealed that age, preoperative red blood cell distribution width, and systemic inflammatory index were independent risk factors for PPCs occurrence and emphasized the importance of early identification and management. Zhang Z. et al. reviewed the literature and noted that the novel tumor suppressor esophageal cancer-related gene-4 (ECRG4) could be used in the treatment of both tumors and arrhythmias, identifying a new possible strategy to reduce the perioperative cardiovascular adverse events in patients with esophageal cancer and gastric cancer. Lei et al. suggested that centralized management of esophageal cancer surgery, while beneficial for patient survival, should ideally not exceed the identified hospital volume threshold, providing evidence-based insights for improved patient care and optimized resource allocation. These findings open doors for further exploration and potential interventions that positively influence patient prognosis.

The collective findings presented within this Research Topic, "*Perioperative management and cancer outcome*," contribute significantly to our understanding of the intricate relationship between perioperative care and cancer prognosis. The insights derived from these articles underscore the importance of personalized, multidisciplinary approaches that consider specific biomarkers, tailored interventions, and risk factor identification. Healthcare professionals can refine perioperative management strategies and optimize cancer treatment outcomes by recognizing the potential impact of factors such as anemia tolerance. Furthermore, the significance of non-surgical interventions, including prehabilitation, targeted pharmacological approaches, and suitable hospital volume, highlights the potential for holistic patient care.

Advancements in perioperative management have the power to significantly influence long-term cancer outcomes. As editors, researchers, and healthcare professionals, let us harness the knowledge presented within this Research Topic to enhance our understanding and implementation of effective perioperative care protocols.

## Author contributions

YT, JPC, WZ and JT: concept and design. YT: drafting of the manuscript. JPC, WZ and JT: critical revision of the manuscript for important intellectual content, administrative, and supervision. All authors contributed to the article and approved the submitted version.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Association of Mu-Opioid Receptor Expression With Long-Term Survival and Perineural Nerve Invasion in Patients Undergoing Surgery for Ovarian Cancer

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**Background:** Opioids are widely used during primary debulking surgery (PDS) for ovarian cancers, and a high mu-opioid receptor (MOR) expression predicts worse cancer outcomes. However, the impact of MOR expression on survival outcomes in ovarian cancers is still not clear.

**Methods:** A retrospective cohort study was conducted in patients who underwent PDS in ovarian cancer patients. MOR expression was measured in tumor and normal tissue. Primary outcomes were overall survival (OS) and disease-free survival (DFS). Secondary outcomes included perineural invasion (PNI), intraoperative sufentanil consumption, length of stay (LOS), and verbal numerical rating scale (VNRS) on postoperative day 1 (POD1), POD3, and POD5.

**Results:** After propensity score matching, a total of 366 patients were finally enrolled in this study. There were no significant differences in OS rates in patients with high versus low levels of MOR (1-year OS: 82.9% versus 83.3%, 3-year: 57.8% versus 59.1%, 5-year: 22.4% versus 23.1%,respectively) in the ovarian cancers. There were no significant differences in DFS between the groups. Intraoperative sufentanil consumption was higher in the MOR high-expression group compared with the MOR low-expression group. Tumors expressing high levels of MOR showed higher rates of PNI. VNRS in the MOR high-expression group was higher on POD1.

**Conclusion:** MOR is not an independent predictor of worse survival in ovarian cancers but is associated with high rates of perineural invasion.

**Keywords: ovarian cancer, mu-opioid receptor, overall survival, disease-free survival, surgery**

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# INTRODUCTION

Ovarian cancer is the third most common gynecological tumor and ranks 5th in all cancer-related deaths in women (1). Although significant progress has been made in the early diagnosis and treatment of ovarian cancer in recent years, the 5-year survival rate of ovarian cancer patients is still lower than 40% (2). This worrisome statistics highlights the need for new therapies.

Primary debulking surgery (PDS) remains the cornerstone in ovarian cancer treatment (3). Primary ovarian cancer surgery is performed to achieve optimal cytoreduction, as the amount of residual tumor is one of the most important prognostic factors for survival of women with high-stage epithelial ovarian cancer (3). Opioids remain the primary analgesics during and after ovarian cancer surgery (4, 5). Opioids mainly exert their analgesic effect by acting as agonists of the mu-opioid receptor (MOR) located in neurons, but it is also expressed on cancer cells (5–7). Previous clinical studies have found that a high tumoral MOR expression is associated with poor prognosis in hepatocellular, laryngeal, and lung cancers (8–10). Furthermore, MOR expression was associated with high perineural nerve invasion (PNI), a clinical predictor of survival in pancreatic and laryngeal cancers (9, 11). In contrast, other studies have found that MOR expression is not a predictor of worse long-term survival in pancreatic and colorectal cancers (11–13).

The association between MOR expression and the long-term prognosis of ovarian cancer is still unclear. Therefore, we conducted a retrospective study and hypothesized that a high expression of MOR is associated with poor prognosis in ovarian cancer. In addition, we determined the impact of MOR expression on length of hospital stay (LOS), intraoperative opioid consumption, and postoperative pain intensity.

# METHODS

## Study Population

This study was conducted at the Fudan University-affiliated hospitals and obtained ethics committee board approval. The inclusion criteria for this study were a) women undergoing PDS for ovarian cancer from January 2015 to December 2018, PDS criteria based on International Federation of Gynecology and Obstetrics (FIGO) stage III or IV ovarian, tubal, and peritoneal cancers diagnosed using clinical findings, including imaging studies (CT, MRI, and chest radiography) and cytology of ascites, pleural effusions, or tumor cyst fluids obtained by tumor centesis; b) aged between 18 and 70 years; c) undergoing surgery under combined general and epidural anesthesia; and d) complete clinical characteristics and follow-up data. Patients were excluded if they met the following exclusion criteria: a) underwent second-time or emergency surgery; b) had a history of other malignancies; c) died within hospital stay after surgery; and d) lost to follow-up. We define surgical complexity based on the number and complexity of the surgical procedures performed. Scores ranging from 1 to 3 were assigned to each surgical procedure based on the complexity of the procedure. We then developed an ordinal scale so that the patients could be stratified into three groups: simple, intermediate, and complex surgery (14).

## Co-Primary Outcomes

The primary outcomes of this study were overall survival (OS) and disease-free survival (DFS). OS was defined from the surgery date to the date of death or last lost follow-up (15). DFS was determined from the surgery date to the date of ovarian cancer recurrence (15). Routine clinical follow-ups were done every 3 months in the first and second years and every 6 months in the third to fifth years. The final follow-up date was January 31, 2020. Cancer recurrence was determined using a combination of computed tomography scan, positron emitted tomography scan, and serum concentrations of CA-125 (16).

## Secondary Outcomes

Secondary outcomes included PNI, length of stay, intraoperative sufentanil consumption, and pain intensity using the verbal numeric rating scale (0: no pain–10: worst pain ever).

## Anesthesia Care

All patients were monitored according to American Society of Anesthesiologists (ASA) guidelines. Induction of general anesthesia was performed with propofol (3.0–4.0 μg/ml, target-controlled infusion protocol (TCI)), sufentanil (0.3–0.5 μg/kg), and rocuronium (o.5 mg/kg). After induction of general anesthesia, patients were tracheal intubated, and general anesthesia was maintained with 2.0%–3.0% sevoflurane in a mixture of oxygen/air. An epidural infusion of 0.375% ropivacaine was used during surgery. After surgery, patients received patient-controlled epidural analgesia (PCEA, 0.1% ropivacaine and 0.5 μg/ml sufentanil, basal infusion: 2–3 ml/h, bolus: 3–4 ml, lockout time: 15 min) for 48 h.

## Immunohistochemistry and PNI

All the samples were retrieved from banked tissue samples. Briefly, immunohistochemistry (IHC) staining was performed in ovarian tumor or normal tissue (ovarian). The primary antibody was the anti-mu opioid receptor (UMB3) C-terminal (ab134054). The antibody was used at a concentration of 1:200. Secondary antibodies anti-Goat Anti-Rabbit IgG H&L (HRP) (ab205718) were used. After staining, two pathologists blinded to clinical data reviewed and scored the sections independently. The IHC score was calculated as previously reported (11). Briefly, the intensity of MOR was graded from 0 to 3, and the percentage of MOR positive was also graded from 0 to 3 (score 0: <25% positive, score 1: 25%–50% positive, score 2: 51%–75% positive, and score 3: >75% positive). A total score from 0 to 6 was calculated (11). PNI was defined as cancer cells that invade the perineural spaces of surrounding nerves (17).

## Statistical Analysis

Patients' characteristics were summarized with descriptive statistics. Continuous data were expressed with mean ±

standard deviation (SD) and analyzed with a t-test. Categorical data were described with n (%) and analyzed with the chi-square test. Chi-square or Fisher's test was used to evaluate associations between categorical variables. The Mann–Whitney U test or t-test was used to assess continuous variables between the groups. The Kaplan–Meier method was used to analyze OS and DFS in the model. Hazard ratios (HR) were calculated with corresponding 95% confidence intervals (CI). Multivariable Cox proportional hazard models were used, including significant covariates. From a recent retrospective study in a similar population of patients (3), the median overall survival time of subjects was 42.3 and 38.5 months, respectively. Assuming that alpha = 0.05, with a two-sided test having power of 80%, a total of 583 participants would be required to detect a 3.8-month difference in overall survival between groups. Because we anticipated a dropout rate of 8%, we planned to enroll 633 patients in the trial. We performed propensity score matching to reduce bias using a 5- to 1-digit Greedy matching algorithm (3). Ten variables were used in the model, including age, body mass index (BMI), ASA class, Charlson comorbidity index (CCI), histologic diagnosis, tumor differentiation, surgical complexity, residual disease, and adjuvant chemotherapy. The standardized differences for all covariates did not exceed 3.45% in the post-matching cohort, suggesting a substantial reduction of bias between the two groups. The mean cutoff values for MOR expression were analyzed with X-Tile software (17). A P-value <0.05 was considered statistically significant. Statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

# RESULTS

A total of 483 patients were included in the study. After the initial examination, 206 patients were grouped in the high MOR expression cohort and 277 in the low-expression group. After propensity score matching, 183 patients remained in each group (MOR high versus MOR low). The baseline characteristics were similar between both groups of patients (**Table 1**).

## Primary Outcome

The median follow-up time in all patients was 45.4 (43.2, 47.3) months. The Kaplan–Meier survival curves for the MOR high expression and MOR low expression are shown in **Figure 1**. There were no significant differences in OS rate at the first, third, and fifth years between the MOR high expression and MOR low expression groups (1-year OS: 82.9%, vs. 83.3%, 3-year OS: 57.8%, vs. 59.1%, and 5- year OS: 22.4% vs. 23.1%, respectively, **Figure 1A**). The univariate analysis indicated that the following covariates were significantly associated with worse OS: age, ASA physical status, CCI, non-serous histology, poor tumor differentiation, residual disease, surgical complexity, ascites, estimated blood loss, and no adjuvant chemotherapy (**Table S1**).

The multivariate analysis after propensity score matching demonstrated that non-serous histology (HR = 1.86, 95% CI: 1.32–2.38, P = 0.018), poor tumor differentiation (HR = 1.26, 95% CI: 1.13–2.73, P < 0.001), residual disease (HR = 1.46, 95% CI: 1.02–1.94, P = 0.023), and no adjuvant chemotherapy (HR =

**TABLE 1** | Patient and treatment characteristics for both groups.

| Variable | Original cohort | | *P* | Matched cohort | | *P* | Standard difference (%) |
|---|---|---|---|---|---|---|---|
| | MOR high expression (n = 206) | MOR low expression (n = 277) | | MOR high expression (n = 183) | MOR low expression (n = 183) | | |
| **Age (years)** | 53.6 ± 8.6 | 54.2 ± 8.2 | 0.436 | 53.2 ± 10.2 | 53.4 ± 10.6 | 0.854 | 1.08 |
| **BMI (kg/m$^2$)** | 25.6 ± 6.3 | 26.3 ± 6.2 | 0.224 | 25.3 ± 6.2 | 26.4 ± 6.3 | 0.093 | 1.65 |
| **ASA (n, %)** | | | 0.857 | | | 0.808 | 2.23 |
| I–II | 151 (73.2%) | 201 (72.6%) | | 137 (75.1%) | 139 (75.8%) | | |
| III–IV | 55 (26.8%) | 76 (27.4%) | | 46 (24.9%) | 44 (24.2%) | | |
| **Patients enrolled** | | | 1.000 | | | 0.775 | |
| 2015 | 49 (23.7%) | 65 (23.5%) | | 42 (23.1%) | 43 (23.5%) | | |
| 2016 | 46 (22.5%) | 63 (23.1%) | | 41 (22.8%) | 42 (23.2%) | | |
| 2017 | 50 (24.3%) | 67 (24.2%) | | 45 (24.8%) | 47 (25.5%) | | |
| 2018 | 61 (29.5%) | 82 (29.2%) | | 55 (29.3%) | 51 (27.8%) | | |
| **CCI (n, %)** | | | 0.667 | | | 0.976 | 3.35 |
| 0 | 36 (17.5%) | 46 (16.8%) | | 32 (17.8%) | 31 (17.3%) | | |
| 1 | 90 (43.7%) | 123 (44.5%) | | 78 (42.6%) | 77 (41.9%) | | |
| ≧2 | 80 (38.8%) | 108 (38.7%) | | 73 (39.6%) | 75 (40.8%) | | |
| **Histologic diagnosis** | | | 0.880 | | | 0.745 | 2.14 |
| Serous histology | 131 (63.6%) | 178 (64.3%) | | 114 (62.5%) | 117 (63.9%) | | |
| Non-serous histology | 75 (36.4%) | 99 (35.7%) | | 69 (37.5%) | 66 (36.1%) | | |
| **Tumor size** | | | 0.830 | | | 0.816 | 1.96 |
| >5 | 121 (58.9%) | 160 (57.8%) | | 106 (58.1%) | 105 (57.4%) | | |
| <5 | 85 (41.1%) | 117 (42.2%) | | 77 (41.9%) | 78 (42.6%) | | |
| **Tumor differentiation** | | | 0.038 | | | 0.575 | 3.45 |
| Well | 19 (9.3%) | 26 (9.5%) | | 17 (9.4%) | 17 (9.3%) | | |
| Moderate | 116 (56.3%) | 164 (59.2%) | | 99 (54.1%) | 97 (53.5%) | | |

*(Continued)*

**TABLE 1** | Continued

| Variable | Original cohort | | *P* | Matched cohort | | *P* | Standard difference (%) |
|---|---|---|---|---|---|---|---|
| | MOR high expression (n = 206) | MOR low expression (n = 277) | | MOR high expression (n = 183) | MOR low expression (n = 183) | | |
| Poor | 71 (34.4%) | 87 (31.3%) | | 67 (36.5%) | 69 (37.2%) | | |
| **Residual disease** | | | 0.550 | | | 0.865 | 3.24 |
| No visible disease | 98 (47.4%) | 129 (46.7%) | | 83 (45.6%) | 85 (46.3%) | | |
| <1-cm residual disease | 70 (34.1%) | 98 (35.4%) | | 64 (35.4%) | 66 (36.3%) | | |
| >1-cm residual disease | 38 (18.5%) | 50 (17.9%) | | 36 (19%) | 32 (17.4%) | | |
| **Surgical complexity** | | | 0.855 | | | 0.873 | |
| Low | 31 (15.4%) | 45 (16.2%) | | 26 (14.2%) | 26 (14.3%) | | |
| Intermediate | 108 (52.6%) | 148 (53.6%) | | 98 (53.6%) | 96 (52.7%) | | |
| High | 67 (32%) | 84 (30.2%) | | 59 (32.2%) | 61 (33%) | | |
| **Surgery time (min)** | 213 ± 63 | 209 ± 59 | 0.474 | 205 ± 61 | 208 ± 62 | 0.641 | |
| **Ascites (ml)** | | | 0.495 | | | 0.849 | |
| <200 | 36 (17.5%) | 51 (18.3%) | | 29 (15.9%) | 28 (15.6%) | | |
| >200 | 29 (14.1%) | 41 (14.8%) | | 26 (14.2%) | 27 (14.5%) | | |
| **Estimated blood loss (n, %)** | | | 0.750 | | | 0.716 | |
| ≤400 ml | 116 (56.3%) | 160 (57.7%) | | 101 (55.4%) | 100 (54.7%) | | |
| >400 ml | 90 (43.7%) | 117 (42.3%) | | 82 (44.6%) | 83 (45.3%) | | |
| **Blood transfusion** | | | 0.798 | | | 0.615 | |
| No | 131 (63.6%) | 173 (62.5%) | | 112 (61.3%) | 111 (60.5%) | | |
| Yes | 75 (36.4%) | 104 (37.5%) | | 71 (38.7%) | 72 (39.5%) | | |
| **Adjuvant Chemotherapy (n, %)** | | | 0.487 | | | 0.811 | 3.36 |
| No | 63 (30.4%) | 93 (33.5%) | | 59 (32.1%) | 60 (32.6%) | | |
| Yes | 143 (69.6%) | 184 (66.5%) | | 124 (67.9%) | 123 (67.4%) | | |

*BMI, body mass index; ASA, American Society of Anesthesiologists score; CCI, Charlson Comorbidity Index; FIGO, Federation International of Gynecology and Obstetrics.*

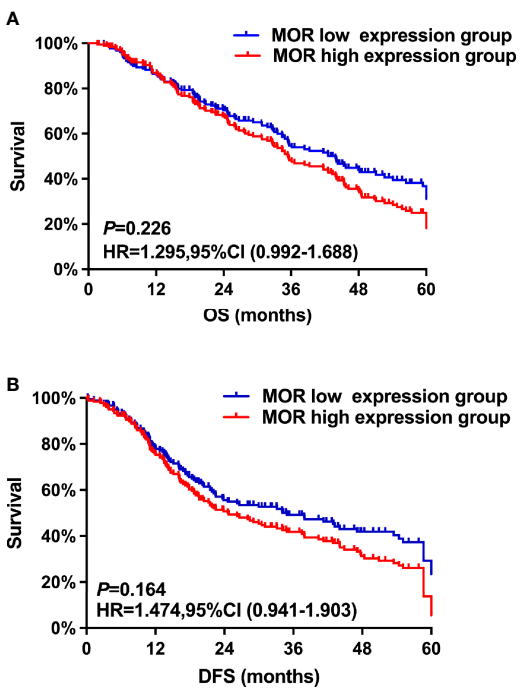


**FIGURE 1** | The study's co-primary outcomes were **(A)** overall survival analysis based on MOR expression and **(B)** disease-free survival based on MOR expression.

1.36, 95% CI: 1.12–1.73, P = 0.026) were associated with worse OS (**Table 2**). A high MOR expression was not a predictor of worse OS (HR = 1.30, 95% CI: 0.99–1.69, P = 0.226).

Similarly, there were no significant differences in first-, third-, and fifth-year DFS rates between the MOR high-expression cohort and the MOR low-expression group of patients (1-year DFS: 77.3%, vs. 78.6%, 3-year DFS: 47.8%, vs. 48.3%, and 5- year DFS: 18.4% vs. 22.1%, respectively, **Figure 1B**). The univariate analysis indicates that the following covariates were significantly associated with worse OS: age, ASA, CCI, non-serous histology, poor tumor differentiation, residual disease, surgical complexity, ascites, estimated blood loss, and adjuvant chemotherapy (**Table S1**).

The multivariate analysis after propensity score matching indicated that non-serous histology (HR = 2.13, 95% CI: 1.74–2.88, P = 0.046), poor tumor differentiation (HR = 1.68, 95% CI: 1.42–2.75, P = 0.035), FIGO stage (HR = 1.53, 95% CI: 1.48–2.28, P < 0.001), residual disease (HR = 1.76, 95% CI: 1.22–2.42, P < 0.001), and no adjuvant chemotherapy (HR = 2.34, 95% CI: 1.12–2.63, P < 0.001) were associated with shorter DFS (**Table 2**). A high MOR expression was not a predictor of worse DFS (HR = 1.47, 95% CI: 0.94–1.90, P = 0.164).

## Secondary Outcomes

The mean intraoperative sufentanil consumption in the MOR high-expression group was significantly higher than in the MOR low-expression group (47.2 ± 4.6 vs. 38.6 ± 4.8, P < 0.001, **Figure 2A**). Pain intensity was higher on POD1 in the MOR

**TABLE 2** | Multivariable Cox proportional of OS and DFS.

| Variables | OS (before matching) | | OS (after matching) | | DFS (before matching) | | DFS (after matching) | |
|---|---|---|---|---|---|---|---|---|
| | HR (95% CI) | *P*-value | HR (95% CI) | *P*-value | HR (95% CI) | *P*-value | HR (95% CI) | *P*-value |
| **Histologic diagnosis (non-serous histology)** | 1.93 (1.22–2.98) | 0.026 | 1.86 (1.32–2.38) | 0.018 | 2.30 (1.62–2.92) | 0.018 | 2.13 (1.74–2.88) | 0.046 |
| **Tumor differentiation (poor)** | 1.44 (1.02–2.78) | 0.011 | 1.26 (1.13–2.73) | <0.001 | 1.76 (1.62–2.88) | 0.023 | 1.68 (1.42–2.75) | 0.035 |
| **FIGO stage (III–IV)** | 1.58 (1.15–2.15) | <0.001 | 1.39 (1.22–1.88) | <0.001 | 1.63 (1.45–2.35) | <0.001 | 1.53 (1.48–2.28) | <0.001 |
| **Residual disease (>1 cm)** | 1.46 (1.23–1.58) | <0.001 | 1.46 (1.02–1.94) | 0.023 | 1.83 (1.62–1.98) | 0.026 | 1.76 (1.22–2.42) | <0.001 |
| **Postop-chemotherapy (no)** | 1.75 (1.41–1.62) | <0.001 | 1.36 (1.12–1.73) | 0.026 | 2.54 (1.32–2.88) | <0.001 | 2.34 (1.12–2.63) | <0.001 |

*OS, overall survival; DFS, disease-free survival.*

high-expression cohort compared with the MOR low-expression group (4.76 ± 1.35 vs. 4.10 ± 1.38, P = 0.024, **Figure 2B**). The mean LOS in the MOR high-expression group was 12.7 (11.3, 13.8) days compared with 12.0 (11.4, 14.2) days in the MOR low-expression group (P = 0.665, **Figure 2C**). There were no differences in MOR expression between tumor and normal tissue (mean: 4.2 vs.4.4, P = 0.551, **Figure 3A**). Interestingly, we observed that a high level of MOR expression was associated with a significantly higher rate of PNI (68.9% vs. 53.4%, P = 0.037, **Figures 3B, C**).

## DISCUSSION

In this study, we evaluated the association between MOR expression and ovarian cancer long-term outcomes in patients undergoing PDS. This study found that MOR expression did not significantly affect OS and DFS.

These findings parallel the results of two previous studies in pancreatic cancer (11, 13), indicating that MOR expression in pancreatic ductal adenocarcinoma (PDAC) patients was not associated with worse OS and DFS. Diaz-Cambronero et al. also observed that high levels of MOR expression did not significantly impact the survival of patients with colorectal cancer (12). In contrast, our previous study found that an increased MOR expression was associated with reduced DFS and OS in subjects with laryngeal squamous cell carcinoma (9). At the *in vitro* level, MOR was found to promote and support tumor growth in lung cancer and hepatocellular carcinoma (18, 19). Furthermore, Gorur et al. observed that downregulating the MOR expression inhibited aggressive cell behaviors in squamous cell carcinoma of the head and neck (20). Fiegl et al. found no benefit of D,L-methadone (opioid agonist) as an adjuvant chemosensitizing anticancer drug in ovarian cancers (21). In their *in vitro* studies, there were no direct anticancer effects found in 2D and 3D cell culture experiments. In addition, the authors observed somewhat contrary results from the 3D cell culture model in which D,L-methadone could either enhance ovarian cancer cell proliferation or counteract the therapeutic effects of cisplatin (22). It is difficult to compare our results with these *in vitro* studies (18–21). The possible reason to explain the discrepancy from *in vitro* studies is bias and confounding owing to unknown and unmeasured variables that might have an impact on the clinical survival outcomes (22–24). Secondly, the difference in the type of cancer, stage of cancer, and the extent of surgical type all may account for the varied effects of MOR and survival outcomes (22–24). Thirdly, different-opioid consumption could have different effects on tumor growth and clinical survival outcomes (25). Our study also showed that tumor differentiation, FIGO stage, residual disease, ascites, and intraoperative and adjuvant chemotherapy

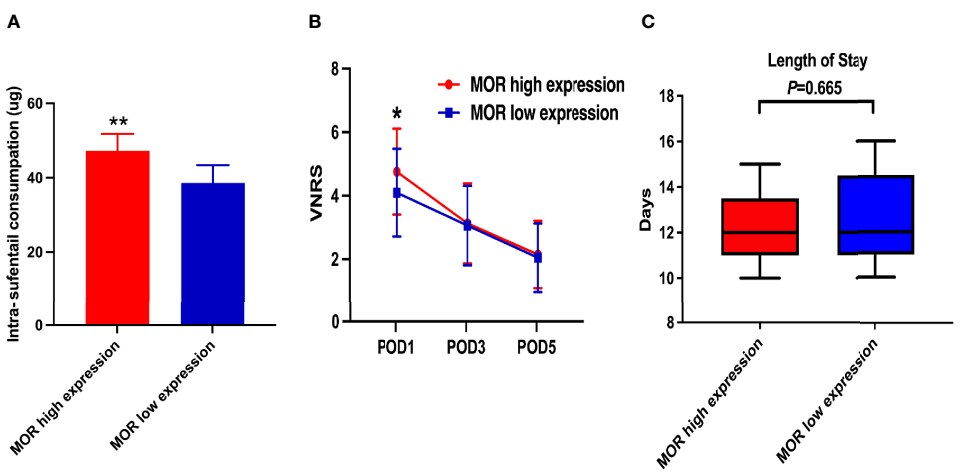


**FIGURE 2** | Secondary outcomes of the study. **(A)** Intraoperative sufentanil consumption according to MOR expression; **(B)** VNRS on POD1, POD3, and POD5 according to MOR expression; and **(C)** LOS according to MOR expression. MOR, mu-opioid receptor; VNRS, verbal numerical rating scale. *$P < 0.05$, **$P < 0.01$.

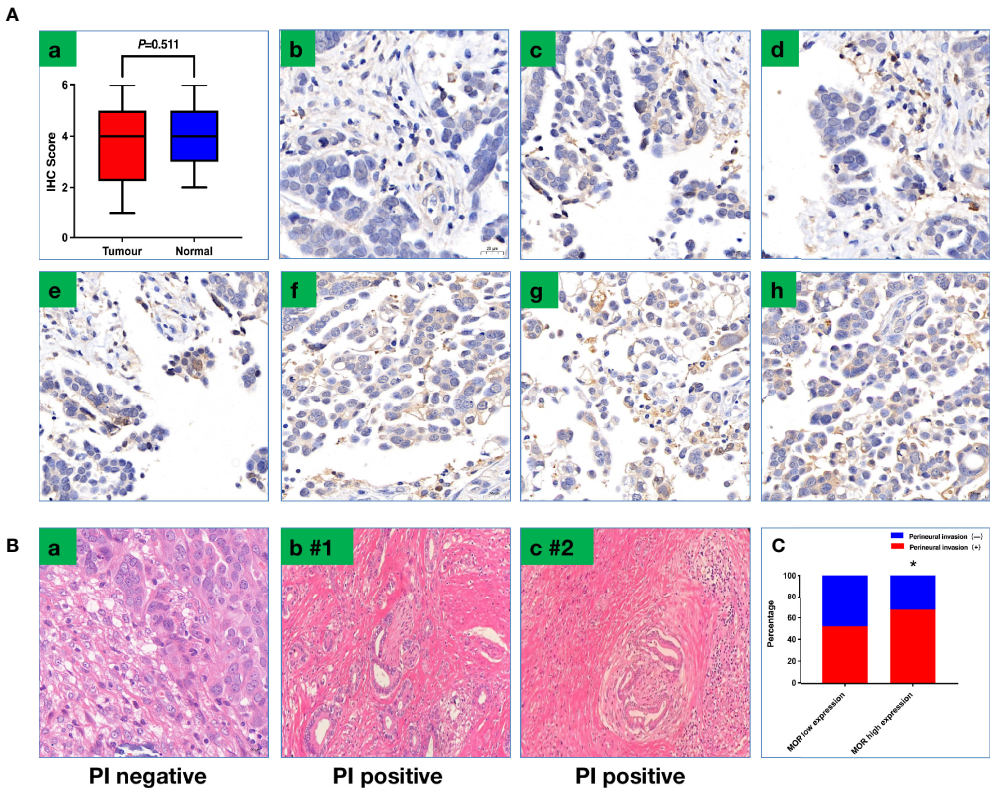


**FIGURE 3** | **(A)** Representative images of IHC to show scoring criteria and MOR expression. (a) MOR expression between tumor tissue and normal tissue; (b) score 0; (c) score 1; (d) score 2; (e) score 3; (f) score 4; (g) score 5; (h) score 6. **(B)** Representative image to show PNI; PNI was defined as cancer cells that invade the perineural spaces of surrounding nerves (a) PNI negative; (b,c) PNI-positive patients (#1–2). **(C)** PNI positive rate based on MOR expression. PNI, perineural invasion, $^{*}P < 0.05$.

were predictors of poor outcomes, as previously reported in other studies (26–29).

Interestingly, we observed that patients with a high expression of MOR also required higher dosages of sufentanil. At least three previous studies reported similar findings in patients with prostate, laryngeal, and pancreatic cancers (9, 11, 13). However, the mechanism by which a higher expression of MOR in tumor specimens is associated with increased consumption of intraoperative opioids is still unclear. PNI is associated with pain and predicts worse outcomes in ovarian cancers (30–32). We can speculate that high levels of tumoral MOR can promote neuronal sensitization in response to an inflammatory tumor microenvironment (33). This is supported by the fact that patients with a higher expression of MOR also had higher pain levels on POD1. Alternatively, elevated concentrations of locally released endorphins in patients with pain could be responsible for a high rate of perineural invasion (34).

In this study, we evaluated the association between MOR expression and survival outcomes in ovarian cancers. Our study has limitations as follows. Firstly, the retrospective design of the study may introduce bias and the negative result that MOR is not associated with OS or DFS could be due to being underpowered. Secondly, while our study shows no association between MOR expression level and outcomes, this does not enable any conclusions regarding the effect of opioids (intraop etc.) on these outcomes. Thirdly, we did not perform a subgroup survival analysis of opioid consumption and MOR expression [high opioid consumption and high MOR expression (HOHM), high opioid consumption and low MOR expression (HOLM), low opioid consumption and high MOR expression (LOHM), low opioid consumption and low MOR expression (LOLM)] since not only MOR expression but further opioid exposure could have impact on the survival outcomes. Last, we did not investigate the mechanism implicated in tumoral MOR expression and perineural invasion.

In conclusion, MOR expression was not associated with OS or DFS in ovarian cancer patients. Our results indicated a high level of MOR expression associated with perineural invasion in ovarian cancers.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Fudan University (No. 20200206). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

HZ, and CM conceived and designed the study. HZ, MQ, ZS, and YW, WX, MS, and TL collected the data. HZ, XZ, MS, and WC interpreted and analyzed the data. HZ were the major contributors in writing the manuscript. HZ, and CM reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.927262/full#supplementary-material

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# Increased MPO in Colorectal Cancer Is Associated With High Peripheral Neutrophil Counts and a Poor Prognosis: A TCGA With Propensity Score-Matched Analysis

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**Background:** Myeloperoxidase (MPO) has been demonstrated to be a local mediator of inflammation in tissue damage in various inflammatory diseases. Given its controversial effect on colorectal cancer (CRC), there has been growing interest in investigating the role of this enzyme in CRC. The mechanism underlying MPO activity and CRC progression requires further clarification.

**Methods:** The expression and function of MPO in CRC were evaluated using TCGA analysis. TCGA, TIMER, and Human Cell Landscape analyses were used to analyze the correlation between MPO expression and neutrophil infiltration in CRC. Spearman's bivariate correlation analysis was used to verify the correlation between MPO levels in CRC and the peripheral neutrophil count. In the clinical analysis, 8,121 patients who underwent elective surgery for CRC were enrolled in this retrospective cohort study from January 2008 to December 2014. Propensity score matching was used to address the differences in baseline characteristics. The Kaplan–Meier method and Cox regression analysis were used to identify independent prognostic factors in patients with CRC.

**Results:** MPO was upregulated in CRC tissues, which is related to malignant progression and worse survival in CRC patients from TCGA analysis. MPO was significantly correlated with the infiltration level of neutrophils in CRC in TCGA, TIMER, and Human Cell Landscape analyses. MPO was positively correlated with the peripheral neutrophil count. Data of the 8,121 patients who underwent CRC surgery were available for analysis. After propensity score matching, 3,358 patients were included in each group. Kaplan–Meier survival curves showed that high preoperative neutrophil levels were associated with decreased overall survival (OS; $P < 0.001$) and disease-free survival (DFS; $P = 0.015$). The preoperative neutrophil count was an independent risk factor for OS

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(hazard ratio [HR], 1.157; 95% confidence interval [CI], 1.055–1.268; P = 0.002) and DFS (HR, 1.118; 95% CI, 1.009–1.238; P = 0.033).

**Conclusions:** Our research indicates that increased MPO levels in CRC are significantly correlated with high preoperative neutrophil counts, and both serve as prognostic indicators for worse survival in CRC patients. Our study suggests that neutrophils may be key players in the mechanism linking MPO levels with poor CRC outcomes.

**Keywords: myeloperoxidase, colorectal cancer, preoperative neutrophil counts, prognosis, TCGA analysis, propensity score-matched analysis**

## INTRODUCTION

Colorectal cancer (CRC) ranks third and second in terms of morbidity and mortality, respectively, among the various cancer types worldwide (1). In China, although CRC ranks fifth as the main cause of cancer-associated death among cancer patients, the mortality accompanying this malignancy has been on the rise in the past few decades (2, 3). Currently, the most common treatment for CRC patients is surgical resection; however, approximately half of the patients relapse within three years after surgery (4). Thus, a prognostic indicator or potential therapeutic target is urgently needed for predicting survival outcomes in CRC patients.

Myeloperoxidase (MPO), a member of the heme peroxidase superfamily, plays a key role in regulating the functions of neutrophils and monocytes (5). MPO is mainly involved in the formation of reactive oxygen species or hypochlorous acid, thus resulting in tissue damage (6–8). An association between MPO and disease has been reported in ovarian and cervical cancers, as well as in CRCs (9, 10). Some studies showed that high preoperative MPO levels improved prognosis in CRC (11, 12), while others reported that MPO promoted malignant phenotypes in CRC patients (13, 14). Given the controversial effect of MPO on CRC, there has been growing interest in investigating the role of this enzyme in CRC. MPO is the most abundant protein expressed by neutrophils and it may also have the greatest potential to damage living cells (15). In autoimmune diseases, MPO exists only in the cytoplasm of the neutrophils (16). Therefore, we were curious about the relationship between MPO levels in CRC and peripheral neutrophil counts.

CRC is a highly heterogeneous tumor, which is closely associated with inflammation and characterized by the infiltration of various immune cells (17). Various peripheral inflammatory markers such as neutrophil and lymphocyte counts are easy to obtain from conventional preoperative laboratory examinations (18, 19). The correlation between a high white blood cell (WBC) count and poor prognosis has been identified in various cancer types, such as oropharyngeal cancer, cervical cancer, and esophageal cancer (20–22). Neutrophils, which are crucial regulators of both inflammation and immune responses, account for 50–70% of leukocytes in circulation and are the major elements of WBCs (23). However, studies on the correlation between preoperative neutrophil count and the prognosis of CRC are controversial. Most evidence shows that neutrophils can promote tumors, and the degree of neutrophil infiltration is related to poor prognosis; however, a few studies have collected evidence that neutrophils can either improve or exert no effect on prognosis (24, 25). We aimed to verify the function of preoperative neutrophils in a large sample cohort. The underlying mechanism behind MPO and the prognosis of patients with CRC have not yet been clarified.

This study aimed to assess the expression and function of MPO in CRC using TCGA analysis and to analyze the potential correlations between MPO in CRC and peripheral neutrophil counts. We further verified the prognostic value of preoperative neutrophil counts for OS and DFS after CRC surgery in our large sample cohort. We speculated that increased MPO levels in CRC were positively correlated with high preoperative peripheral neutrophil counts, both of which predicted worse survival outcomes in CRC patients undergoing elective surgery.

## MATERIALS AND METHODS

### RNA-Sequencing Data and Bioinformatics Analysis

Gene expression data with clinical information from Colon adenocarcinoma (COAD) patients (521 cases, workflow type: HTSeq-TPM) were collected from TCGA using the R package "TCGAbiolinks". The exclusion criteria were normal colorectal samples and an OS of < 30 days. The TPM data from 521 cases were used for further analyses. Of the 521 samples, 480 were tumor tissues and 41 were normal tissues. Normal tissue is the tissue adjacent to a tumor, specifically at a distance of 2 cm from the tumor. Patient characteristics including sex, age, BMI, TNM stage, pathological stage, primary therapy outcome, residual tumor, CEA level, perineural invasion, lymphatic invasion, history of colon polyps, presence of colon polyps, OS event, disease-specific survival (DSS) event, and progression-free interval (PFI) event were recorded. Unavailable or unknown clinical features were considered missing values. This study met the TCGA publication guidelines. All data used in the study were obtained from TCGA.

### Immune Infiltration Analysis Using ssGSEA

Immune infiltration analysis of CRC was performed using the single-sample Gene Set Enrichment Analysis (ssGSEA) method using the GSVA package in R (version 3.6.3) for 24 types of

immune cells in tumor samples, including neutrophils, mast cells, eosinophils, macrophages, natural killer (NK) cells, CD56dim NK cells, CD56bright NK cells, dendritic cells (DCs), immature DCs (iDCs), activated DCs (aDCs), plasmacytoid DCs (pDCs), T cells, CD8+ T cells, T helper cells, Th1 cells, Th2 cells, Th17 cells, T follicular helper cells (Tfhs), Tregs, effector memory T cells (Tems), central memory CD4+ T cells (Tcms), γδT cells (Tgd), cytotoxic cells, and B cells. The correlation between MPO and these immune cells was analyzed using the Spearman correlation test, and the infiltration of immune cells between the high and low MPO-expression groups was analyzed using the Wilcoxon rank-sum test.

### GEPIA2

GEPIA2 is a website developed by Zhang Zemin's laboratory at Peking University. It can analyze the RNA-seq expression data of 9736 tumor samples and 8587 normal samples from TCGA and GTEx projects (26).

### OncoLnc

Using OncoLnc (OncoLnc), we collected the survival data of 8647 patients with 21 kinds of tumors from TCGA and the corresponding mRNA and miRNA expression profile data. Simultaneously, the lncRNA expression data from the MiTranscriptome project were collected to perform survival analysis which can be easily used to explore survival-related genes in various tumors.

## TIMER

TIMER (http://cistrome.org/TIMER/) was used to systematically analyze the infiltration of immune cells in different types of cancer. The abundance of six types of immunoreactive substances (B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, macrophages, and DCs) was estimated using the TIMER algorithm. The partial correlation coefficient indicates the relationship between variables. A partial correlation coefficient greater than 0.7 implies a very close relationship; that in the range 0.4–0.7 indicates a close relationship; that in the range 0.2–0.4 indicates a moderate relationship; and that lower than 0.2 indicates a distant relationship.

## Human Cell Landscape

The Human Cell Landscape database (http://bis.zju.edu.cn/HCL/) is a public single-cell RNA sequencing database that contains the cell type composition of major human organs and a basic scheme for the Human Cell Landscape. The evaluation of the relationship between MPO and neutrophils was performed using the data analyzed in this database.

## Immunohistochemical Staining

Paraffin-embedded tissues were stained with an MPO antibody (ab208670, Abcam, Cambridge, UK). The staining score was determined by two experienced pathologists at the Zhongshan Hospital (China). Six high-power fields (HPFs; ×200 magnification) were randomly counted by the two independent pathologists (each with three fields). The IHC score ranged from 0 to 300, according to the sum of the percentage of stained cells.

## RNA Separation and Real-Time Quantitative PCR

Total RNA was extracted using the TRIzol reagent (Invitrogen, Waltham, MA, USA). cDNA was obtained by reverse transcription using the PrimeScript RT kit (Takara, Shiga, Japan). The expression of candidate genes and the housekeeping gene GAPDH was evaluated *via* quantitative real-time PCR using the ABI 7900HT real-time PCR system (Applied Biosystems, Carlsbad, CA, USA). The relative transcription levels were calculated using the $2-\Delta\Delta CT$ method. GAPDH (human) primer sequences: 5'-GGAGCGAGATCC CTCCAAAAT-3'; 5'-GGCTGTTGTCATACTTCTCATGG-3'. MPO (human) primer sequences: 5'-TGCTGCCCTTTG ACAACCTG-3'; 5'-TGCTCCCGAAGTAAGAGGGT-3'.

## Clinical Study Design

This clinical retrospective study was performed at the Shanghai Cancer Center, Fudan University, Shanghai, China. The study was approved by the center's Ethics Committee (IRB2105235-6) and informed consent was obtained from all subjects involved in the study.

## Clinical Study Population and Data Sources

Of the 13,721 patients who underwent elective surgery for CRC between January 2008 and December 2014, a total of 8,121 with clinical features and survival data were included in this study. The inclusion criteria were as follows: CRC diagnosed by histological evidence, patients undergoing elective radical surgery for CRC, and patients older than 20 years of age. We excluded patients with incomplete medical records, benign tumors or carcinomas in situ, emergency operations, an ASA physical status score > 3, metastasis at initial visit and a history of malignant tumors. Sixty-nine pairs of CRC tissues and their matched adjacent non-cancerous tissues from 8,121 patients were used for IHC and qPCR analyses. These 69 patients underwent elective surgery for CRC in December 2014.

Patients were divided into two groups according to their preoperative neutrophil counts. Those with preoperative neutrophils > $3.5\times10^9$/L were defined as the high preoperative neutrophil group. The cut-off value for neutrophils was calculated using the receiver operating characteristic (ROC) curve; the threshold was associated with an increased risk of postoperative mortality and was within the normal range of the neutrophil count.

## Variables and Outcomes

We reviewed and recorded the following data from the clinical information system of the Shanghai Cancer Center: sex, age, preoperative adjuvant chemotherapy, surgical approach, tumor histology, tumor differentiation, surgical margin positivity, TNM stage, infiltrating lymph nodes > 12, number of cancer nodules >

1, surgery again within 30 days, death, intraoperative transfusion, and blood loss.

The primary endpoints were OS and DFS; OS was defined as the interval between the date of diagnosis and the date of death for any reason, while DFS was defined as the interval between the date of diagnosis and the date of recurrence, metastasis, secondary primary tumor, or death.

The relationship between the Mismatch Repaire-status and neutrophil counts, and the effect of MMR-status on survival in 668 patients with CRC.

We analyzed 668 patients with MMR-status. The chi-squared test was used to evaluate the differences in MMR-status between high/low preoperative neutrophil counts. Kaplan-Meier method was used to evaluate the overall survival differences between MMR-proficient and MMR-deficient patients.

## Statistical Analyses

In TCGA analysis, all statistical analyses were conducted and plots were generated using the R software version 3.4.4 (R Foundation for Statistical Computing, Austria). The Wilcoxon rank-sum and Wilcoxon signed-rank tests were used to analyze the expression of MPO in non-paired and paired samples, respectively. The Kruskal–Wallis test, Wilcoxon signed-rank test, and logistic regression were used to evaluate the relationships between clinicopathological features and MPO expression. The median MPO expression level was regarded as the cut-off value. Cox regression analyses and the Kaplan–Meier method were used to evaluate prognostic factors. Accordingly, a univariate Cox analysis was used to compare the effect of MPO expression on survival and other clinical features. An ROC curve was used to further evaluate the value of the biomarker, and nomograms were constructed to predict the 1-, 3-, and 5-year survival probabilities. Spearman's correlation and Wilcoxon signed-rank tests were used to analyze the correlation between MPO expression and neutrophil counts. In correlation analyses, the correlation coefficient indicates the relationship between variables. A correlation coefficient above 0.7 shows that the relationship is very close; that in the range 0.4–0.7 shows a moderate relationship; and that in the range 0.2–0.4 shows a low correlation.

In the clinical study, analyses were performed using IBM SPSS Statistics 25.0 (SPSS Corp., Armonk, NY, USA). The chi-squared test was used to evaluate the differences in baseline patient characteristics between the two groups. To reduce possible confounding factors, propensity score matching was performed. The key confounders including sex, preoperative adjuvant chemotherapy, tumor differentiation, tumor histology, surgical margin positive, lymph node invasion > 12, and number of cancer nodule ≥ 1 were matched. We used the R package "MatchIt" for propensity score matching.

In the propensity-matched cohort, the Kaplan–Meier method was used to compare OS and DFS using the log-rank test. Cox proportional hazards models were used to confirm the independent prognostic factors for CRC patients. All variables were adjusted using a univariate Cox proportional hazards model. Variables with $P < 0.05$ were included in the multivariate analysis. A multivariate Cox proportional hazards model was used in a stepwise manner to select the prognostic factors. The hazard ratio (HR) and corresponding 95% confidence interval (CI) were calculated. In all tests, P-values < 0.05 were considered statistically significant. Differences were considered significant at * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

# RESULTS

## TCGA Analysis: MPO Is Upregulated in CRC Tissues

To analyze the relationship between MPO expression and CRC, the MPO expression data and detailed clinical characteristics of 478 CRC patients were downloaded from TCGA, including TNM stage, pathological stage, primary therapy outcome, sex, age, BMI, residual tumor, CEA level, perineural invasion, lymphatic invasion, history of colon polyps, presence of colon polyps, and mortality (**Table 1**).

We used the Wilcoxon signed-rank test to compare the expression of MPO in CRC tissues and normal tissues in TCGA. MPO expression levels in 480 tumor tissues were markedly higher than those in 41 normal tissues ($P = 0.001$; **Figure 1A**). Correspondingly, analysis of MPO expression in 41 paired CRC tissues and their matched non-cancerous tissues also showed a significant upregulation of MPO in patients with CRC ($P = 0.002$; **Figure 1B**). MPO is also highly expressed in certain cancers, such as colon adenocarcinoma, pancreatic adenocarcinoma, and acute myeloid leukemia, as inferred from the TIMER2 database (**Figure 1C**).

## TCGA Analysis: The Upregulation of MPO Is Related to the Malignant Progression of CRC Patients

Under the Kruskal–Wallis test and Wilcoxon signed-rank test in our current study, a higher level of MPO expression was significantly correlated with a higher M stage ($P = 0.001$), a higher pathological stage (stage I vs. IV, $P = 0.001$; stage III vs. IV, $P = 0.002$), and a higher CEA level ($P = 0.005$; **Figures 1D–F**). Furthermore, higher MPO expression was associated with higher mortality in terms of OS ($P = 0.041$), DSS ($P = 0.005$), and PFI ($P = 0.004$) (**Figures 1G–I**). However, the comparison of MPO expression in the patient characteristics including T stage, N stage, history of colon polyps, presence of colon polyps, lymphatic invasion, perineural invasion, residual tumor, BMI, age, and sex was not statistically significant ($P > 0.05$; **Supplementary Figures 1A–J**).

To further analyze the role of MPO in CRC, we divided the patients into two groups based on MPO expression. The median expression level was used as the cut-off point for grouping. As shown in **Table 1**, high expression of MPO was strongly associated with a more advanced M stage ($P = 0.004$), higher pathological stage ($P = 0.003$), higher CEA level ($P = 0.010$), more OS death events ($P = 0.045$), more DSS death events ($P = 0.011$), and more PFI death events ($P = 0.005$); in contrast, it was not associated with the T stage, N stage, primary therapy

**TABLE 1 |** The relationship between MPO expression and the clinicopathological features of CRC in TCGA.

| Characteristic | Low expression of MPO | High expression of MPO | p |
|---|---|---|---|
| n | 239 | 239 | |
| T stage, n (%) | | | 0.208 |
| T1 | 6 (1.3%) | 5 (1%) | |
| T2 | 48 (10.1%) | 35 (7.3%) | |
| T3 | 160 (33.5%) | 163 (34.2%) | |
| T4 | 24 (5%) | 36 (7.5%) | |
| N stage, n (%) | | | 0.739 |
| N0 | 146 (30.5%) | 138 (28.9%) | |
| N1 | 51 (10.7%) | 57 (11.9%) | |
| N2 | 42 (8.8%) | 44 (9.2%) | |
| M stage, n (%) | | | 0.004 |
| M0 | 182 (43.9%) | 167 (40.2%) | |
| M1 | 21 (5.1%) | 45 (10.8%) | |
| Pathological stage, n (%) | | | 0.003 |
| Stage I | 49 (10.5%) | 32 (6.9%) | |
| Stage II | 92 (19.7%) | 95 (20.3%) | |
| Stage III | 74 (15.8%) | 59 (12.6%) | |
| Stage IV | 21 (4.5%) | 45 (9.6%) | |
| Primary therapy outcome, n (%) | | | 0.402 |
| PD | 10 (4%) | 15 (6%) | |
| SD | 2 (0.8%) | 2 (0.8%) | |
| PR | 5 (2%) | 8 (3.2%) | |
| CR | 113 (45.2%) | 95 (38%) | |
| Sex, n (%) | | | 0.234 |
| Female | 120 (25.1%) | 106 (22.2%) | |
| Male | 119 (24.9%) | 133 (27.8%) | |
| Age, n (%) | | | 0.926 |
| <=65 | 96 (20.1%) | 98 (20.5%) | |
| >65 | 143 (29.9%) | 141 (29.5%) | |
| BMI, n (%) | | | 0.724 |
| <25 | 42 (16.4%) | 45 (17.6%) | |
| >=25 | 87 (34%) | 82 (32%) | |
| Residual tumor, n (%) | | | 0.215 |
| R0 | 172 (46%) | 174 (46.5%) | |
| R1 | 3 (0.8%) | 1 (0.3%) | |
| R2 | 8 (2.1%) | 16 (4.3%) | |
| CEA level, n (%) | | | 0.010 |
| <=5 | 107 (35.3%) | 89 (29.4%) | |
| >5 | 41 (13.5%) | 66 (21.8%) | |
| Perineural invasion, n (%) | | | 0.372 |
| NO | 68 (37.6%) | 67 (37%) | |
| YES | 19 (10.5%) | 27 (14.9%) | |
| Lymphatic invasion, n (%) | | | 0.054 |
| NO | 139 (32%) | 127 (29.3%) | |
| YES | 71 (16.4%) | 97 (22.4%) | |
| History of colon polyps, n (%) | | | 0.989 |
| NO | 133 (32.6%) | 129 (31.6%) | |
| YES | 75 (18.4%) | 71 (17.4%) | |
| Colon polyps present, n (%) | | | 0.963 |
| NO | 82 (32.9%) | 80 (32.1%) | |
| YES | 43 (17.3%) | 44 (17.7%) | |
| OS event, n (%) | | | 0.045 |
| Alive | 197 (41.2%) | 178 (37.2%) | |
| Dead | 42 (8.8%) | 61 (12.8%) | |
| DSS event, n (%) | | | 0.011 |
| Alive | 209 (45.2%) | 189 (40.9%) | |
| Dead | 22 (4.8%) | 42 (9.1%) | |
| PFI event, n (%) | | | 0.005 |
| Alive | 189 (39.5%) | 161 (33.7%) | |
| Dead | 50 (10.5%) | 78 (16.3%) | |

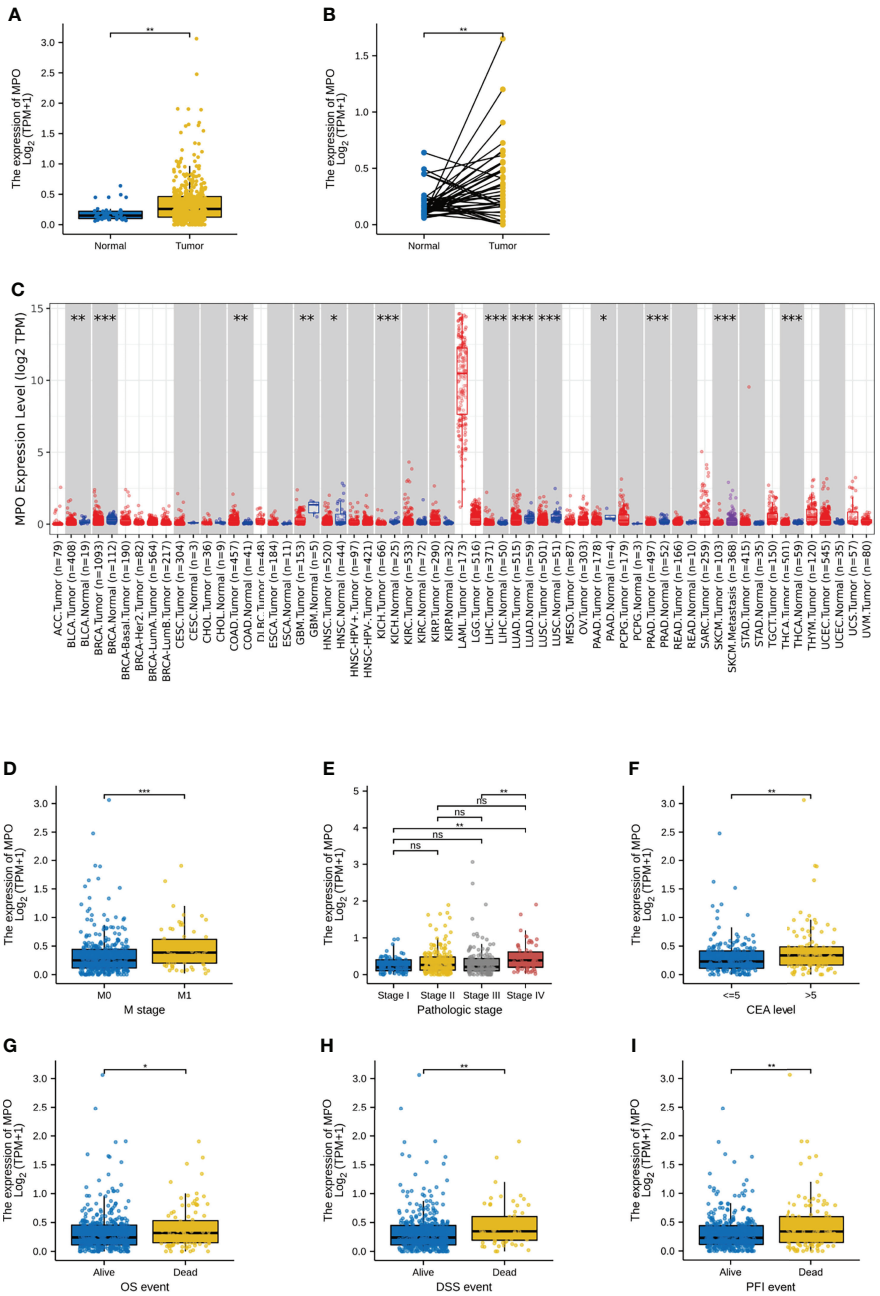



**FIGURE 1** | MPO was upregulated in CRC and correlated with the malignant progression of CRC patients. **(A)** The MPO expression levels in 480 tumor tissues and 41 normal tissues. **(B)** The expression of MPO in 41 normal and matched tumor tissues. **(C)** MPO expression in several cancers in the TIMER2 database. **(D–F)** The association with elevated MPO and clinicopathological characteristics, including M stages, pathological stage, and CEA level. **(G–I)** The association with elevated MPO and death events, including OS events, DSS events, and PFI events. The difference was considered significant at * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$. ns, no significance.

outcome, sex, age, BMI, residual tumor, perineural invasion, lymphatic invasion, history of colon polyps, or colon polyps present (all $P > 0.05$) (**Table 1**).

Importantly, logistic regression analysis of MPO expression and clinicopathological features also confirmed the relationship between highly expressed MPO and the pathological stage (OR: 1.479; 95% CI: 1.024–2.141; $P = 0.037$ for stage III & IV vs. stage I & II, respectively), age (OR: 0.636; 95% CI: 0.439–0.917; $P = 0.016$ for > 65 vs. ≤ 65), and lymphatic invasion (OR: 1.495; 95% CI: 1.015–2.208; $P = 0.042$ for Yes vs. No); see **Table 2**. Together, our evaluation revealed that high MPO expression is related to the malignant progression of CRC.

**TABLE 2** | The relationship between increased MPO expression and clinicopathological features by logistic regression.

| Characteristics | Total (N) | Odds Ratio (OR) | P value |
|---|---|---|---|
| T stage (T3&T4 vs. T1&T2) | 477 | 0.848 (0.539-1.333) | 0.476 |
| N stage (N1&N2 vs. N0) | 478 | 1.321 (0.916-1.907) | 0.136 |
| M stage (M1 vs. M0) | 415 | 1.531 (0.903-2.623) | 0.116 |
| Pathological stage (Stage III&Stage IV vs. Stage I&Stage II) | 467 | 1.479 (1.024-2.141) | 0.037 |
| Sex (Male vs. Female) | 478 | 0.874 (0.610-1.252) | 0.464 |
| Age (>65 vs. <=65) | 478 | 0.636 (0.439-0.917) | 0.016 |
| BMI (>=25 vs. <25) | 256 | 1.115 (0.663-1.874) | 0.680 |
| Residual tumor (R1&R2 vs. R0) | 374 | 1.864 (0.851-4.305) | 0.128 |
| CEA level (>5 vs. <=5) | 303 | 0.929 (0.579-1.492) | 0.760 |
| Perineural invasion (YES vs. NO) | 181 | 1.604 (0.804-3.311) | 0.188 |
| Lymphatic invasion (YES vs. NO) | 434 | 1.495 (1.015-2.208) | 0.042 |
| History of colon polyps (YES vs. NO) | 408 | 0.767 (0.510-1.150) | 0.200 |
| Colon polyps present (YES vs. NO) | 249 | 1.165 (0.690-1.977) | 0.569 |

## TCGA Analysis: The Upregulation of MPO Is Related to Worse Survival in CRC Patients

To gain a deeper insight into the correlation between MPO expression and the prognosis of CRC patients in TCGA, Kaplan–Meier survival analyses were conducted for OS, PFI, and DSS events in CRC patients. We observed that high MPO expression was associated with a shorter OS (HR: 1.62; 95% CI: 1.09–2.41; P = 0.018), worse DSS (HR: 2.06; 95% CI: 1.22–3.47; P = 0.007) and poorer PFI (HR: 1.71; 95% CI: 1.20–2.44; P = 0.003); see **Figures 2A–C**. This suggests that a higher expression of MPO is related to worse survival in CRC patients. We also used the Gene Expression Profiling Interactive Analysis2 (GEPIA2), OncoLnc, and TIMER2 databases to analyze TCGA data sets. The results of the survival analysis performed using these three database showed that a high expression of MPO is associated with worse prognosis (**Figures 2D–F**).

Moreover, we performed a univariate analysis of prognostic factors for OS using the Cox regression model (**Table 3** and **Figure 3A**). High MPO expression was associated with worse OS (HR, 1.618; CI: 1.087–2.407; P = 0.018). In addition, a higher T stage (HR, 3.072; CI: 1.423–6.631, P = 0.004), higher N stage (HR, 2.592; CI: 1.743–3.855; P < 0.001), higher M stage (HR, 4.193; 95% CI: 2.683–6.554; P < 0.001), higher pathological stage (HR, 2.947; 95% CI: 1.942–4.471; P < 0.001), older age (HR, 1.610; 95% CI: 1.052–2.463; P = 0.028), higher BMI (HR, 0.549; 95% CI: 0.311–0.969; P = 0.038), residual tumor (HR, 4.364; 95% CI: 2.401–7.930; P < 0.001), higher CEA level (HR, 3.128; 95% CI: 1.788–5.471; P < 0.001) and lymphatic invasion (HR, 2.450; 95% CI: 1.614–3.720; P < 0.001) were also associated with poor OS (**Table 3**). We also conducted univariate analyses for DSS and PFI using the Cox regression model (**Tables 4**, **5**; **Figures 3B, C**). Similarly, MPO levels were correlated with poorer PFI (HR, 1.711; 95% CI: 1.198–2.443; P = 0.003) and DSS (HR, 2.060; 95% CI: 1.223–3.467; P = 0.007). In summary, MPO is an independent risk factor for OS, PFI, and DSS in patients with CRC.

In addition, an ROC curve was generated to further evaluate the value of MPO as a biomarker for CRC (**Figure 2G**). MPO exhibited a good predictive ability in patients with CRC (AUC: 0.650; CI: 0.584–0.717). By combining the expression levels of MPO and clinical variables, nomograms were constructed to predict the 1-, 3-, and 5-year survival (OS, PFI, and DSS) probability of patients (**Figures 3D–F**). Overall, MPO has a good predictive ability in patients with CRC.

## TCGA, TIMER, and Human Cell Landscape Analyses: The Relationship Between MPO and Neutrophils in CRC

We investigated the relationship between MPO and the infiltration of different immune cells. Under the assessment, the MPO expression was demonstrated to positively correlate with the dominant immune cell type in tumors, containing macrophages, neutrophils, mast cells, eosinophils, DC, pDC, Tems, iDCs, NK cells, and other cells (**Figure 4A**). Next, we analyzed the correlation between MPO expression and T cells, B cells, CD8+ T cells, cytotoxic cells, DC, macrophages, mast cells, neutrophils, NK cells, Th1 cells, Th17 cells, Th2 cells, and Tregs using ssGSEA, which confirmed that a higher MPO expression is significantly linked with higher infiltration levels of immune cells (such as cytotoxic cells (P = 0.024), DC (P < 0.001), macrophages (P < 0.001), mast cells (P < 0.001) neutrophils (P < 0.001), NK cells (P < 0.001), Th1 cells (P < 0.001), and Tregs (P=0.008)) (**Figure 4D**). Considering that neutrophils are a vital part of nonspecific immunity and play a significant role during the process of pro- and antitumor immunity, we next performed Spearman correlation and Wilcoxon signed-rank tests to investigate the correlation between MPO expression and neutrophils, which proved that a higher MPO expression was linked with higher infiltration levels of neutrophils (**Figures 4B, C**). The TIMER database analysis among six types of immune cells showed that the expression of MPO was significantly correlated with the infiltration level of neutrophils (r = 0.173, P = 4.98e-4), macrophages (r = 0.247, P = 5.00e-7), DCs (r = 0.185, P = 1.91e-4), and CD4+ T cells (r = 0.146, P = 3.44e-3), but not with B cells and CD8+ T cells (**Figure 4E**). Further evaluation of the relationship between MPO and neutrophils was conducted using the data analyzed in a single-cell RNA sequencing database, Human Cell Landscape (http://bis.zju.edu.cn/HCL/). The results revealed that MPO was highly expressed at the single neutrophil level in the fetal

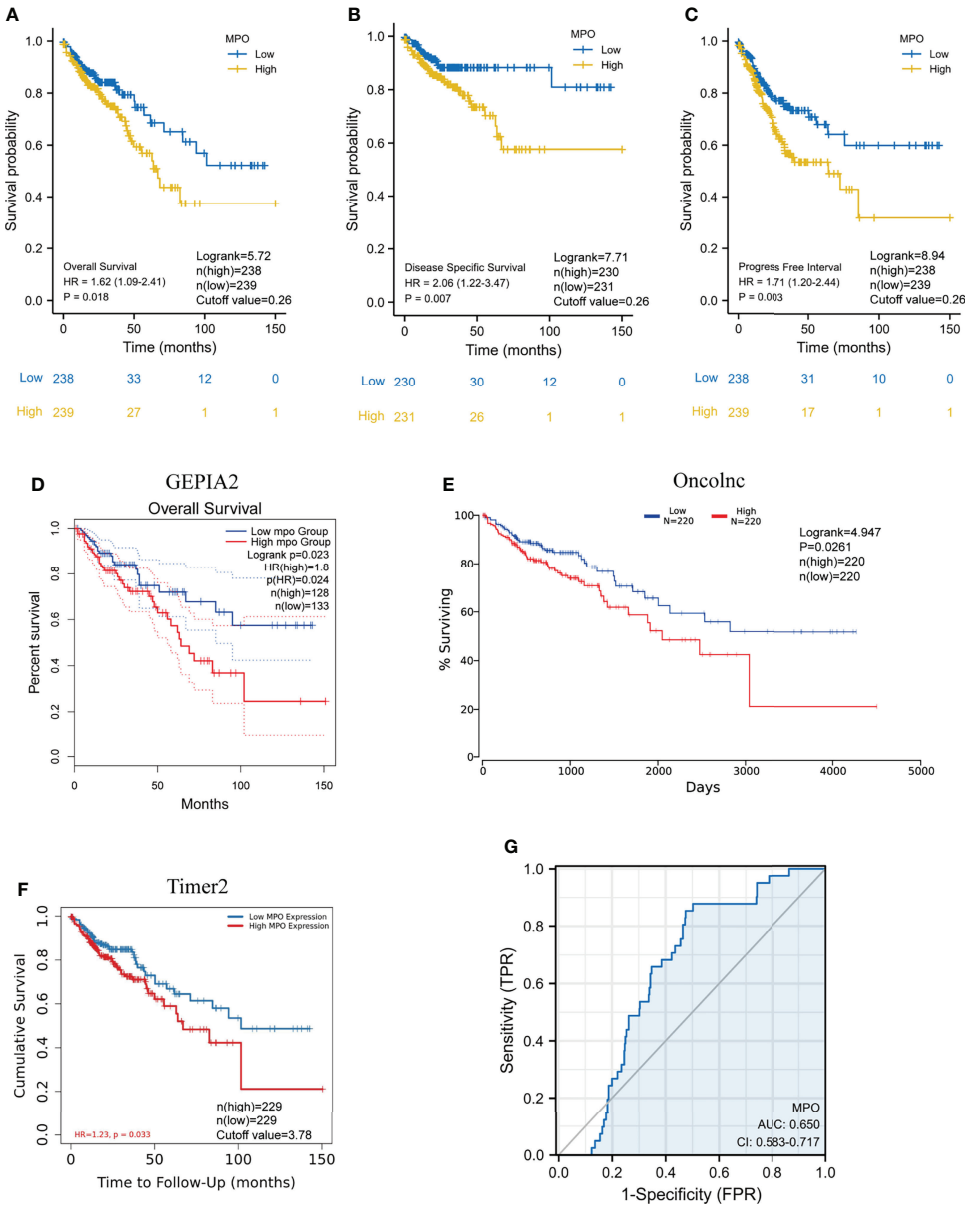



**FIGURE 2** | High expression of MPO is related to poor prognosis in CRC patients. **(A–C)** Kaplan–Meier analyses of OS, PFI, and DSS between the low- and high-MPO groups in TCGA. OS: Logrank=5.72, P=0.018, n(high)=238, n(low)=239, cutoff value=0.26; DSS: Logrank=7.71, P=0.007, n(high)=230, n(low)=231, cutoff value=0.26; PFI: Logrank=8.94, P=0.003, n(high)=238, n(low)=239, cutoff value=0.26. **(D–F)** Gene Expression Profiling Interactive Analysis2 (GEPIA2), OncoLnc, and TIMER2 databases were used to analyze overall survival between high and low MPO groups, respectively. GEPIA2 OS: P=0.023, n(high)=128, n(low)=133; OncoLnc OS: Logrank=4.947, P=0.0261, n(high)=220, n(low)=220, cutoff value=3.78; TIMER2 OS: P=0.033, n(high)=229, n(low)=229. **(G)** ROC analysis with respect to the MPO expression.

intestine (**Figures 4F–H**). Taken together, MPO levels were significantly correlated with neutrophil infiltration in CRC.

## Correlation Between MPO in CRC and Peripheral Neutrophil Counts

To investigate the expression of MPO in CRC, we performed immunohistochemical staining in 69 pairs of CRC tissues and their matched adjacent non-cancerous tissues. MPO is mainly expressed in the lysosome, vesicles, and; therefore nucleoplasm. Tumor tissue is inflamed and granulocyte-rich as expected, the immunohistochemical positivity of MPO is much higher in tumor tissues than that in their matched adjacent non-cancerous tissues (**Figures 5A, B**). The mRNA expression data of MPO in 69 pairs of CRC tissues and their matched adjacent non-cancerous tissues followed the same pattern (**Figure 5C**).

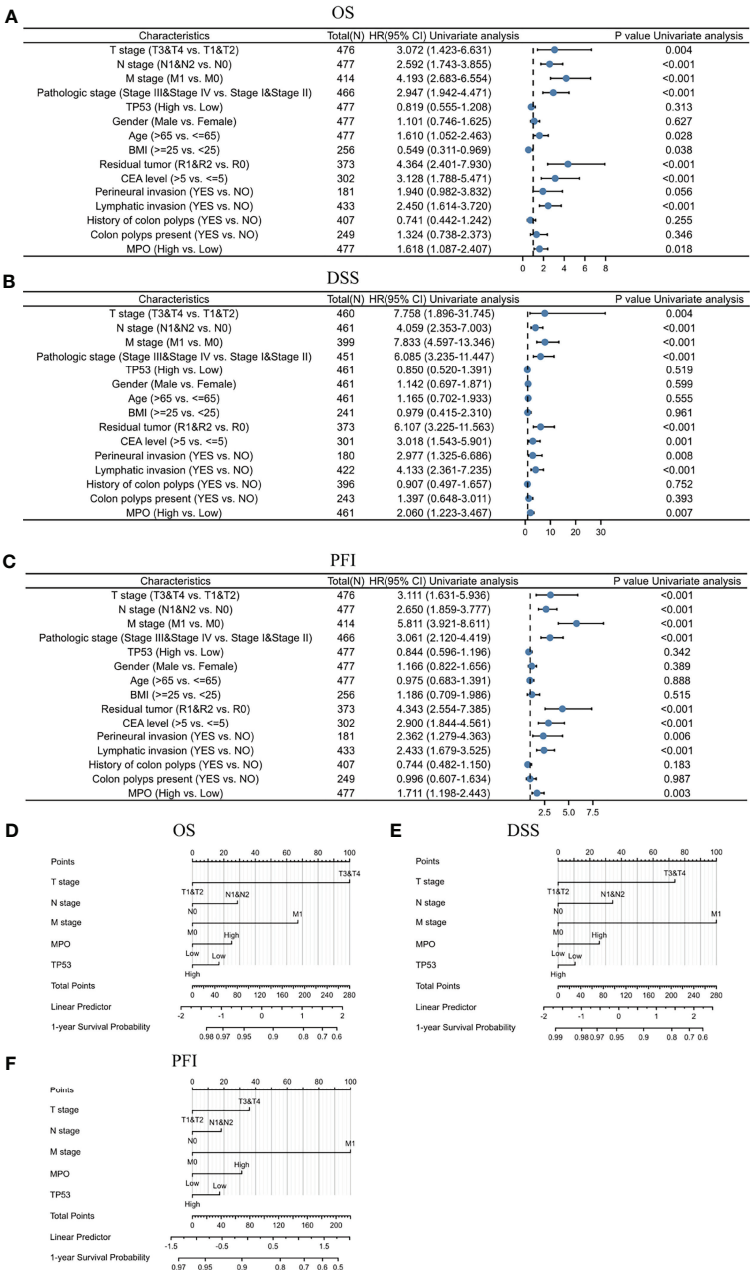



**FIGURE 3** | Univariate analysis of MPO for survival using the Cox regression model and nomogram based on MPO and clinical variables for survival in CRC. **(A)** Univariate analysis of prognostic factors for OS using the Cox regression model. **(B)** Univariate analysis of prognostic factors for DSS using the Cox regression model. **(C)** Univariate analysis of prognostic factors for PFI using the Cox regression model. **(D)** Nomogram for OS prognosis in CRC. **(E)** Nomogram for DSS prognosis in CRC. **(F)** Nomogram for PFI prognosis in CRC.

We further examined whether there was a relationship between MPO expression in CRC and the peripheral neutrophil counts of CRC patients. Consistent with the TCGA analysis, Spearman's bivariate correlation analysis showed positive correlations between MPO IHC staining scores and peripheral neutrophil counts ($r = 0.406$, $P < 0.001$; **Figure 5D**). Overall, the results revealed that MPO was upregulated in CRC and positively correlated with peripheral neutrophil counts, but the correlation was moderate.

## Clinical Data Validation: Kaplan–Meier Survival and Cox regression Proportional Hazard Survival for OS and DFS Between Patients With High and Low Preoperative Neutrophil Counts

A total of 8,121 patients were included in our data analysis (**Figure 6**), where the median postoperative follow-up period was

**TABLE 3 |** Univariate analysis of prognostic factors for OS with the Cox regression model.

| Characteristics | Total (N) | Univariate analysis HR (95% CI) | Univariate analysis P value |
|---|---|---|---|
| T stage (T3&T4 vs. T1&T2) | 476 | 3.072 (1.423-6.631) | 0.004 |
| N stage (N1&N2 vs. N0) | 477 | 2.592 (1.743-3.855) | <0.001 |
| M stage (M1 vs. M0) | 414 | 4.193 (2.683-6.554) | <0.001 |
| Pathological stage (Stage III&Stage IV vs. Stage I&Stage II) | 466 | 2.947 (1.942-4.471) | <0.001 |
| TP53 (High vs. Low) | 477 | 0.819 (0.555-1.208) | 0.313 |
| Sex (Male vs. Female) | 477 | 1.101 (0.746-1.625) | 0.627 |
| Age (>65 vs. <=65) | 477 | 1.610 (1.052-2.463) | 0.028 |
| BMI (>=25 vs. <25) | 256 | 0.549 (0.311-0.969) | 0.038 |
| Residual tumor (R1&R2 vs. R0) | 373 | 4.364 (2.401-7.930) | <0.001 |
| CEA level (>5 vs. <=5) | 302 | 3.128 (1.788-5.471) | <0.001 |
| Perineural invasion (YES vs. NO) | 181 | 1.940 (0.982-3.832) | 0.056 |
| Lymphatic invasion (YES vs. NO) | 433 | 2.450 (1.614-3.720) | <0.001 |
| History of colon polyps (YES vs. NO) | 407 | 0.741 (0.442-1.242) | 0.255 |
| Colon polyps present (YES vs. NO) | 249 | 1.324 (0.738-2.373) | 0.346 |
| MPO (High vs. Low) | 477 | 1.618 (1.087-2.407) | 0.018 |

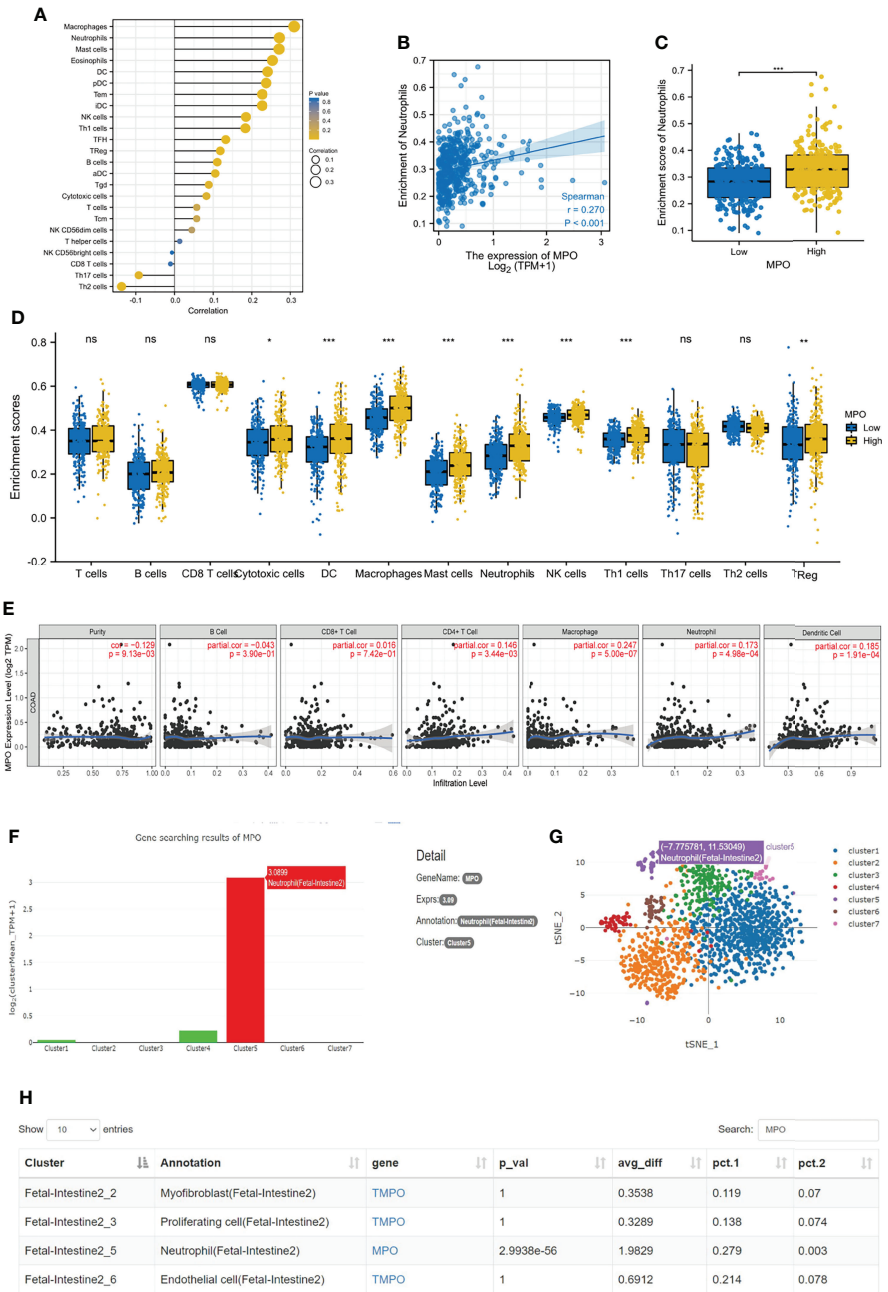
**TABLE 4 |** Univariate analysis of prognostic factors for DSS with the Cox regression model.

| Characteristics | Total (N) | Univariate analysis HR (95% CI) | Univariate analysis P value |
|---|---|---|---|
| T stage (T3&T4 vs. T1&T2) | 460 | 7.758 (1.896-31.745) | 0.004 |
| N stage (N1&N2 vs. N0) | 461 | 4.059 (2.353-7.003) | <0.001 |
| M stage (M1 vs. M0) | 399 | 7.833 (4.597-13.346) | <0.001 |
| Pathological stage (Stage III&Stage IV vs. Stage I&Stage II) | 451 | 6.085 (3.235-11.447) | <0.001 |
| TP53 (High vs. Low) | 461 | 0.850 (0.520-1.391) | 0.519 |
| Sex (Male vs. Female) | 461 | 1.142 (0.697-1.871) | 0.599 |
| Age (>65 vs. <=65) | 461 | 1.165 (0.702-1.933) | 0.555 |
| BMI (>=25 vs. <25) | 241 | 0.979 (0.415-2.310) | 0.961 |
| Residual tumor (R1&R2 vs. R0) | 373 | 6.107 (3.225-11.563) | <0.001 |
| CEA level (>5 vs. <=5) | 301 | 3.018 (1.543-5.901) | 0.001 |
| Perineural invasion (YES vs. NO) | 180 | 2.977 (1.325-6.686) | 0.008 |
| Lymphatic invasion (YES vs. NO) | 422 | 4.133 (2.361-7.235) | <0.001 |
| History of colon polyps (YES vs. NO) | 396 | 0.907 (0.497-1.657) | 0.752 |
| Colon polyps present (YES vs. NO) | 243 | 1.397 (0.648-3.011) | 0.393 |
| MPO (High vs. Low) | 461 | 2.060 (1.223-3.467) | 0.007 |

**TABLE 5 |** Univariate analysis of prognostic factors for PFI with the Cox regression model.

| Characteristics | Total (N) | Univariate analysis HR (95% CI) | Univariate analysis P value |
|---|---|---|---|
| T stage (T3&T4 vs. T1&T2) | 476 | 3.111 (1.631-5.936) | <0.001 |
| N stage (N1&N2 vs. N0) | 477 | 2.650 (1.859-3.777) | <0.001 |
| M stage (M1 vs. M0) | 414 | 5.811 (3.921-8.611) | <0.001 |
| Pathological stage (Stage III&Stage IV vs. Stage I&Stage II) | 466 | 3.061 (2.120-4.419) | <0.001 |
| TP53 (High vs. Low) | 477 | 0.844 (0.596-1.196) | 0.342 |
| Sex (Male vs. Female) | 477 | 1.166 (0.822-1.656) | 0.389 |
| Age (>65 vs. <=65) | 477 | 0.975 (0.683-1.391) | 0.888 |
| BMI (>=25 vs. <25) | 256 | 1.186 (0.709-1.986) | 0.515 |
| Residual tumor (R1&R2 vs. R0) | 373 | 4.343 (2.554-7.385) | <0.001 |
| CEA level (>5 vs. <=5) | 302 | 2.900 (1.844-4.561) | <0.001 |
| Perineural invasion (YES vs. NO) | 181 | 2.362 (1.279-4.363) | 0.006 |
| Lymphatic invasion (YES vs. NO) | 433 | 2.433 (1.679-3.525) | <0.001 |
| History of colon polyps (YES vs. NO) | 407 | 0.744 (0.482-1.150) | 0.183 |
| Colon polyps present (YES vs. NO) | 249 | 0.996 (0.607-1.634) | 0.987 |
| MPO (High vs. Low) | 477 | 1.711 (1.198-2.443) | 0.003 |

69.4 months (95% CI: 68.7–70.0). The enrolled patients were divided into two groups according to their preoperative neutrophil counts, and the cut-off value of neutrophils ($3.5\times10^9$/L) was calculated using an ROC curve. We observed that 51.04% (4,145 out of 8,121) of patients had high preoperative neutrophil counts. As shown in **Table 6**, higher preoperative neutrophil counts were correlated with clinicopathological characteristics, including male sex (P <

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| Cluster | Annotation | gene | p_val | avg_diff | pct.1 | pct.2 |
|---|---|---|---|---|---|---|
| Fetal-Intestine2_2 | Myofibroblast(Fetal-Intestine2) | TMPO | 1 | 0.3538 | 0.119 | 0.07 |
| Fetal-Intestine2_3 | Proliferating cell(Fetal-Intestine2) | TMPO | 1 | 0.3289 | 0.138 | 0.074 |
| Fetal-Intestine2_5 | Neutrophil(Fetal-Intestine2) | MPO | 2.9938e-56 | 1.9829 | 0.279 | 0.003 |
| Fetal-Intestine2_6 | Endothelial cell(Fetal-Intestine2) | TMPO | 1 | 0.6912 | 0.214 | 0.078 |

**FIGURE 4** | MPO was significantly correlated with the infiltration level of neutrophils in CRC. **(A)** MPO expression has a significant correlation with many immune cells infiltration. **(B, C)** The correlation between MPO and neutrophils by the Wilcoxon signed-rank and Spearman correlation tests. **(D)** We analyzed the correlation between MPO expression and T cells, B cells, CD8+ T cells, cytotoxic cells, DC, macrophages, mast cells, neutrophils, NK cells, Th1 cells, Th17 cells, Th2 cells, and Tregs using ssGSEA. **(E)** The relationship between different immune cells and MPO was analyzed in TIMER 2.0, including B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and DCs. **(F–H)** The relationship between MPO and neutrophils in a single-cell RNA sequencing database. The difference was considered significant at * P < 0.05, ** P < 0.01, or *** P < 0.001. ns, no significance.

0.001), not prechemotherapy (P < 0.001), mucinous adenocarcinoma and signet-ring cell carcinoma (P < 0.001), poorer tumor differentiation (P < 0.001), more positive surgical margin (P < 0.001), advanced TNM stage (P < 0.001), more infiltrating lymph nodes > 12 (P < 0.001), more number of cancer nodules ≥ 1 (P = 0.029), more death events (P < 0.001), and more blood transfusions (P = 0.011). It showed that a high preoperative neutrophil level was likely to correlate with more malignant clinicopathological features, more blood transfusion, and poor prognosis in CRC patients.

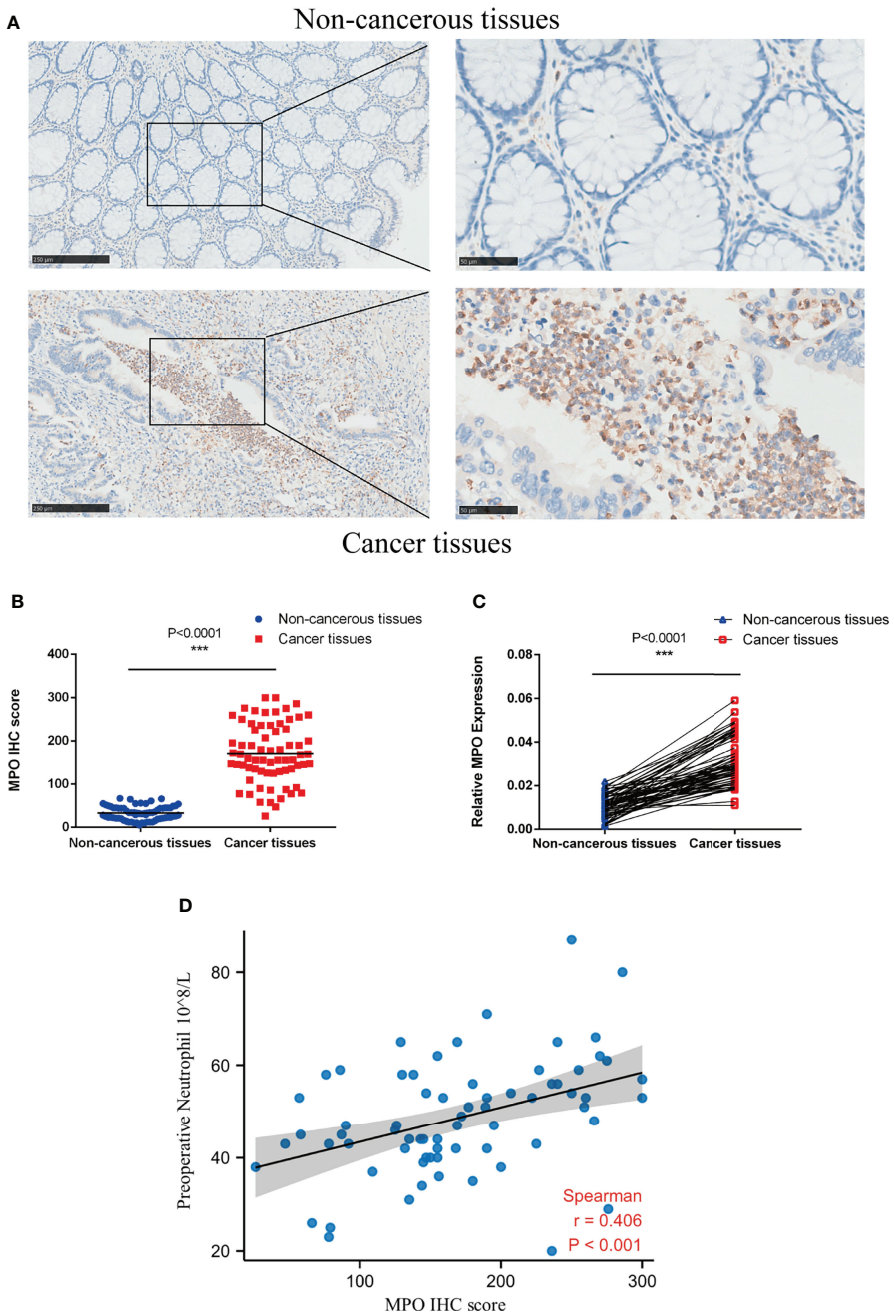


**FIGURE 5** | MPO was upregulated in CRC and positively correlated with peripheral neutrophil counts. **(A)** IHC was used to detect MPO protein expression in CRC and non-cancerous tissues (69 pairs); left, scale bar = 250 μm; right, scale bar = 50 μm. **(B)** Quantitative analysis of MPO IHC scores in CRC and non-cancerous tissues (69 pairs). **(C)** qPCR was used to evaluate the relative expression of MPO mRNA in CRC and adjacent non-cancerous tissues (69 pairs). **(D)** The relationship between MPO levels in CRC and peripheral neutrophil counts was determined using Spearman's correlation test (r = 0.406, P < 0.001, n = 69). Differences were considered significant at * P < 0.05, ** P < 0.01, or *** P < 0.001.

The propensity score matching was performed to reduce the imbalance due to the differences in baseline characteristics between the two groups. After matching, 3,358 pairs remained for each group. There were no significant differences in patient characteristics between the two groups in the matched cohort, except for the TNM stage (**Table 6**).

Kaplan–Meier survival analyses were further conducted to investigate patient prognosis in terms of OS and DFS after propensity score matching. The OS and DFS in the high preoperative neutrophil group were shorter than those in the low preoperative neutrophil group (OS, Logrank=13.743, P < 0.001; DFS, Logrank= 5.910, P = 0.015; **Figure 7**), demonstrating

that high preoperative neutrophil levels elicited poorer prognosis in patients with CRC after matching.

After adjustment, multivariable Cox regression showed that a high preoperative neutrophil count was strongly associated with poorer OS (HR, 1.157; 95% CI, 1.055–1.268; P = 0.002) and worse DFS (HR, 1.118; 95% CI, 1.009–1.238; P = 0.033); see **Tables 7**, **8**. Other variables that significantly influenced the risk of death after multivariate analysis were older age, preoperative neoadjuvant chemotherapy, tumor differentiation, surgical margin positivity, advanced TNM stage, infiltrating lymph node > 12, number of cancer nodules ≥ 1, and blood transfusion; all of which were independent predictors of poorer OS or DFS. Overall, the preoperative neutrophil count was independently associated with increased overall mortality and cancer recurrence after CRC surgery.

## The Relationship Between the MMR-Status and Neutrophil Counts, and the Effect of MMR-Status on Durvival in 668 Patients With CRC

It is well known that the type of inflammatory infiltrate is different between MMR-proficient and MMR-deficient CRCs. It may be very interesting to investigate whether this difference is reflected in peripheral neutrophil counts. Meanwhile, the MMR-status is strongly related to the prognosis in the literature. We analyzed 668 patients with MMR-status. Of these 668 patients, 307 (46.0%) patients presented with low preoperative neutrophils and 361 (54%) patients showed high preoperative neutrophils. In patients with low preoperative neutrophil, there were 16(5.2%) patients with MMR-deficient, and 291(94.8%) patients with MMR-proficient. In patients with high preoperative neutrophil, there were 32(8.9%) patients with MMR-deficient, and 329(91.1%) patients with MMR-proficient. There was no significant difference in MMR-status between two groups (P=0.068) (**Supplementary Table 1**). There was no significant difference in overall survival between MMR-deficient and MMR-proficient groups (Logrank=0.008, P=0.928) (**Supplementary Figure 2**).

# DISCUSSION

In this study, MPO was demonstrated to be upregulated in CRC patients by evaluating the expression profile and function of MPO through TCGA, which is related to malignant progression and survival of patients with CRC. In addition, our study validated that MPO was strongly correlated with the peripheral neutrophil count of CRC patients. Furthermore, this large, retrospective study confirmed that high preoperative

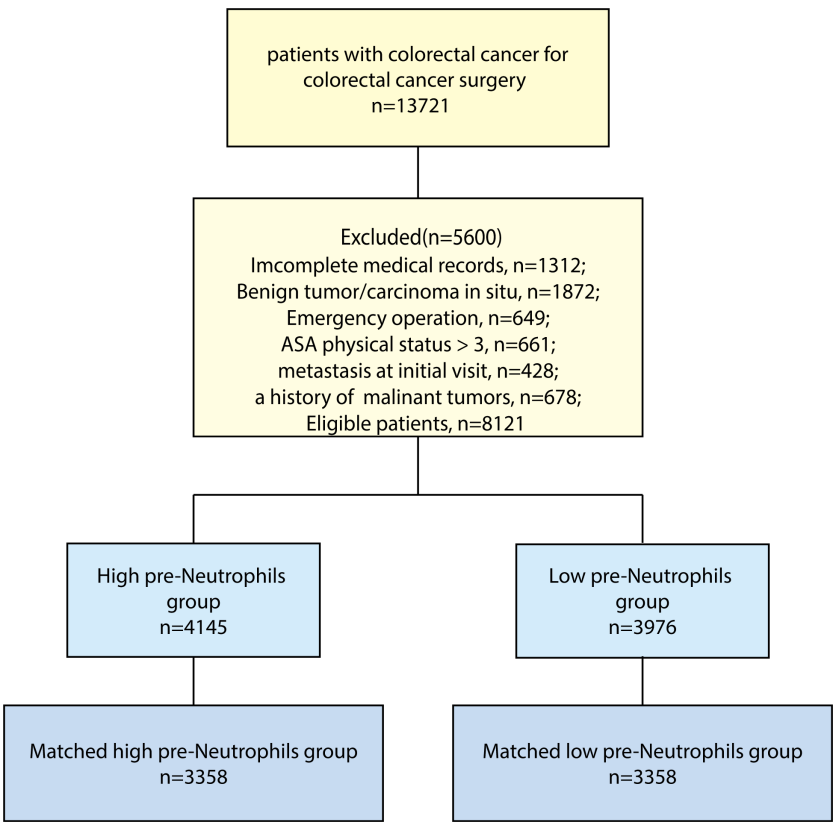


**FIGURE 6** | Flow chart of patient selection. Flow chart of patient selection.

**TABLE 6 |** Patients' baseline characteristics in the total study cohort and the propensity-matched cohort.

| Variables | Overall patients | | P value | Matched patients | | P value |
|---|---|---|---|---|---|---|
| | Low pre-Neutrophils (n=3976) | High pre-Neutrophils (n=4145) | | Low pre-Neutrophils (n=3358) | High pre-Neutrophils (n=3358) | |
| **Sex, n (%)** | | | <0.001 | | | 0.843 |
| Male | 2147 (54.0) | 2665 (64.3) | | 1979 (58.9) | 1987 (59.2) | |
| Female | 1829 (46.0) | 1480 (35.7) | | 1379 (41.1) | 1371 (40.8) | |
| **Age, n (%)** | | | 0.369 | | | 0.721 |
| ≤44 | 531 (13.4) | 537 (13.0) | | 445 (13.3) | 436 (13.0) | |
| 45-54 | 805 (20.2) | 828 (20.0) | | 651 (19.4) | 672 (20.0) | |
| 55-64 | 1453 (36.5) | 1462 (35.3) | | 1223 (36.4) | 1174 (35.0) | |
| 65-74 | 836 (21.0) | 910 (22.0) | | 721 (21.5) | 745 (22.2) | |
| >75 | 351 (8.8) | 480 (9.8) | | 318 (9.5) | 331 (9.9) | |
| **Pre-chemotherapy, n (%)** | | | <0.001 | | | 0.920 |
| No | 3518 (88.5) | 3929 (94.8) | | 3145 (93.7) | 3147 (93.7) | |
| Yes | 458 (11.5) | 216 (5.2) | | 213 (6.3) | 211 (6.3) | |
| **Surgical approach, n (%)** | | | 0.406 | | | 0.366 |
| Laparotomy | 3666 (92.2) | 3801 (91.7) | | 3102 (92.4) | 3082 (91.8) | |
| Laparoscopy | 310 (7.8) | 344 (8.3) | | 256 (7.6) | 276 (8.2) | |
| **Tumor histology, n (%)** | | | <0.001 | | | 0.658 |
| Adenocarcinoma | 3530 (88.8) | 3509 (84.7) | | 2937 (87.5) | 2913 (86.7) | |
| Mucinous adenocarcinoma | 401 (10.1) | 559 (13.5) | | 378 (11.3) | 402 (12.0) | |
| Signet-ring cell carcinoma | 45 (1.1) | 77 (1.9) | | 43 (1.3) | 43 (1.3) | |
| **Tumor differentiation, n (%)** | | | <0.001 | | | 0.447 |
| Poor | 726 (18.3) | 955 (23.0) | | 672 (20.0) | 719 (21.4) | |
| Moderate | 2699 (67.9) | 2788 (67.3) | | 2324 (69.2) | 2264 (67.4) | |
| Well | 84 (2.1) | 88 (2.1) | | 72 (2.1) | 72 (2.1) | |
| Unknown | 467 (11.7) | 314 (7.6) | | 290 (8.6) | 303 (9.0) | |
| **Surgical Margin positive, n (%)** | | | 0.001 | | | 0.759 |
| No | 3929 (98.8) | 4058 (97.9) | | 3311 (98.6) | 3308 (98.5) | |
| Yes | 47 (1.2) | 87 (2.1) | | 47 (1.4) | 50 (1.5) | |
| **TNM stage, n (%)** | | | <0.001 | | | <0.001 |
| 0-1 | 787 (19.8) | 657 (15.9) | | 649 (19.3) | 563 (16.8) | |
| II | 1053 (26.5) | 1190 (28.7) | | 884 (26.3) | 1001 (29.8) | |
| III | 1560 (39.2) | 1592 (38.4) | | 1363 (40.6) | 1232 (36.7) | |
| IV | 435 (10.9) | 634 (15.3) | | 379 (11.3) | 492 (14.7) | |
| Unknown | 141 (3.5) | 72 (1.7) | | 83 (2.5) | 70 (2.1) | |
| **Infiltrating Lymph nodes > 12, n (%)** | | | <0.001 | | | 0.093 |
| No | 970 (24.4) | 826 (19.9) | | 733 (21.8) | 677 (20.2) | |
| Yes | 3006 (75.6) | 3319 (80.1) | | 2625 (78.2) | 2681 (79.8) | |
| **Number of Cancer nodule≥1, n (%)** | | | 0.029 | | | 0.849 |
| No | 3399 (85.5) | 3470 (83.8) | | 2827 (84.2) | 2831 (84.4) | |
| Yes | 576 (14.5) | 673 (16.2) | | 531 (15.8) | 525 (15.6) | |
| **Results** | | | | | | |
| **Surgery again within 30days, n (%)** | | | 0.800 | | | 0.514 |
| No | 3903 (98.2) | 4072 (98.2) | | 3296 (98.2) | 3303 (98.4) | |
| Yes | 73 (1.8) | 73 (1.8) | | 62 (1.8) | 55 (1.6) | |
| **Death, n (%)** | | | <0.001 | | | 0.003 |
| No | 2957 (74.4) | 2883 (69.6) | | 2478 (73.8) | 2370 (70.6) | |
| Yes | 1019 (25.6) | 1262 (30.4) | | 880 (26.2) | 988 (29.4) | |
| **Blood Transfusion, n (%)** | | | 0.011 | | | 0.629 |
| No | 3891 (97.9) | 4019 (97.0) | | 3276 (97.6) | 3282 (97.7) | |
| Yes | 85 (2.1) | 126 (3.0) | | 82 (2.4) | 76 (2.3) | |
| **Blood loss, n (%)** | | | 0.679 | | | 0.114 |
| <400ml | 3939 (99.1) | 4110 (99.2) | | 3323 (99.0) | 3335 (99.3) | |
| ≥400ml | 37 (0.9) | 35 (0.8) | | 35 (1.0) | 23 (0.7) | |

neutrophil count is an independent prognostic indicator for predicting OS and DFS in patients with CRC. Overall, we demonstrated that increased MPO expression was prominently correlated with a high peripheral neutrophil count, and both of these variables were independently linked with worse outcomes in CRC patients.

MPO is a cationic heme-containing peroxidase found primarily in neutrophils and minorly in monocytes (27). The bactericidal capacities of activated leukocytes have been attributed, at least partially, to the actions of MPO. MPO catalyzes the formation of reactive oxygen intermediates, including hypochlorite (HOCl), which play an important role in the killing of microorganisms

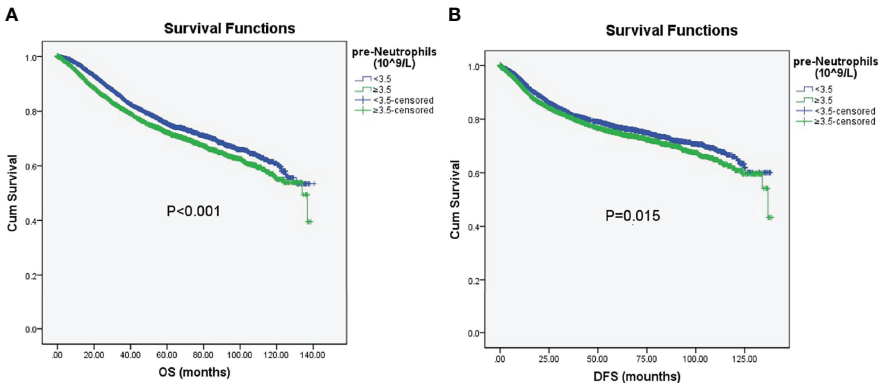


**FIGURE 7** | Kaplan–Meier analysis of OS and DFS. **(A)** Kaplan–Meier survival curve for OS according to preoperative neutrophils (pre-neutrophils) in the propensity score-matched cohort (OS, Logrank=13.743, P < 0.001). **(B)** Kaplan–Meier survival curve for DFS according to preoperative neutrophils (pre-neutrophils) in the propensity score-matched cohort (DFS, Logrank= 5.910, P = 0.015). Statistical significance was set at P < 0.05.

(27–29); however, excessive MPO activity aggravates inflammation, leading to tissue damage. MPO is a local mediator of inflammation and also an important target for the treatment of inflammatory diseases (30); although, recent studies have shown that MPO deficiency leads to an exaggeration of the inflammatory response and affects neutrophil function, including the production of cytokines (27). For example, MPO knockout mice exhibited an enhanced response of CD4+ T cells in lymph nodes, aggravating arthritis (31). The causal link between MPO oxidation and disease is complex, and overexpression and loss of MPO expression are associated with worse outcomes. Given these contradictory reports, we were more concerned about the role of MPO in cancer. High preoperative MPO levels may improve the prognosis of postoperative CRC patients with liver metastasis (11). The high tumor infiltration of MPO-expressing cells in colorectal and breast cancers is associated with a significant improvement in prognosis (32, 33). In contrast, the MPO promoter single nucleotide polymorphism (SNP) rs2333227 enhances the malignant phenotype of CRC by regulating MPO transcriptional activity (13). Additionally, MPO overexpression in human tumors may be related to the enhancement of cell invasion and migration (14, 34). Thus, the association between MPO gene variation and CRC risk is inconsistent and warrants further investigation. In our study, bioinformatic analysis using high-throughput RNA-sequencing data from TCGA revealed that MPO expression in CRC tissues was higher than that in normal tissues. In addition, high MPO levels are associated with malignant progression. Moreover, MPO overexpression resulted in shorter OS, PFI, and DSS in this study. Therefore, MPO was validated as an independent prognostic factor for OS, PFI, and DSS based on the univariate Cox regression analysis. These findings suggest that MPO may serve as a potential prognostic marker and therapeutic target for CRC. Using IHC and qRT-PCR analyses, we confirmed that the relative expression level of MPO in CRC tissues was significantly higher than that in the matched adjacent non-cancerous tissues.

Neutrophils are one of the earliest innate immune cells to be recruited to inflammatory tissues (35). When neutrophils are activated, MPO proteins are emptied into phagosomes or secreted by degranulation. In phagosomes, MPO produces highly active hypohalogenate and nitrogen dioxide, which react easily to form different reactive oxygen species and key bactericidal and immunomodulatory products of the neutrophil MPO-halide system (36, 37). Interestingly, neutrophil aggregation and elevated levels of pro-inflammatory cytokines are associated with disease severity in patients with COVID-19, and proteomic analysis confirmed that the level of MPO in the nasopharyngeal tissue of COVID-19 patients was also increased (37). Furthermore, neutrophils treated with stimuli are rich in MPO-DNA complexes (38) and, in turn, MPO can regulate the function and immune response of neutrophils. Recent research has shown that MPO can affect the degranulation of neutrophils and improve their phagocytosis (39), and it can also "break" the migration of neutrophils and prevent their accumulation (15). The relationship between MPO in CRC and preoperative neutrophil counts is yet to be reported. In this study, we confirmed that MPO in CRC was significantly correlated with the infiltration level of neutrophils in CRC through TCGA, TIMER, and Human Cell Landscape analyses. We also validated that MPO levels in CRC positively correlated with peripheral neutrophil counts. This finding implies a potential mechanism underlying MPO and poor cancer outcomes.

Many inflammatory cells represent innate and acquired immune responses in the microenvironment of solid malignant tumors (40), where neutrophils account for a large proportion of these inflammatory cells. Tumor-associated macrophages (TAMs) can be divided into the M1 antitumor and M2 tumor-promoting phenotypes. New evidence shows that tumor-associated neutrophils (TAN) can differentiate into the N1 anti-tumor or N2 pro-tumor phenotype. Neutrophils can be transformed into a tumor-promoting state in the microenvironment (41) and, in addition to cytotoxicity, they can promote the spread of tumor cells by secreting matrix metalloproteinases and elastase to degrade the extracellular matrix. The tumor-promoting state also regulates immunosuppression by secreting reactive oxygen species and arginase-1, thus limiting T cell-dependent antitumor immunity.

**TABLE 7 |** Univariate and multivariate Cox regression analysis for OS in the propensity-matched cohort.

| Variables | Univariate HR (95% CI) | P Value | Multivariate HR (95% CI) | P Value |
|---|---|---|---|---|
| **Pre-Neutrophils classification** | | | | |
| <3.5×10^9/L | 1 | | 1 | |
| ≥3.5×10^9/L | 1.187 (1.084-1.300) | <0.001 | 1.157(1.055-1.268) | 0.002 |
| **Sex** | | | | |
| Male | 1 | | | |
| Female | 1.020 (0.931-1.119) | 0.667 | | |
| **Age** | | <0.001 | | <0.001 |
| ≤44 | 1 | | 1 | |
| 45-54 | 0.936 (0.788-1.112) | 0.451 | 1.102 (0.926-1.311) | 0.276 |
| 55-64 | 0.964 (0.825-1.126) | 0.643 | 1.197 (1.023-1.400) | 0.025 |
| 65-74 | 1.221 (1.038-1.435) | 0.016 | 1.696 (1.437-2.002) | <0.001 |
| >75 | 1.910 (1.602-2.276) | <0.001 | 2.892 (2.416-3.460) | <0.001 |
| **Preoperative Neoadjuvant chemotherapy** | | | | |
| No | 1 | | 1 | |
| Yes | 1.507 (1.279-1.776) | <0.001 | 1.594 (1.333-1.907) | <0.001 |
| **Surgical approach** | | | | |
| Laparotomy | 1 | | | |
| Laparoscopy | 0.911 (0.759-1.093) | 0.316 | | |
| **Tumor histology** | | <0.001 | | |
| Adenocarcinoma | 1 | | | |
| Mucinous adenocarcinoma | 1.252 (1.097-1.429) | 0.001 | | |
| Signet-ring cell carcinoma | 2.574 (1.901-3.486) | <0.001 | | |
| **Tumor Differentiation** | | <0.001 | | <0.001 |
| Well | 1 | | 1 | |
| Moderate | 1.484 (1.006-2.189) | 0.047 | 0.930(0.628-1.379) | 0.719 |
| Poor | 2.986 (2.016-4.425) | <0.001 | 1.267(0.848-1.895) | 0.248 |
| Unknown | 1.595 (1.048-2.425) | 0.029 | 1.083(0.706-1.663) | 0.715 |
| **TNM stage** | | <0.001 | | <0.001 |
| 0-1 | 1 | | 1 | |
| II | 1.472 (1.200-1.807) | <0.001 | 1.398 (1.136-1.721) | 0.002 |
| III | 3.250 (2.704-3.906) | <0.001 | 2.354 (1.939-2.857) | <0.001 |
| IV | 13.686 (11.323-16.542) | <0.001 | 9.819 (8.011-12.035) | <0.001 |
| Unknown | 0.701 (0.379-1.298) | 0.258 | 0.564 (0.299-1.066) | 0.078 |
| **Surgical margin positive** | | | | |
| No | 1 | | 1 | |
| Yes | 2.990 (2.302-3.885) | <0.001 | 1.678 (1.284-2.193) | <0.001 |
| **Infiltrating Lymph nodes > 12** | | | | |
| No | 1 | | 1 | |
| Yes | 0.718 (0.648-0.796) | <0.001 | 0.826 (0.741-0.920) | 0.001 |
| **Number of cancer nodule≥1** | | | | |
| No | 1 | | 1 | |
| Yes | 2.911 (2.632-3.220) | <0.001 | 1.434 (1.284-1.601) | <0.001 |
| **Blood Transfusion** | | | | |
| No | 1 | | 1 | |
| Yes | 1.699 (1.331-2.168) | <0.001 | 1.420 (1.109-1.817) | 0.005 |
| **Blood loss** | | | | |
| <400ml | 1 | | | |
| ≥400ml | 1.545 (1.024-2.331) | 0.038 | | |
| **Surgery again within 30days** | | | | |
| No | 1 | | | |
| Yes | 1.049 (0.751-1.466) | 0.778 | | |

These tumor-promoting effects may serve as potential targets for cancer therapy (42). Neutrophils play a tumor-promoting role through the formation of neutrophil extracellular traps (NETs) within the tumor, known as NETosis (43). NETs are involved in the deterioration of a variety of diseases such as cancer, autoimmune diseases, and thrombosis (44). Most evidence shows that neutrophils can promote tumors, and the degree of neutrophil infiltration is related to poor prognosis; however, a few studies have observed that neutrophils can improve or did not affect prognosis (24, 25, 45). Our study confirmed that a higher preoperative neutrophil count was correlated with poorer OS and DFS in CRC. High preoperative neutrophil count is an independent prognostic indicator for predicting OS and DFS in patients with CRC. Yamamoto et al. also reported that a high neutrophil count was independently associated with worse survival in patients with metastatic CRC with wild-type RAS (46). Wculek et al. have suggested that the deletion of Smad4 in CRC promotes the expression of CCL15 and recruits more CCR1+ TANs and matrix

**TABLE 8 |** Univariate and multivariate Cox regression analysis for DFS in the propensity-matched cohort.

| Variables | UnivariateHR (95% CI) | P Value | Multivariate HR (95% CI) | P Value |
|---|---|---|---|---|
| **Pre-Neutrophils classification** | | | | |
| <3.5×10^9/L | 1 | | 1 | |
| ≥3.5×10^9/L | 1.134 (1.025-1.256) | 0.015 | 1.118 (1.009-1.238) | 0.033 |
| **Sex** | | | | |
| Male | 1 | | | |
| Female | 0.974 (0.878-1.080) | 0.616 | | |
| **Age** | | <0.001 | | <0.001 |
| ≤44 | 1 | | 1 | |
| 45-54 | 0.994 (0.814-1.213) | 0.951 | 1,192 (0.976-1.457) | 0.086 |
| 55-64 | 0.985 (0.822-1.180) | 0.866 | 1.174 (0.978-1.409) | 0.085 |
| 65-74 | 1.345 (1.117-1.619) | 0.002 | 1.748 (1.448-2.111) | <0.001 |
| >75 | 2.161 (1.774-2.631) | <0.001 | 2.877 (2.356-3.514) | <0.001 |
| **Preoperative Neoadjuvant chemotherapy** | | | | |
| No | 1 | | 1 | |
| Yes | 1.612 (1.345-1.933) | <0.001 | 1.892 (1.556-2.302) | <0.001 |
| **Surgical approach** | | | | |
| Laparotomy | 1 | | | |
| Laparoscopy | 0.849 (0.689-1.045) | 0.123 | | |
| **Tumor histology** | | <0.001 | | |
| Adenocarcinoma | 1 | | | |
| Mucinous adenocarcinoma | 1.292 (1.117-1.495) | 0.001 | | |
| Signet-ring cell carcinoma | 2.676 (1.929-3.712) | <0.001 | | |
| **Tumor Differentiation** | | <0.001 | | <0.001 |
| Well | 1 | | 1 | |
| Moderate | 1.381 (0.913-2.089) | 0.126 | 0.880 (0.579-1.339) | 0.551 |
| Poor | 2.718 (1.787-4.135) | <0.001 | 1.190 (0.774-1.830) | 0.428 |
| Unknown | 1.565 (1.001-2.448) | 0.050 | 1.056 (0.667-1.672) | 0.816 |
| **TNM stage** | | <0.001 | | <0.001 |
| 0-1 | 1 | | 1 | |
| II | 1.491 (1.215-1.830) | <0.001 | 1.400 (1.137-1.725) | 0.002 |
| III | 3.354 (2.790-4.032) | <0.001 | 2.351 (1.930-2.864) | <0.001 |
| IV | 15.001 (12.099-18.598) | <0.001 | 9.237 (7.315-11.664) | <0.001 |
| Unknown | 0.713(0.386-1.320) | 0.282 | 0.520 (0.274-0.986) | 0.045 |
| **Surgical Margin positive** | | | | |
| No | 1 | | 1 | |
| Yes | 3.075 (2.284-4.139) | <0.001 | 1.578 (1.163-2.141) | 0.003 |
| **Infiltrating Lymph nodes > 12** | | | | |
| No | 1 | | 1 | |
| Yes | 0.700(0.624-0.786) | <0.001 | 0.781 (0.693-0.880) | <0.001 |
| **Number of cancer nodule ≥1** | | | | |
| No | 1 | | 1 | |
| Yes | 2.861(2.549-3.212) | <0.001 | 1.449 (1.274-1.648) | <0.001 |
| **Blood Transfusion** | | | | |
| No | 1 | | 1 | |
| Yes | 1.732(1.317-2.279) | <0.001 | 1.562 (1.185-2.059) | 0.002 |
| **Blood loss** | | | | |
| <400ml | 1 | | | |
| ≥400ml | 1.615 (1.015-2.571) | 0.043 | | |
| **Surgery again within 30days** | | | | |
| No | 1 | | | |
| Yes | 0.970 (0.658-1.429) | 0.876 | | |

metalloproteinase-9 to the metastatic site to form the premetastatic niche of disseminated tumor cells (47). Presently, the underlying mechanism behind MPO and the prognosis of patients with CRC have not yet been clarified; our research, however, suggests that neutrophils are potential key players in the mechanism linking MPO levels with poor CRC outcomes.

In our study, there was no significant difference in MMR-status between high/low preoperative neutrophil counts. There was no significant difference in overall survival between MMR-deficient and MMR-proficient patients. These results may be due the insufficient sample size. We will investigate the correlation between MMR-status and peripheral neutrophil counts in our future study. Zhu et al. reported that patients with CRC who had lost at least one MMR protein (MLH1, MSH2, MSH6, or PMS2) had a better prognosis. An unexpected finding was that there was a correlation between MMR-deficiency and elevated CD66b+ TAN levels (48). Park et al. reported that MMR-deficiency was associated with increased peritoneal involvement and poor tumor differentiation. C-reactive protein, neutrophil count, neutrophil count > $7.5\times10^9$/L and NPS (neutrophil/platelet score) were higher in patients with MMR-

deficient CRC than that in patients with MMR-proficient CRC before operation. In multivariate survival analysis, there was no significant correlation between MMR-deficiency and cancer-specific survival, while NPS was independently related to survival. When the analysis was limited to patients with II/III disease, NPS was still associated with survival, while MMR-status was still not associated with survival (49). Overall, the relationship between MMR-status and systemic inflammatory responses remains unclear and needs further investigation.

Our study addressed an important concern and confirmed that MPO is upregulated in patients with CRC and is related to malignant progression and survival of the patients. Furthermore, neutrophils were identified as potential key players in the mechanism linking MPO levels with poor CRC outcomes. It may be challenging to directly apply MPO in clinical settings, but the bridging role played by neutrophils can facilitate the transformation of basic research into clinical practice. One of the advantages of our clinical study is that the overall sample size (>8000) and allocation (>3000) in each group in this study were much larger than those of previous studies, and the data were obtained from one of the largest cancer centers in China. Another advantage is that the median postoperative follow-up period in this study was more than 5 years (median: 69.6 months), and we focused on the long-term outcomes of CRC patients. In addition, we used propensity score matching and multivariate Cox regression analysis to correct for confounding factors. It is usually difficult to assess the expression of MPO before operation, but the expression of neutrophils from peripheral blood can easily be determined; this aspect is the greatest contribution of our study. The prognosis of patients with CRC could be predicted using a simple and feasible method before operation. In light of this, it is suggested that anesthesiologists and surgeons pay more attention to the patients with a higher preoperative inflammation status and take measures to inhibit perioperative stress response and inflammation, so as to improve the prognosis of patients (50–52). In this regard, the present study findings can have reasonable clinical applications.

Our study had several limitations. First, the clinical validation part of the study was retrospective and non-randomized, and patient information was obtained from a single cancer center. Second, owing to various perioperative factors associated with a high neutrophil count, although we utilized propensity score matching, we still could not eliminate the potential influence of unmeasured confounders. In addition, although we found that MPO expression and preoperative neutrophil counts are involved in CRC progression, the potential mechanism needs to be further studied.

In conclusion, our study showed that increased MPO is positively correlated with high peripheral neutrophil counts, both of which serve as potential risk indicators for malignant progression and worse survival in CRC. Our study suggests that neutrophils are key players in the mechanism linking MPO levels with poor CRC outcomes, which implies the clinical applicability of our study results. In the future, well-designed prospective and basic studies are warranted to explore the underlying mechanisms further.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved. This clinical retrospective study was performed at the Shanghai Cancer Center, Fudan University, Shanghai, China. The study was approved by the center's Ethics Committee (IRB2105235-6). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

MW, and CM designed the study. MW, DW, YY, MG, and CS performed the study. MW, CZ, and QL analyzed the data. MW, DW, and YY wrote the paper. MW, MS and DZ revised the paper. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.940706/full#supplementary-material

**Supplementary Figure 1 |** The association between MPO and clinicopathological characteristics. **(A–J)** There was no association between MPO and clinicopathological characteristics, including T stages, N stages, history of colon polyps, colon polyps present, lymphatic invasion, perineural invasion, residual tumor, BMI, age, and sex.

**Supplementary Figure 2 |** Kaplan-Meier analysis of OS according to MMR-status. **(A)** Kaplan-Meier survival curve for OS according to MMR-status in 668 patients with CRC. (Logrank=0.008, P = 0.928).

**Supplementary Table 1 |** The relationship between MMR-status and high/low preoperative neutrophil counts.

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# High preoperative white blood cell count determines poor prognosis and is associated with an immunosuppressive microenvironment in colorectal cancer

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**Background:** The correlation between high white blood cell (WBC) count and poor prognosis has been identified in various types of cancer; however, the clinical significance and immune context of WBC count in colorectal cancer remains unclear.

**Methods:** Between February 2009 and November 2014, 7,433 patients at the Shanghai Cancer Center who had undergone elective surgery for colorectal cancer were enrolled in this retrospective cohort study. Patients were divided into two groups: low and high preoperative WBC groups. Propensity score matching was used to address the differences in baseline characteristics. The Kaplan–Meier method and Cox regression analysis were used to identify independent prognostic factors in colorectal cancer patients. Tumor-infiltrating immune cells in the high and low preoperative WBC groups were compared using immunohistochemical staining.

**Results:** Of the 7,433 patients who underwent colorectal cancer surgery and were available for analysis, 5,750 were included in the low preoperative WBC group, and 1,683 were included in the high preoperative WBC group. After propensity score matching, 1,553 patients were included in each group. Kaplan–Meier survival curves showed that a high preoperative WBC count was associated with a decreased overall survival (P = 0.002) and disease-free survival (P = 0.003), and that preoperative WBC count was an independent risk factor for overall survival (hazard ratio, 1.234; 95% confidence interval, 1.068–1.426; P = 0.004) and disease-free survival (hazard ratio, 1.210; 95% confidence interval, 1.047–1.397, P = 0.01). Compared to the low preoperative WBC group, the high preoperative WBC group exhibited higher expression of regulatory T cells (P = 0.0034), $CD68^+$ macrophages (P = 0.0071), and $CD66b^+$ neutrophils

(P = 0.0041); increased expression of programmed cell death protein 1 (P = 0.005) and programmed cell death ligand 1 (P = 0.0019); and lower expression of $CD8^+$ T cells (P = 0.0057) in colorectal cancer patients.

**Conclusions:** Our research indicates that a high preoperative WBC count is a prognostic indicator in colorectal cancer patients and is associated with an immunosuppressive tumor microenvironment, which could aid in future risk stratification.

KEYWORDS

high preoperative WBC, preoperative leukocytosis, long-term prognosis, colorectal cancer, propensity score matching, tumor microenvironment

## Introduction

Colorectal cancer (CRC) is the third most common tumor in the world and one of the leading causes of cancer-related deaths worldwide (1). In China, CRC ranks fifth among the main causes of death caused by cancer among men and women; however, the death rates from CRC have been on the rise in recent decades (2, 3). Although great progress has been made in surgery, chemotherapy, and radiotherapy, the mortality rate of CRC remains high. A large proportion of CRC patients still develop resistance to chemotherapy and eventually relapse within two years after undergoing surgery (4). Thus, a prognostic indicator is urgently needed to predict the outcome of CRC patients, which is very important for risk stratification and determining treatment strategies (5).

CRC is an inflammation-related tumor characterized by the infiltration of heterogeneous immune cells into tumor microenvironment and peripheral hematological disorders (6), which create a complex microenvironment that allows for the development of tumors (7). During acute inflammation, white blood cells (WBCs) are considered the first line of defense against microbial infection, protecting the host from pathogens (8). However, in the state of long-term activation, continuous production of growth factors and reactive oxygen species may lead to permanent genomic changes and hinder the recruitment of lymphocytes by interacting with the DNA of the proliferating epithelium. Moreover, leukocytosis inhibits the activation of $CD8^+$ tumor infiltrating lymphocytes by upregulating programmed death protein 1 (PD-1) on T lymphocytes and myeloid cells (7, 9). In malignant diseases, leukocytes and neutrophils can dilate locally and throughout the body, promoting anti-cancer treatment resistance and tumor progression through angiogenesis, invasion, and inhibitory factors (10). Tumor-infiltrating immune cells and inflammatory cells are the main components of the tumor microenvironment (TME) and are critical for the immune function of the host and the biological behavior of the tumor (11). Chronic inflammation is thought to lead to the development of various malignant lesions and an increased risk of recurrence (12).

As indicators of prognosis, various serum molecular markers, such as basophil, neutrophil, and lymphocyte counts, are easy to acquire from conventional preoperative examinations and are useful in diagnosing and evaluating treatment and predicting the prognosis of CRC patients (13–15). The correlation between a high WBC count and poor prognosis has been identified in various cancer types, such as oropharyngeal, cervical, and esophageal cancer (16–18). Although several groups have reported the adverse effects of peripheral leukocytosis on the prognosis of patients with various malignant tumors, this is limited by a retrospective mismatch, uneven treatment options, short follow-up, lack of multivariable analysis, and a small number of patients (5, 19, 20). The mechanism underlying this phenomenon has not yet been clarified.

Therefore, the purpose of this study was to investigate the prognostic value of preoperative WBC count on overall survival (OS) and disease-free survival (DFS) after CRC surgery in a larger matched sample cohort and the functional relevance of WBC in an immunological context. We speculated that high preoperative WBC count predicts a poorer survival outcome in CRC patients undergoing selective surgery and is associated with an immunosuppressive TME in CRC.

## Materials and methods

### Study design

This retrospective study was approved by the Ethics Committee of the Shanghai Cancer Center at Fudan University in Shanghai, China (IRB2105235-6). All the participants signed an informed consent form.

## Study population

Between February 2009 and November 2014, 12,636 patients underwent elective surgery for CRC, and 7,433 patients with clinical characteristic data, OS records, and DFS records were included in this study (Figure 1). The inclusion criteria were as follows CRC diagnosed according to histological evidence, patients undergoing elective radical surgery for CRC, and patients older than 20 years.

We excluded patients with incomplete medical information, benign tumors or carcinoma *in situ*, emergency surgery, an American Society of Anesthesiologists physical status of > 3, or those with a history of other malignant tumors during the initial visit (Figure 1). According to the preoperative WBC count, patients were classified into a high preoperative WBC group (WBC count ≥ 7,000/μL) and low preoperative WBC group (WBC count < 7,000/μL). The cut-off value for WBC count was calculated using the receiver operating characteristic (ROC) curve; the threshold was associated with an increased risk of postoperative mortality and was within the normal range of the WBC count (21).

## Variables and outcomes

We reviewed and recorded the following variables from the clinical information system of the Shanghai Cancer Center: sex, age, preoperative adjuvant chemotherapy, surgical approach, tumor location, tumor histology, vascular tumor thrombus, nerve invasion, surgical margin positivity, T, N, M, and TNM stages, infiltrating lymph nodes > 12, number of cancer nodules ≥1, preoperative hemoglobin, surgery again within 30 days, death, intraoperative transfusion, and blood loss.

The main endpoints of the study were OS and DFS. OS was defined as the interval between the date of diagnosis and the date of death from any cause. DFS was defined as the interval between the date of diagnosis and the date of recurrence, metastasis, occurrence of a secondary primary tumor, or death.

## Immunohistochemical staining

Among patients underwent elective surgery for CRC in 2014, 30 patients were randomly selected from the high preoperative WBC group and 30 patients from the low preoperative WBC group. These 60 CRC tissues were used for IHC. Paraffin-embedded tissues were stained with antibodies. Two experienced pathologists determined the staining score. Six high-power fields (×200 magnification) were randomly counted by two independent pathologists (each experts in three fields), and the densities of $CD8^+$ T cells, $Foxp3^+$ regulatory T (Treg), $CD68^+$ macrophages, $CD66b^+$

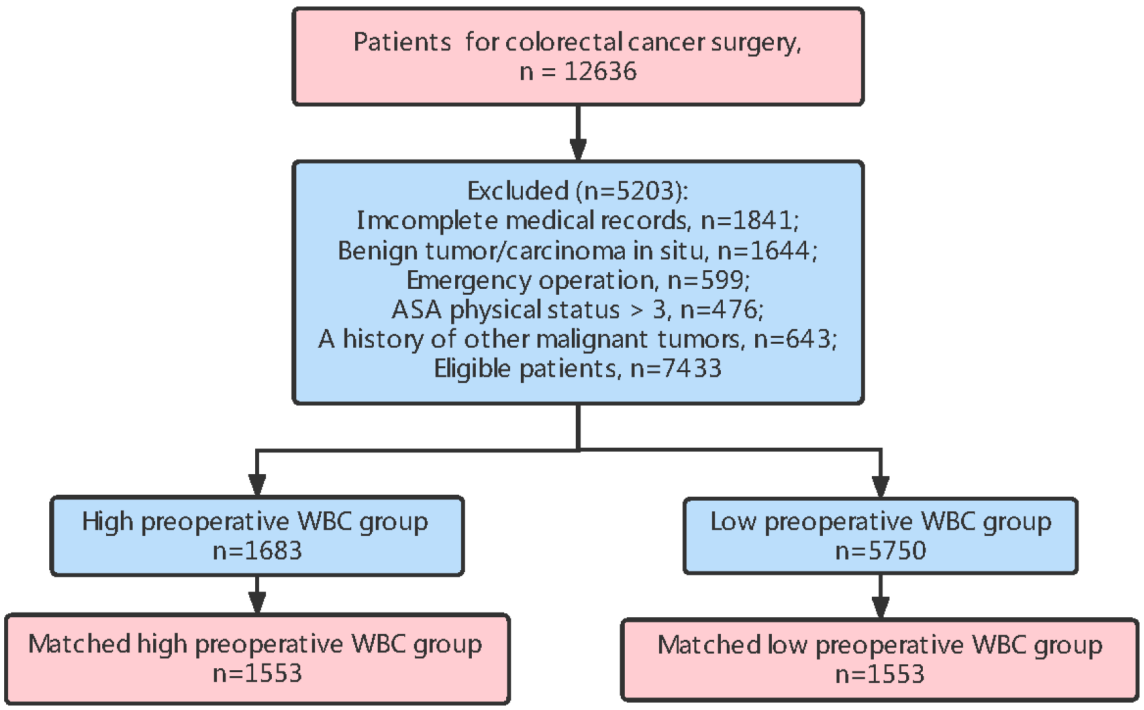


FIGURE 1
A. Flow chart of patient selection.

neutrophils, PD-1$^+$ cells, and programmed cell death ligand 1 (PD-L1)$^+$ cells were recorded. The immunohistochemical antibodies used are listed in Supplementary Table 1.

## Statistical analysis

In this study, the baseline characteristics of the patients were expressed by the values and percentages of the classified variables. The chi-square test was used to evaluate differences in baseline characteristics between the two groups. To reduce the possible confounding effect of each variable and the difference in baseline characteristics between the two groups, propensity score matching was performed. The paired variables were sex, age, preoperative adjuvant chemotherapy, tumor histology, TNM stage, and infiltrating lymph nodes > 12. The R software package "MatchIt" was used to match the propensity score. After matching, 1,553 patients were included in each group (Figure 1; Table 1).

In the propensity score matched cohort, the Kaplan–Meier method was used to compare OS and DFS using the log-rank test. The Cox proportional hazards model was used to identify independent prognostic factors in CRC patients. All variables were adjusted using a univariate Cox proportional hazards model. A multivariate Cox proportional hazard model was used in stepwise entry to select prognostic factors. Meanwhile, the hazard ratio and the corresponding 95% confidence interval (CI) were calculated. The densities of infiltrating immune cells between two groups were evaluated using an independent t-test or Mann-Whitney U test. All analyses were performed using IBM SPSS Statistics 25 (SPSS Inc., USA). Statistical significance was set at $P < 0.05$.

# Results

The results are presented in Figure 1 and Table 1. Our median postoperative follow-up period was 69.4 months (95% CI: 68.7–70.0) for all the patients, 69.5 months (95% CI: 68.8–70.2) for the low preoperative WBC group, and 69.0 months (95% CI: 67.5–70.4) for the high preoperative WBC group ($P = 0.677$).

Generally, a high WBC classification is defined as a preoperative WBC count of ≥ 7,000/μL. Based on this definition, 22.6% (1,683/7,433) of patients were in the high preoperative WBC group and 77.4% (5,750/7,433) were in the low preoperative WBC group. The patient characteristics are shown in Table 1. More men (65.0% *vs.* 57.7%, $P < 0.001$) were in the high preoperative WBC group than in the low preoperative WBC group. Patients in the high preoperative WBC group were less likely to undergo preoperative adjuvant chemotherapy (5.7% *vs.* 8.9%, $P < 0.001$) and were more prone to have a tumor located on the left-side of the colon (23.0% *vs.* 20.6%, $P < 0.001$), right-side colon (27.0% *vs.* 23.8%, $P < 0.001$), and the transverse colon (can't tell right or left) (1.6% *vs.* 1.2%, $P < 0.001$), had more mucinous adenocarcinoma (14.3% *vs.* 11.1%, $P < 0.001$), signet-ring cell carcinoma (2.0% *vs.* 1.3%, $P < 0.001$), and positive surgical margins (2.0% *vs.* 1.3%, $P < 0.001$). Furthermore, the high preoperative WBC group also had had a worse T ($P < 0.001$) and M stage ($P = 0.002$), a higher TNM stage ($P < 0.001$), more infiltrating lymph nodes > 12 (81.6% *vs.* 77.3%, $P = 0.001$) and more hemoglobin < 90 g/L (8.9% *vs.* 5.8%, $P < 0.001$). There were no significant differences in age, surgical approach, vascular tumor thrombus, nerve invasion, N stage, or the number of cancer nodules ≥ 1 ($P > 0.05$). The results indicated that a high preoperative WBC count was more likely to correlate with more malignant clinicopathological features in CRC patients.

Preoperative WBC counts, neutrophil counts, lymphocyte counts, monocyte counts, neutrophil-lymphocyte ratio (NLR), systemic immune-inflammation index (SII) was markedly higher in the high preoperative WBC group than in the low preoperative WBC group (all $P<0.0001$, Figures 2A–F). However, preoperative lymphocyte-monocyte ratio (LMR) was significantly higher in the low preoperative WBC group than in the high preoperative WBC group ($P<0.0001$, Figure 2G). There were no significant differences in platelet-lymphocyte ratio (PLR) between two groups (Figure 2H). It showed that a high preoperative WBC count was associated with more elevated inflammation-related biomarkers.

The propensity score matching was chosen to reduce the imbalance due to the differences in baseline characteristics between the two groups. After matching, 1,553 patients remained in each group. There were no significant differences in patient characteristics between the two groups in the matched cohort, except for surgical margin positivity and preoperative hemoglobin (Table 1). In the propensity score matched cohort, the overall mortality rate was significantly higher in the high preoperative WBC count group (26.3% *vs.* 22.2%, $P = 0.007$) than in the low preoperative WBC count group during follow-up for more than 5 years. Furthermore, a greater percentage of patients in the high preoperative WBC count group required blood transfusion (3.9% *vs.* 1.8%, $P = 0.001$, Table 1). There was no significant difference in blood loss between the two groups (0.8% *vs.* 0.8%, $P=1.000$, Table 1). However, the occurrence of blood transfusion is significantly different, which may be related to the different degree of anemia between the two groups before operation. Summarizing this propensity score matched cohort, a high preoperative WBC count was associated with a higher mortality rate and more blood transfusions after CRC surgery.

To assess the association between preoperative WBC count and prognosis, we performed a Kaplan–Meier survival analysis for OS and DFS after propensity score matching. The OS and DFS in the high preoperative WBC group were shorter than those in the low preoperative WBC group (Figures 3A, B; median survival time in OS: 136.933 months *vs.* 134.667 months; 5-year OS rate: 79.9% *vs.* 75.7%; $P = 0.002$; median survival time in DFS: 136.933 months *vs.* 134.667 months; 5-year

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TABLE 1 Patients baseline Characteristics in the total study cohort and the propensity score matched cohort.

| Variables | Overall patients | | P value | Matched patients | | P value |
|---|---|---|---|---|---|---|
| | Low pre-WBC (n=5750) | High pre-WBC (n=1683) | | Low pre-WBC (n=1553) | High pre-WBC (n=1553) | |
| **Sex, n (%)** | | | <0.001 | | | 0.472 |
| Male | 3315 (57.7) | 1094 (65.0) | | 1031 (66.4) | 1012 (65.2) | |
| Female | 2435 (42.3) | 589 (35.0) | | 522 (33.6) | 541 (34.8) | |
| **Age, n (%)** | | | 0.446 | | | 0.917 |
| ≤44 | 799 (12.8) | 269 (14.3) | | 174 (11.2) | 175 (11.3) | |
| 45-54 | 1247 (20.0) | 386 (20.5) | | 338 (21.8) | 322 (20.7) | |
| 55-64 | 2263 (36.3) | 652 (34.6) | | 569 (36.6) | 563 (36.3) | |
| 65-74 | 1342 (21.5) | 404 (21.4) | | 335 (21.6) | 346 (22.3) | |
| >75 | 585 (9.4) | 174 (9.2) | | 137 (8.8) | 147 (9.5) | |
| **Pre-chemotherapy, n (%)** | | | <0.001 | | | 0.673 |
| No | 5239 (91.1) | 1587 (94.3) | | 1477 (95.1) | 1482 (95.4) | |
| Yes | 511 (8.9) | 96 (5.7) | | 76 (4.9) | 71 (4.6) | |
| **Surgical approach, n (%)** | | | 0.805 | | | 0.546 |
| Laparotomy | 5296 (92.1) | 1547 (91.9) | | 1437 (92.5) | 1428 (92.0) | |
| Laparoscopy | 454 (7.9) | 136 (8.1) | | 116 (7.5) | 125 (8.0) | |
| **Tumor location, n (%)** | | | <0.001 | | | 0.070 |
| Rectum | 3381 (54.2) | 909 (48.2) | | 820 (52.8) | 754 (48.6) | |
| Left-side colon | 1283 (20.6) | 433 (23.0) | | 326 (21.0) | 353 (22.7) | |
| Right-side colon | 1483 (23.8) | 509 (27.0) | | 393 (25.3) | 422 (27.2) | |
| Entire colon | 12 (0.2) | 4 (0.2) | | 0 (0.0) | 2 (0.1) | |
| Transverse colon (can't tell right or left) | 77 (1.2) | 30 (1.6) | | 14 (0.9) | 22 (1.4) | |
| **Tumor histology, n (%)** | | | <0.001 | | | 0.424 |
| Adenocarcinoma | 5034 (87.5) | 1410 (83.8) | | 1331 (85.7) | 1340 (86.3) | |
| Mucinous adenocarcinoma | 641 (11.1) | 240 (14.3) | | 199 (12.8) | 198 (12.7) | |
| Signet-ring cell carcinima | 75 (1.3) | 33 (2.0) | | 23 (1.5) | 15 (1.0) | |
| **Vascular tumor thrombus, n (%)** | | | 0.080 | | | 0.276 |
| Negative | 4549 (79.1) | 1298 (77.1) | | 1229 (79.1) | 1204 (77.5) | |
| Positive | 1201 (20.9) | 385 (22.9) | | 324 (20.9) | 349 (22.5) | |
| **Nerve invasion, n (%)** | | | 0.691 | | | 0.707 |
| Negative | 4722 (82.1) | 1375 (81.7) | | 1282 (82.5) | 1274 (82.0) | |
| Positive | 1028 (17.9) | 308 (18.3) | | 271 (17.5) | 279 (18.0) | |
| **Surgical Margin positivity, n (%)** | | | 0.035 | | | 0.038 |
| No | 5677 (98.7) | 1650 (98.0) | | 1537 (99.0) | 1523 (98.1) | |
| Yes | 73 (1.3) | 33 (2.0) | | 16 (1.0) | 30 (1.9) | |
| **T stage, n (%)** | | | <0.001 | | | 0.748 |
| T1 | 486 (8.4) | 98 (5.8) | | 105 (6.7) | 91 (5.8) | |
| T2 | 1121 (19.5) | 254 (15.1) | | 257 (16.5) | 238 (15.3) | |
| T3 | 246 (4.3) | 86 (5.1) | | 73 (4.7) | 75 (4.8) | |
| T4 | 3657 (63.6) | 1201 (71.4) | | 1080 (69.5) | 1115 (71.8) | |
| Tx | 240 (4.2) | 44 (2.6) | | 38 (2.4) | 34 (2.2) | |
| **N stage, n (%)** | | | 0.421 | | | 0.431 |
| N0 | 3204 (55.7) | 912 (54.2) | | 864 (55.6) | 848 (54.6) | |
| N1 | 1643 (28.6) | 487 (28.9) | | 422 (27.2) | 453 (29.3) | |
| N2 | 903 (15.7) | 284 (16.9) | | 267 (17.2) | 252 (16.2) | |

*(Continued)*

TABLE 1 Continued

| Variables | Overall patients | | P value | Matched patients | | P value |
|---|---|---|---|---|---|---|
| | Low pre-WBC (n=5750) | High pre-WBC (n=1683) | | Low pre-WBC (n=1553) | High pre-WBC (n=1553) | |
| **M stage, n (%)** | | | 0.002 | | | 0.389 |
| M0 | 5465 (95.0) | 1567 (93.1) | | 1472 (94.8) | 1461 (94.1) | |
| M1 | 285 (5.0) | 116 (6.9) | | 81 (5.2) | 92 (5.9) | |
| **TNM stage, n (%)** | | | <0.001 | | | 0.923 |
| I | 1183 (20.6) | 259 (15.4) | | 244 (15.7) | 242 (15.6) | |
| II | 1661 (28.9) | 576 (34.2) | | 555 (35.7) | 545 (35.1) | |
| III | 2439 (42.4) | 705 (41.9) | | 653 (42.0) | 656 (42.2) | |
| IV | 285 (5.0) | 116 (6.9) | | 81 (5.2) | 92 (5.9) | |
| Unknown | 182 (3.2) | 27 (1.6) | | 20 (1.3) | 18 (1.2) | |
| **Infiltrating lymph nodes > 12, n (%)** | | | <0.001 | | | 0.176 |
| No | 1303 (22.7) | 310 (18.4) | | 294 (18.9) | 265 (17.1) | |
| Yes | 4447 (77.3) | 1373 (81.6) | | 1259 (81.1) | 1288 (82.9) | |
| **Number of cancer nodules ≥ 1, n (%)** | | | 0.600 | | | 0.836 |
| No | 4969 (86.4) | 1446 (85.9) | | 1334 (85.9) | 1338 (86.2) | |
| Yes | 781 (13.6) | 237 (14.1) | | 219 (14.1) | 215 (13.8) | |
| **Preoperative Hemoglobin, g/L** | | | <0.001 | | | 0.005 |
| <90 | 331 (5.8%) | 150 (8.9%) | | 93 (6.0%) | 134 (8.6%) | |
| ≥90 | 5419 (94.2%) | 1533 (91.1%) | | 1460 (94.0%) | 1419 (91.4%) | |
| **Results** | | | | | | |
| **Surgery again within 30 days, n (%)** | | | 0.468 | | | 0.289 |
| No | 5646 (98.2) | 1657 (98.5) | | 1520 (97.9) | 1528 (98.4) | |
| Yes | 104 (1.8) | 26 (1.5) | | 33 (2.1) | 25 (1.6) | |
| **Death, n (%)** | | | 0.003 | | | 0.007 |
| No | 4391 (76.4) | 1225 (72.8) | | 1208 (77.8) | 1144 (73.7) | |
| Yes | 1359 (23.6) | 458 (27.2) | | 345 (22.2) | 409 (26.3) | |
| **Blood Transfusion, n (%)** | | | <0.001 | | | 0.001 |
| No | 5635 (98.0) | 1618 (96.1) | | 1525 (98.2) | 1493 (96.1) | |
| Yes | 115 (2.0) | 65 (3.9) | | 28 (1.8) | 60 (3.9) | |
| **Blood loss, n (%)** | | | 0.954 | | | 1.000 |
| <400ml | 5703 (99.2) | 1669 (99.2) | | 1540 (99.2) | 1540 (99.2) | |
| ≥400ml | 47 (0.8) | 14 (0.8) | | 13 (0.8) | 13 (0.8) | |

DFS rate: 77.5% *vs.* 73.5%; P = 0.003). After matching, the high preoperative WBC count group exhibited worse outcomes than the low preoperative WBC count group.

In the propensity score matched cohort, the Cox proportional hazards model was created to evaluate the association between the preoperative WBC count and survival, as shown in Tables 2 and 3. OS and DFS were compared in a univariate Cox model and later in a multivariate Cox regression. After adjustment, multivariable Cox regression showed that a high preoperative WBC count was strongly associated with poorer OS (hazard ratio, 1.234; 95% CI, 1.068–1.426; P = 0.004) and worse DFS (hazard ratio, 1.210; 95% CI, 1.047–1.397, P = 0.01) when compared to the low preoperative WBC count group. Other adverse prognostic factors for OS and DFS after multivariate analysis were age (≥65 years), preoperative neoadjuvant chemotherapy, tumor histology (signet-ring cell carcinoma), vascular tumor thrombus, nerve invasion, surgical margin positivity, TNM stage (III and IV), infiltrating lymph nodes > 12, number of cancer nodules ≥ 1, and intraoperative blood transfusion. In summary, high preoperative WBC count is an independent predictor of OS and DFS in CRC patients.

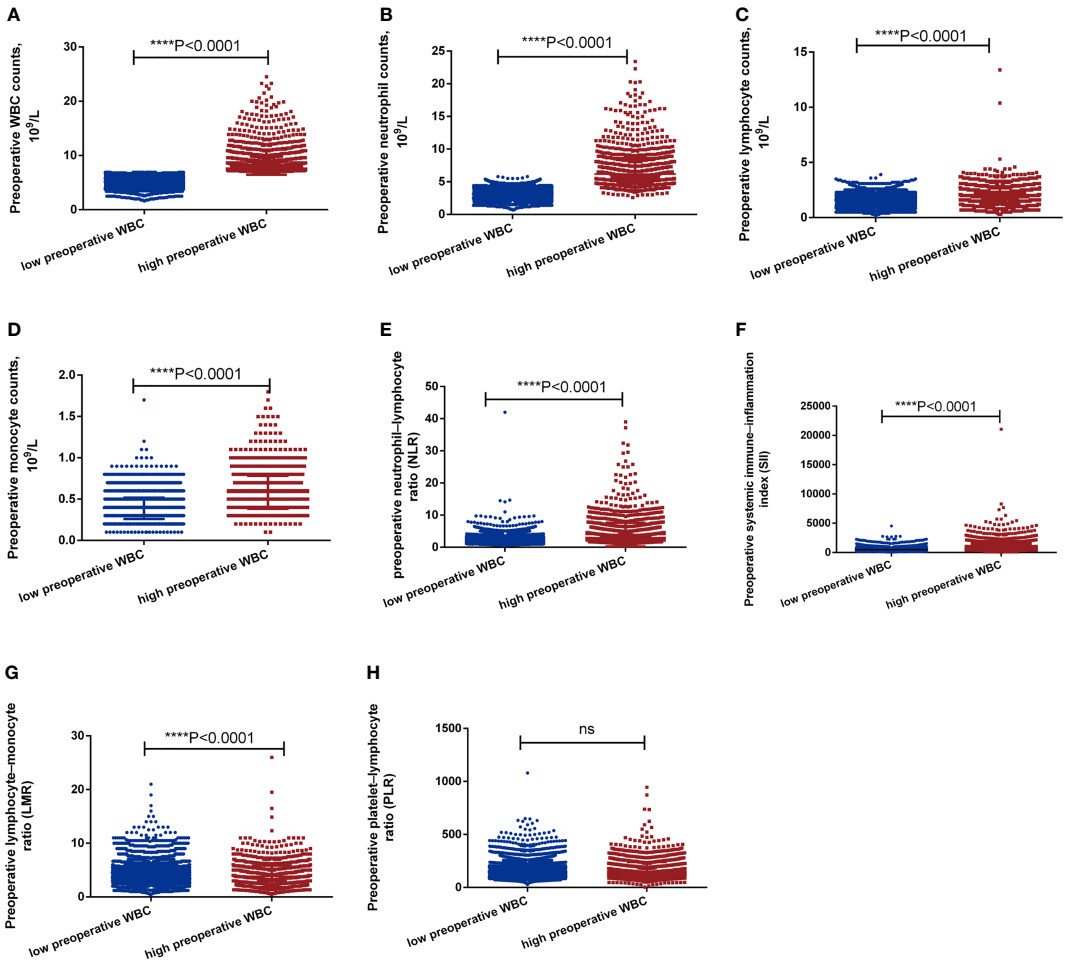
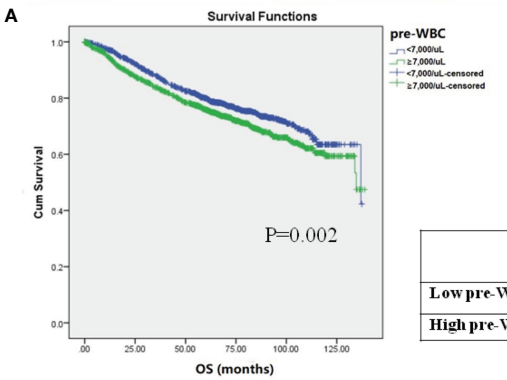



FIGURE 2
The level of inflammation-related biomarkers between high preoperative WBC group and low preoperative WBC group before matching. **(A-H)**. The level of WBC, neutrophils, lymphocyte, monocyte, NLR, PLR, LMR, SII between high preoperative WBC group and low preoperative WBC group before matching. Differences were considered significant at ****$P < 0.0001$, compared to the low preoperative WBC group. ns, no significance.

To explore the potential mechanism, we performed immunohistochemical staining of tumor-infiltrating immune cells in the high and low preoperative WBC groups (n = 60). Compared with the low preoperative WBC group, the high preoperative WBC group showed higher expression of Treg cells (P = 0.0034), $CD68^+$ macrophages (P = 0.0071), and $CD66b^+$ neutrophils (P = 0.0041), but lower expression of $CD8^+$ T cells (P = 0.0057), suggesting a more immunosuppressive TME with increased Treg cells, macrophages, and neutrophil infiltration in the high preoperative WBC group (Figures 4A–D, G–J). We also found that the expression of PD-1 and PD-L1 increased in the high preoperative WBC count group (P = 0.005; P = 0.0019) (Figures 4E–F, K–M). Taken together, these data suggest that a high preoperative WBC count is associated with an immunosuppressive environment in CRC.

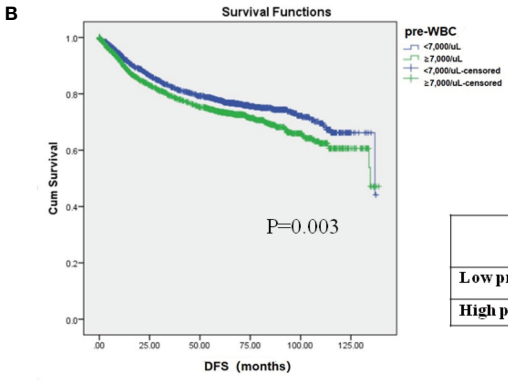
## Discussion

Even after the use of propensity score matching, our findings suggested that the high preoperative WBC count group exhibited worse outcomes than the low preoperative WBC count group. We confirmed that high preoperative WBC count is an independent prognostic indicator for predicting OS and DFS in CRC patients. Additionally, the analysis showed that age (≥65 years), preoperative neoadjuvant chemotherapy, tumor type (signet-ring cell carcinoma), vascular tumor thrombus, nerve invasion, positive surgical margin, stage of TNM (III and IV), infiltrating lymph nodes > 12, number of cancer nodules ≥ 1, and intraoperative blood transfusion were also independent risk factors for worse survival outcomes. A high preoperative WBC count was associated with an immunosuppressive environment in CRC, with higher infiltration




| | Median survival time (months) |
|---|---|
| Low pre-WBC | 136.993 |
| High pre-WBC | 134.667 |

| | Follow up months | 0 | 20 | 40 | 60 | 80 | 100 | 136 | 140 |
|---|---|---|---|---|---|---|---|---|---|
| Low pre-WBC | Number at risk | 1553 | 1351 | 1145 | 917 | 384 | 181 | 2 | 0 |
| | Survival rate | | 94.2% | 85.8% | 79.9% | 75.2% | 70.9% | 42.3% | |
| High pre-WBC | Number at risk | 1553 | 1278 | 1094 | 841 | 363 | 175 | 8 | 0 |
| | Survival rate | | 89.9% | 82.2% | 75.7% | 70.8% | 65.6% | 47.5% | |




| | Median survival time (months) |
|---|---|
| Low pre-WBC | 136.993 |
| High pre-WBC | 134.667 |

| | Follow up months | 0 | 20 | 40 | 60 | 80 | 100 | 134 | 140 |
|---|---|---|---|---|---|---|---|---|---|
| Low pre-WBC | Number at risk | 1553 | 1223 | 1014 | 807 | 385 | 159 | 2 | 0 |
| | Survival rate | | 88.7% | 81.2% | 77.5% | 75.0% | 71.8% | 44.1% | |
| High pre-WBC | Number at risk | 1553 | 1159 | 974 | 753 | 313 | 151 | 7 | 0 |
| | Survival rate | | 84.9% | 77.9% | 73.5% | 70.4% | 65.5% | 47.1% | |

FIGURE 3
**(A)** Kaplan–Meier survival curve for overall survival (OS) according to preoperative WBC (pre-WBC) in the propensity score-matched cohort. The OS rates, median survival time, and number at risk are shown. **(B)** Kaplan–Meier survival curve for disease-free survival (DFS) according to preoperative WBC (pre-WBC) in the propensity score-matched cohort. The DFS rates, median survival time, and number at risk are shown. Significance with $P < 0.05$.

of Treg cells, neutrophils, and macrophages and increased levels of PD-1 and PD-L1, but less infiltration of $CD8^+$ T cells. Overall, this study demonstrated that a high WBC count was independently linked with worse outcomes and an immunosuppressive environment in CRC.

Research has shown that up to 50% of cancers may be associated with inflammation, which is involved in the initiation, promotion, malignant progression, invasion, and metastasis of cancer (22). Our study indicated that leukocytosis before surgery was present in 22.6% of CRC patients and was strongly associated with worse OS and DFS. Several studies have shown that multiple types of malignant tumors are related to inflammation or infection, such as lung, gastric, and skin cancer (23–25). In addition, the host

TABLE 2 Univariable and multivariable cox regression analysis for overall survival in the propensity score matched cohort.

| Variables | UnivariablesHR(95% CI) | P Value | MultivariablesHR((95% CI) | P Value |
|---|---|---|---|---|
| **Pre-WBC classification** | | | | |
| <7,000/uL | 1 | | 1 | |
| ≥7,000/uL | 1.247 (1.081-1.440) | 0.003 | 1.234 (1.068-1.426) | 0.004 |
| **Sex** | | | | |
| Male | 1 | | | |
| Female | 0.917 (0.787-1.067) | 0.262 | | |
| **Age** | | | | |
| ≤44 | 1 | | 1 | |
| 45-54 | 0.989 (0.742-1.318) | 0.939 | 1,197 (0.896-1.601) | 0.224 |
| 55-64 | 1.040 (0.797-1.357) | 0.773 | 1.237 (0.945-1.620) | 0.122 |
| 65-74 | 1.298 (0.984-1.711) | 0.065 | 1.684 (1.270-2.233) | <0.001 |
| >75 | 2.222 (1.657-2.979) | <0.001 | 3.147 (2.331-4.248) | <0.001 |
| **Preoperative Neoadjuvant chemotherapy** | | | | |
| No | 1 | | 1 | |
| Yes | 1.375 (1.017-1.859) | 0.039 | 1.703 (1.227-2.365) | 0.001 |
| **Surgical approach** | | | | |
| Laparotomy | 1 | | | |
| Laparoscopy | 1.003 (0.756-1.330) | 0.983 | | |
| **Tumor location** | | | | |
| Rectum | 1 | | | |
| Left-side colon | 1.102 (0.919-1.320) | 0.294 | | |
| Right-side colon | 1.068 (0.900-1.267) | 0.45 | | |
| Entire colon | 0.000 (0-8.452E+60) | 0.916 | | |
| Transverse colon (cant tell right or left) | 1.116 (0.554-2.248) | 0.76 | | |
| **Tumor histology** | | | | |
| Adenocarcinoma | 1 | | 1 | |
| Mucinous adenocarcinoma | 0.984 (0.792-1.222) | 0.885 | 1.021 (0.808-1.290) | 0.861 |
| Signet-ring cell carcinoma | 3.996 (2.656-6.012) | <0.001 | 1.818 (1.177-2.809) | 0.007 |
| **Vascular tumor thrombus** | | | | |
| Negative | 1 | | 1 | |
| Positive | 2.598 (2.241-3.012) | <0.001 | 1.520 (1.281-1.803) | <0.001 |
| **Nerve invasion** | | | | |
| Negative | 1 | | 1 | |
| Positive | 2.355 (2.013-2.754) | <0.001 | 1.601 (1.349-1.900) | <0.001 |
| **TNM stage** | | | | |
| I | 1 | | 1 | |
| II | 1.515 (1.115-2.059) | 0.008 | 1.337 (0.980-1.824) | 0.067 |
| III | 3.190 (2.395-4.248) | <0.001 | 2.066 (1.522-2.806) | <0.001 |
| IV | 12.179 (8.778-16.898) | <0.001 | 7.408 (5.205-10.545) | <0.001 |
| Unknown | 1.009 (0.365-2.788) | 0.986 | 0.926 (0.320-2.683) | 0.887 |
| **Surgical margin positivity** | | | | |
| No | 1 | | 1 | |
| Yes | 4.591 (3.214-6.558) | <0.001 | 2.599 (1.798-3.756) | <0.001 |
| **Infiltrating lymph nodes > 12** | | | | |
| No | 1 | | | |
| Yes | 0.758 (0.639-0.900) | 0.002 | 0.828 (0.691-0.994) | 0.043 |
| **Number of cancer nodule≥1** | | | | |
| No | 1 | | | |

*(Continued)*

TABLE 2 Continued

| Variables | UnivariablesHR(95% CI) | P Value | MultivariablesHR((95% CI) | P Value |
|---|---|---|---|---|
| Yes | 2.681 (2.276-3.159) | <0.001 | 1.421 (1.183-1.706) | <0.001 |
| **Blood Transfusion** | | | | |
| No | 1 | | 1 | |
| Yes | 1.759 (1.240-2.493) | 0.002 | 1.596 (1.120-2.276) | 0.01 |
| **Blood loss** | | | | |
| <400ml | 1 | | | |
| ≥400ml | 1.304 (0.619-2.745) | 0.485 | | |
| **Surgery again within 30 days** | | | | |
| No | 1 | | | |
| Yes | 0.804 (0.464-1.392) | 0.435 | | \ |

TABLE 3 Univariable and multivariable cox regression analysis for disease-free survival in the propensity score matched cohort.

| Variables | UnivariablesHR(95% CI) | P Value | Multivariable HR((95% CI) | P Value |
|---|---|---|---|---|
| **Pre-WBC classification** | | | | |
| <7,000/uL | 1 | | 1 | |
| ≥7,000/uL | 1.241 (1.075-1.432) | 0.003 | 1.210 (1.047-1.397) | 0.01 |
| **Sex** | | | | |
| Male | 1 | | | |
| Female | 0.906 (0.778-1.055) | 0.205 | | |
| **Age** | | | | |
| ≤44 | 1 | | 1 | |
| 45-54 | 0.998 (0.749-1.329) | 0.987 | 1.255 (0.939-1.679) | 0.125 |
| 55-64 | 1.048 (0.803-1.367) | 0.732 | 1.306 (0.997-1.710) | 0.053 |
| 65-74 | 1.273 (0.965-1.678) | 0.087 | 1.696 (1.278-2.249) | <0.001 |
| 75+ | 2.132 (1.590-2.859) | <0.001 | 2.925 (2.167-3.949) | <0.001 |
| **Preoperative Neoadjuvant chemotherapy** | | | | |
| No | 1 | | 1 | |
| Yes | 1.403 (1.038-1.897) | 0.028 | 1.837 (1.322-2.552) | <0.001 |
| **Surgical approach** | | | | |
| Laparotomy | 1 | | | |
| Laparoscopy | 0.965 (0.727-1.279 | 0.803 | | |
| **Tumor location** | | | | |
| Rectum | 1 | | | |
| Left-side colon | 1.105 (0.922-1.324) | 0.28 | | |
| Right-side colon | 1.052 (0.887-1.247) | 0.563 | | |
| Entire colon | 0.000 (0-1.463E+58) | 0.912 | | |
| Transverse colon (cant tell right or left) | 1.069 (0.531-2.154) | 0.852 | | |
| **Tumor histology** | | | | |
| Adenocarcinoma | 1 | | 1 | |
| Mucinous adenocarcinoma | 0.984 (0.792-1.221) | 0.881 | 1.015 (0.803-1.283) | 0.9 |
| Signet-ring cell carcinima | 3.670 (2.440-5.521) | <0.001 | 1.820 (1.174-2.821) | 0.007 |
| **Vascular tumor thrombus** | | | | |
| Negative | 1 | | 1 | |
| Positive | 2.675 (2.307-3.102) | <0.001 | 1.520 (1.280-1.806) | <0.001 |
| **Nerve invasion** | | | | |
| Negative | 1 | | 1 | |

*(Continued)*

TABLE 3 Continued

| Variables | UnivariablesHR(95% CI) | P Value | Multivariable HR((95% CI) | P Value |
|---|---|---|---|---|
| Positive | 2.405 (2.056-2.813) | <0.001 | 1.622 (1.365-1.927) | <0.001 |
| **TNM stage** | | | | |
| I | 1 | | 1 | |
| II | 1.536 (1.130-2.086) | 0.006 | 1.367 (1.001-1.865) | 0.049 |
| III | 3.334 (2.503-4.441) | <0.001 | 2.146 (1.579-2.917) | <0.001 |
| IV | 12.929 (9.314-17.947) | <0.001 | 7.754 (5.442-11.047 | <0.001 |
| Unknown | 1.005 (0.364-2.778) | 0.992 | 0.938 (0.323-2.719) | 0.906 |
| **Surgical Margin positivity** | | | | |
| No | 1 | | 1 | |
| Yes | 4.398 (3.079-6.282) | <0.001 | 2.177 (1.504-3.149) | <0.001 |
| **Infiltrating Lymph nodes > 12** | | | | |
| No | 1 | | 1 | |
| Yes | 0.726 (0.612-0.862) | <0.001 | 0.809 (0.675-0.969) | 0.022 |
| **Number of cancer nodules ≥1** | | | | |
| No | 1 | | 1 | |
| Yes | 2.754 (2.338-3.245) | <0.001 | 1.426 (1.187-1.715) | <0.01 |
| **Blood Transfusion** | | | | |
| No | 1 | | 1 | |
| Yes | 1.745 (1.231-2.475) | 0.002 | 1.692 (1.186-2.414) | 0.004 |
| **Blood loss** | | | | |
| <400ml | 1 | | | |
| ≥400ml | 1.398 (0.664-2.942) | 0.378 | | |
| **Surgery again within 30 days** | | | | |
| No | 1 | | | |
| Yes | 0.818 (0.472-1.417) | 0.474 | | |

inflammation reaction can inhibit antitumor immune function, thereby leading to poor prognosis of patients (26). At present, the correlation between higher levels of circulating inflammatory markers and prognosis has been revealed in various malignant tumors (27–30). Inflammation is an immune response characterized by a dramatic increase in the number of leukocytes in circulation and infectious tissue (31). Various studies concluded that inflammatory markers such as the neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, and C-reactive protein can all predict the prognosis of CRC (32, 33). Although several groups have reported adverse effects of peripheral leukocytosis on the prognosis of patients with various malignant tumors, this is limited by a retrospective mismatch, uneven treatment options, short follow-up, lack of multivariable analysis, and a small number of patients (5, 15, 19, 34). Our larger sample-matched cohort validated that a higher preoperative WBC count was correlated with poorer OS and DFS in CRC, which was in line with previous research (7, 35). Multivariate analysis validated that a high preoperative WBC count was an independent prognostic marker for malignancy in CRC. Our results showed that the mortality rate of patients with leukocytosis increased by 4.1%. Moreover, preoperative leukocytosis is associated with increased mortality, morbidity, and postoperative complications in CRC surgery (36, 37). Therefore, based on the potential role of leukocytosis in predicting prognosis, it could be used for disease management and follow-up to improve the OS of CRC patients.

Immunomodulatory cytokines and systemic inflammatory markers play a key role in the occurrence and development of cancer. The mechanism underlying the relationship between systemic inflammation and survival outcomes in CRC patients remains unclear. The TME may be one of the main factors involved in its pathogenesis. Many inflammatory cells represent innate and acquired immune responses in the microenvironment of solid malignant tumors (38). Our study used CD8 as a cytotoxic T cell marker, Foxp3 as a Treg cell marker, CD66b as a neutrophil marker, and CD68 as a macrophage marker, which are the key components of the TME. Our study showed that a high preoperative WBC count was associated with an immunosuppressive environment in CRC, with higher infiltration of Treg cells, neutrophils, and macrophages and increased levels of PD-1 and PD-L1, but less infiltration of $CD8^+$ T cells. Several studies have reported that a high baseline WBC count is associated with a low infiltration of $CD8^+$ T cells in tumors, which is consistent with the findings of our study (5, 7).

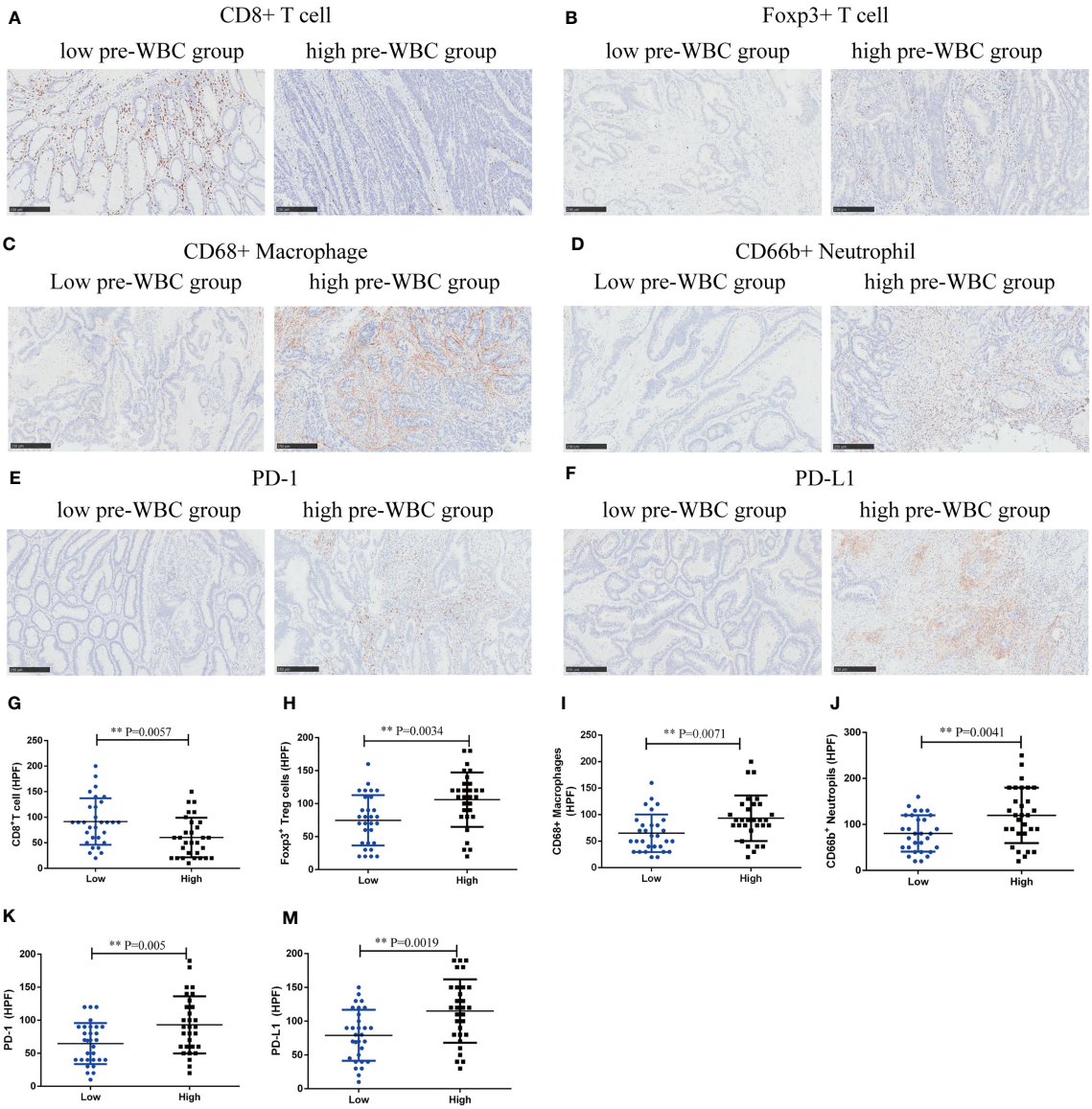



FIGURE 4
High preoperative WBC group was associated with immunosuppressive contexture in CRC. **(A–F)**. Representative immunohistochemical (IHC) staining of $CD8^+$ T cells, $Foxp3^+$ Tregs, $CD68^+$ Macrophages, $CD66b^+$ Neutrophils, and immunosuppressive checkpoints (PD-1, PD-L1) between high and low preoperative WBC groups. **(G–M)** Comparison of $CD8^+$ T cells, $Foxp3^+$ Tregs, $CD68^+$ Macrophages, $CD66b^+$ neutrophils and immunosuppressive checkpoints (PD-1, PD-L1) between two groups. n=30 in each group. Differences were considered significant at $^{**}P < 0.01$, compared to the low preoperative WBC group.

However, to the best of our knowledge, there are no reports on the functional relevance of leukocytosis in the immunosuppressive TME in CRC; thus, our research fills this gap. Simultaneously, we provided a reasonable explanation for the relationship between leukocytosis and poor prognosis.

Several studies have shown that the immune microenvironment in tumors is closely related to clinical outcomes and therapeutic drug resistance (39). Treg cells play a role in the promotion of tumors by inhibiting adaptive antitumor immunity (40). Tumor growth factors, pro-inflammatory cytokines, pro-angiogenic factors, and reactive oxygen species rich in the TME, together with a large number of Treg cells, can lead to cytotoxic T-lymphocyte dysfunction and poor prognosis (41). Tumor-associated macrophages, which tend to be pro-tumor M2 subsets, may induce cancer cell proliferation by secreting growth factors and angiogenesis. They may also promote tumor growth by secreting matrix metalloproteinases (42, 43). Neutrophils are crucial regulators of both inflammation and immune responses,

accounting for 50–70% of leukocytes in the circulation, and are the major elements of WBC (44). Neutrophils can be transformed into a tumor-promoting state in the TME (45). In addition to cytotoxicity, neutrophils can promote the spread of tumor cells by secreting metalloproteinases and elastase to degrade the extracellular matrix. It also regulates immunosuppression by secreting reactive oxygen species and arginase-1, thus limiting T cell-dependent antitumor immunity (46). It is an immunosuppressive TME that leads to poor prognosis in CRC.

Clinically, the TNM staging system is the most used indicator for risk stratification of CRC patients and guides treatment decisions (47). However, TNM staging is often performed postoperatively, and it is difficult to predict survival preoperatively and choose further treatment strategies. Moreover, TNM staging can only reflect the biological behavior of tumors. However, patients tend to have different survival outcomes even at the same TNM stage (48). The prognosis of tumors is not only related to the clinicopathological characteristics of the tumor but is also affected by tumor-host interactions, including inflammatory and immune responses (49). Recently, owing to the repeatable, inexpensive, and convenient features of hematological indices, inflammatory markers established on the basis of blood cell counts have been regarded as potential prognostic indicators for CRC patients (50). These inflammatory indicators are helpful for anesthesiologists and surgeons for comprehensive evaluation before surgery. The first contribution of our study is that we can predict the prognosis of CRC patients using a simple and feasible method before surgery. At present, anesthesiologists and surgeons are paying increasing attention to the short- and long-term prognosis of patients (51–53). Enhanced recovery after surgery also requires anesthesiologists to focus on these factors (54). This suggests that anesthesiologists and surgeons should pay more attention to patients with a higher preoperative inflammation status and take measures to inhibit perioperative stress response and inflammation to improve the prognosis of patients (55–57). This implies good clinical application of these implications. The second contribution of our research is that we are the first to report that leukocytosis is associated with an immunosuppressive microenvironment in CRC, which will provide a better understanding of the relationship between leukocytosis and worse outcomes and help us tailor more precise strategies for CRC patients.

Thus, our research has important implications. The advantage of our clinical research is that our overall sample size (>7000) and allocation (>1500) in each group is much larger than those in previous studies (5, 10, 15, 19, 20), with data from one of the largest cancer centers in China. Another advantage of this study is that we focused on the long-term prognosis of CRC patients. Our median postoperative follow-up period was >5 years (median: 69.6 months), which is much longer than that in previous studies (5, 15, 19). Additionally, we used propensity score matching and multiple Cox regression analyses to correct for confounding factors. Therefore, our study provides high-quality evidence. Previous studies have suggested that leukocytosis is associated with decreased levels of $CD8^+$ T cells in the CRC TME; however, the evaluation of other immunosuppressive cells has not been reported. We found that the preoperative WBC count was correlated with several immunosuppressive cells, shaping an immunosuppressive TME. Our study not only explored a new mechanism behind the clinical significance of preoperative WBC count in CRC malignancy but also stratified patients for personalized treatment. Further research is needed to determine whether leukocytosis could be an immunological biomarker in patients who are sensitive to immunotherapy.

Our study had some limitations. First, the study was retrospective and non-randomized, and patient information was obtained from a cancer center. Second, due to the various perioperative factors associated with leukocytosis, we were unable to eliminate the potential effects of unmeasured confounding factors. Third, although we have determined the effect of leukocytosis on the tumor immunosuppressive microenvironment in the malignant process of CRC, the potential mechanism of these immune cell interactions needs to be further studied. Well-designed prospective and basic studies are helpful to explore the clinical significance and immune environment of preoperative leukocytosis in the long-term prognosis of CRC patients.

In conclusion, preoperative leukocytosis was independently associated with increased overall mortality and cancer recurrence after CRC surgery, and it was associated with an immunosuppressive TME, which might be useful for risk stratification and follow-up scheduling.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

## Ethics statement

This retrospective study was approved by the ethics committee of the Shanghai Cancer Center at Fudan University in Shanghai, China (IRB2105235-6). The patients/participants provided their written informed consent to participate in this study.

## Author contributions

MW, MS, DZ, and CM designed the study; MW, YY, MG, and WZ performed the study; KN and QL analyzed the data; and MW, WZ, and MG wrote the paper. MW and CM revised the paper. All

authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.943423/full#supplementary-material

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# The effect of prehabilitation on the postoperative outcomes of patients undergoing colorectal surgery: A systematic review and meta-analysis

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**Study objective:** Prehabilitation is analogous to marathon training and includes preoperative preparation for exercise, as well as nutrition and psychology. However, evidence-based recommendations to guide prehabilitation before colorectal surgery are limited. We aimed to evaluate the effect of prehabilitation on the postoperative outcomes of patients undergoing colorectal surgery.

**Design:** This study is a systematic review and meta-analysis.

**Methods:** The PubMed, Embase, and Cochrane databases were searched for studies reporting the effect of prehabilitation strategies versus standard care or rehabilitation in patients undergoing colorectal surgery. The primary outcomes were overall postoperative complications and length of hospital stay (LOS), and the secondary outcome was functional capacity (measured using the 6-min walk test [6MWT]) at 4 and 8 weeks after surgery.

**Main results:** Fifteen studies with 1,306 participants were included in this meta-analysis. The results showed no significant reduction in the number of overall postoperative complications (risk ratio = 1.02; 95% confidence interval [CI] = 0.79–1.31; $p$ = 0.878) or LOS (standardized mean difference = 0.04; 95% CI = −0.11 to 0.20; $p$ = 0.589) in patients who underwent colorectal surgery with or without prehabilitation strategy. Additionally, there were no significant differences in the functional capacity estimated using the 6MWT at 4 and 8 weeks postoperatively.

**Conclusions:** Prehabilitation did not significantly affect the number of postoperative complications, LOS, or functional capacity of patients undergoing colorectal surgery. Whether prehabilitation should be recommended deserves further consideration.

**Systematic Review Registration:** https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=290108, identifier CRD42021290108

KEYWORDS

colorectal surgery, complications, functional capacity, meta-analysis, prehabilitation, systematic review

# Introduction

Colorectal cancer (CRC) ranks third in terms of global cancer incidence and is the second leading cause of cancer-related mortality according to Global Cancer Statistics 2020. More than 1.9 million CRC cases were diagnosed and over 900,000 CRC-related deaths occurred in 2020 (1). Surgery is the primary curative treatment for CRC. However, adverse outcomes following colorectal surgery are common and costly despite advances in surgical techniques, perioperative care, enhanced recovery after surgery (ERAS) protocols, and rehabilitation strategies (2).

Prehabilitation was recently proposed to optimize preoperative conditions, thereby improving postoperative outcomes. Unlike ERAS and rehabilitation, which mainly focus on the postoperative period, prehabilitation can help patients enhance their physiological reserves and improve their functional capacity before surgery (3, 4) using interventions focusing on nutrition, exercise, and psychosocial components. Thus, prehabilitation can be thought of as training before a marathon owing to the multidimensional aspects of preoperative preparation, which may enable patients to optimize their surgical eligibility and improve their surgical outcomes (5).

Several previous studies have reported the potential advantages of prehabilitation for various surgical procedures (6, 7). However, the number of meta-analyses on the prehabilitation of patients undergoing colorectal surgery is currently limited (8–10). These studies also reported conflicting results regarding the relationship between prehabilitation and length of hospital stay (LOS). Thus, whether prehabilitation strategies are beneficial and which detailed type of prehabilitation strategies can affect the outcomes of patients undergoing colorectal surgery positively remain unknown. Therefore, generating and evaluating the best evidence for prehabilitation strategies concerning colorectal surgery is imperative.

This systematic review and meta-analysis aimed to determine the effect of prehabilitation on the postoperative outcomes of patients undergoing colorectal surgery. Our findings may support evidence-based medical practices and guide clinicians' decisions.

# Methods

## Study design

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and registered in the International Prospective Register of Systematic Reviews (CRD42021290108) (11).

## Literature search

A systematic literature search of PubMed, Embase, and Cochrane databases for papers published from inception to 25 January 2022, was performed without language limitation. We sought to include studies exploring prehabilitation strategies in patients undergoing colorectal surgery. The search was constructed using the PICO (patient, intervention, comparison, and outcome) framework: patient (adults undergoing colorectal surgery), intervention (prehabilitation strategies), comparator (standard care or rehabilitation only), and outcome (primary: overall complication rates and LOS). The full literature search strategy is presented in Table S1. A database of privately and publicly funded clinical studies conducted worldwide was also sought by screening trial registries (https://www.clinicaltrials.gov/ and https://trialsearch.who.int/). Manual backward searches of references from the primary studies and other relevant systematic reviews were also conducted. After the database search and sourcing of the manuscripts were complete, all original publications were downloaded into a single reference list, and duplicates were removed.

## Study selection criteria

Studies that allocated adult participants (aged ≥18 years) undergoing colorectal surgery to receive prehabilitation strategies versus standard care or rehabilitation were eligible for inclusion in this study. The inclusion criteria were as follows: (1) studies involving patients undergoing colorectal surgery; (2)

prehabilitation intervention included exercise, nutritional optimization, or psychological support alone or in combination as defined by original studies; (3) control groups included standard care, placebo, or postoperative rehabilitation only; and (4) randomized controlled trials (RCTs) and quasi-RCTs, such as those that allocate participants to groups based on the location of residence or date of assessment. The exclusion criteria were as follows: (1) no available full-text article, (2) reviews or protocol manuscript, (3) secondary analysis, (4) no defined outcomes, and (5) duplicate records.

## Data extraction

The data extraction form was piloted by all reviewers and revised by consensus. Two authors (XZ and SW) independently and parallelly screened all titles and abstracts. Articles were considered for full-text review if they met the study inclusion criteria or could not be excluded based on the abstract alone. Discrepancies were addressed by a discussion with a third reviewer (LB) to reach a consensus.

The data extraction form gathered the following information: author's name, country, publication year, type of study design, study aim and design, participants' data, details of prehabilitation intervention and comparison groups, overall complications, LOS, and 6-minute walk test (6MWT) at 4 and 8 weeks.

## Assessment of methodological quality and risk of bias

Two authors independently assessed the quality of the included articles using the Cochrane Collaboration tool for risk of bias assessment. Each study was rated as unclear, low risk, or high risk for random sequence generation, allocation concealment, blinding, attrition, and selective outcome reporting. In cases of disagreement, a consensus was reached through discussion.

Publication bias was visually assessed using funnel plots and quantitatively calculated using the Egger's, Begg's, and Harbord's tests (12). The certainty of the evidence for outcomes was examined using the grading of recommendations assessment, development, and evaluation approach (13).

## Primary and secondary outcomes

The primary outcomes were overall postoperative complications and LOS. Postoperative complications (e.g., pneumonia, urinary tract infection, and hemorrhage) following colorectal surgery and postoperative LOS, which was calculated from the date of surgery until hospital discharge, were assessed.

The secondary outcome was functional capacity assessed using 6MWT performed 4 and 8 weeks postoperatively. Patients were instructed to walk back and forth at a certain length of the hallway for 6 min at a pace that would tire them by the end of the walk. The distance in meters reflects the physical function of the patients (14).

## Statistical analysis and data management

Outcome data were pooled using the Mantel–Haenszel method based on a random- or fixed-effects model when available from at least two trials. Heterogeneity between studies was quantified using the $I^2$ statistic. Random-effects models were prioritized if $I^2 > 40\%$ or $p < 0.10$ for significant heterogeneity. Statistical significance was set at two-sided $p < 0.05$.

Forest and funnel plots were generated using Stata 13.1 (StataCorp LLC, College Station, Texas, United States) and RevMan 5.3 software (The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark). The principal summary measures were risk ratios and standard mean differences (SMDs) with 95% confidence intervals (CIs) for dichotomous and continuous variables, respectively. Where means and standard deviations could not be extracted from the included trials, they were estimated from medians and interquartile ranges using methods described by Wan and others (15). Funnel plots were constructed to detect publication biases. There was no significant publication bias if the two sides were symmetrical; otherwise, a publication bias was possible.

For the primary outcomes, sensitivity analyses were performed using Stata 13.1 with a "leave-one-out" approach, in which all studies were iteratively removed one at a time to analyze their influence on both pooled estimates and heterogeneity. For the overall complication rate, the source of heterogeneity was further explored with a meta-regression, and the possible covariants (year of publication, age, type of control, or geographical location) were tested. Subgroup analyses were also conducted based on the exact type of control and intervention strategies to identify potential influencing factors.

Trial sequential analysis (TSA) was performed for both dichotomous and continuous primary outcomes to reduce the possible risks of random errors owing to insufficient sample size and repeated significance testing of pooled data. TSA software version 0.9.5.10 beta (Copenhagen Trial Unit, Centre for Clinical Intervention Research, Copenhagen, Denmark) was used to perform the analysis and estimate the required information size (RIS) for this meta-analysis. Monitoring boundaries were used to determine whether the $p$-values in the meta-analyses sufficiently demonstrated the anticipated effect.

# Results

## Study selection

The literature search identified 653 non-duplicate citations (Figure 1), of which 573 were excluded after the abstract screening. Thus, 80 full-text articles were retrieved and assessed for eligibility, of which 65 were excluded because of the ineligible study population ($n$ = 9), no utilization of prehabilitation strategy ($n$ = 5), ineligible comparator ($n$ = 1), lack of outcome assessment ($n$ = 3), incorrect study design ($n$ = 13), or unavailability of the full text ($n$ = 34). In total, 15 trials were included in the final quantitative analysis (16–30).

## Study characteristics

The characteristics of the studies included in the meta-analysis are presented in Table 1. The 15 trials (16–30) included 1,306 participants, of whom 685 underwent prehabilitation strategies and 621 received standard care or rehabilitation only. The average age of patients in both groups was 70 years. Eight, three, and four studies included patients undergoing multimodal prehabilitation (17, 19–21, 23, 26, 27, 30), exercise (16, 25, 29), and nutrition optimization, respectively (18, 22, 24, 28).

## Risk of bias in the included studies

The risk of bias is summarized in the Supplementary Material (Figure S1). One of the 15 included studies was a prospective study (20), which was not included in the subsequent assessment. Of the remaining 14 trials, 1 was open-labeled (22), 12 (16–19, 21–25, 27–29) used appropriate random sequence generation, and 10 (16–19, 21–25, 27, 29) used allocation concealment. Only two trials used double-blinded methods (16, 24), five trials were single-blinded (17–19, 23, 25), and one trial was unblinded (28). Others were open-label or failed to state blinding methods. Seven of the RCTs reported using blinded assessors for outcome indicators. No reporting bias was observed in this study. As the studies in abstract form and meeting reports were not eligible in this meta-analysis, no other bias was considered. Overall, 10 studies (16–19, 21, 23–25, 27, 29) were deemed high quality, whereas 4 (22, 26, 28, 30) were graded as having a high risk of bias.

## Effect of prehabilitation on overall complications

We examined the effects of prehabilitation on postoperative complications. The risk ratio in overall complications was 1.02

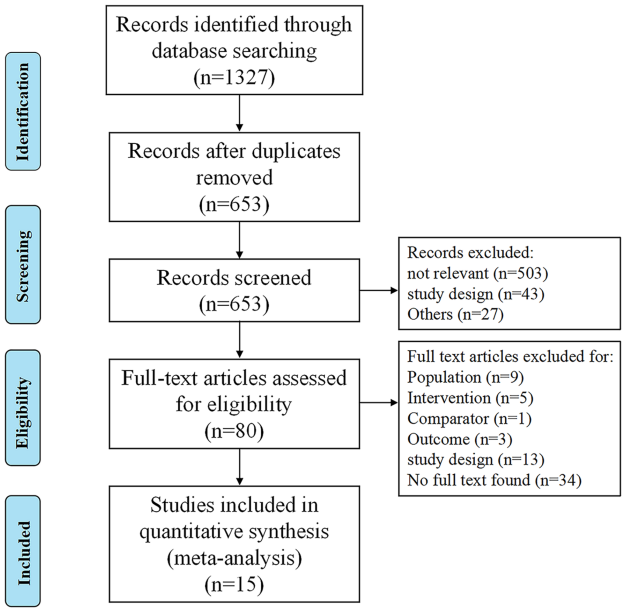


FIGURE 1
PRISMA flow diagram of the selection and inclusion process.

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TABLE 1 Characteristics of included studies comparing prehabilitation versus standard care or rehabilitation among patients undergoing colorectal surgery.

| Study | Year | Country | Study design | Prehabilitation strategies | Control type | Numbers of participants | | Age | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Intervention | Control | Intervention | Control |
| Berkel (16) | 2021 | The Netherlands | RCT | Exercise | Usual care | 28 | 29 | 74 | 73 |
| Bousquet-Diona (17) | 2018 | Canada | RCT | Multimodal | Rehabilitation | 37 | 26 | 74 | 71 |
| Burden (18) | 2011 | UK | RCT | Nutrition | Usual care | 54 | 62 | 64.5 | 65.3 |
| Carli (19) | 2020 | Canada | RCT | Multimodal | Rehabilitation | 55 | 55 | 78 | 82 |
| Chia (20) | 2015 | Singapore | Prospective study | Multimodal | Usual care | 57 | 60 | 79 | 80.5 |
| Fulop (21) | 2020 | Hungary | RCT | Multimodal | Usual care | 77 | 72 | 70 | 70 |
| Gilbert (22) | 2021 | France | Stepped wedge trial | Nutrition | Usual care | 74 | 73 | 80.5 | 79.2 |
| Gillis (23) | 2014 | Canada | RCT | Multimodal | Rehabilitation | 38 | 39 | 65.7 | 66 |
| Gillis (24) | 2015 | Canada | RCT | Nutrition | Usual care | 22 | 21 | 67.6 | 69.1 |
| Hernon (25) | 2021 | UK | RCT | Exercise | Usual care | 137 | 63 | 67.1 | 69.1 |
| Li (26) | 2012 | Canada | RCT | Multimodal | Usual care | 42 | 45 | 67.4 | 66.4 |
| López-Rodríguez-Arias (27) | 2021 | Spain | RCT | Multimodal | Usual care | 10 | 10 | 66.5 | 66 |
| MacFie (28) | 2000 | UK | RCT | Nutrition | Usual care | 24 | 25 | 68 | 64 |
| Northgraves (29) | 2019 | UK | RCT | Exercise | Usual care | 10 | 11 | 64.1 | 63.5 |
| van Rooijen (30) | 2019 | The Netherlands | RCT | Multimodal | Usual care | 20 | 30 | 75 | 71 |

(95% CI = 0.79–1.31; $p = 0.878$; Figure 2), indicating no significant reduction in the risk of clinically important postoperative complications following prehabilitation. There was a moderate level of heterogeneity ($I^2 = 46.7\%$; $p = 0.028$). We then performed a meta-regression to explore the potential sources of heterogeneity (Table S2). The results indicated that year of publication ($p = 0.718$), age ($p = 0.829$), type of control ($p = 0.877$), and geographical location ($p = 0.255$) did not significantly influence the results of meta-analysis regarding the overall complications. Furthermore, the detailed type of prehabilitation strategies was assessed by subgroup analysis for exercise, nutrition, or trimodal prehabilitation (Figure S2). Subgroup analysis results demonstrated that the risk ratios for postoperative complications in studies concerning exercise, nutrition, and trimodal prehabilitation were 1.22 (95% CI = 0.22–6.86), 1.47 (95% CI = 0.81–2.66), and 1.02 (95% CI = 0.79–1.31), respectively. No significant differences were found between subgroups.

As shown in the TSA (Figure 3), the RIS was calculated as 1,975 patients for overall complications, whereas the *z*-curve crossed the adjusted TSA boundary favoring the intervention and control groups, indicating no need for further trials to validate the conclusions.

## Effect of prehabilitation on LOS

Nine studies investigated the LOS, and the pooled results showed no significant reduction (SMD = 0.04; 95% CI = −0.11 to 0.20; $p = 0.589$; Figure 4). Heterogeneity ($I^2 < 0.001\%$; $p = 0.439$) among the studies reporting this outcome was low. TSA revealed that the *z*-curve did not cross traditional boundaries. However, the boundary RIS was not available because of insufficient information use (3.65%). A detailed graph is shown in Figure S3.

## Effect of prehabilitation on functional capacity

Four studies examined the effect of prehabilitation on functional capacity as measured by the 6MWT. There was no significant difference in functional capacity at 4 weeks (SMD = 0.16; 95% CI = −0.06 to 0.38; $p = 0.144$; Figure S4) or 8 weeks postoperatively (SMD = 0.18; 95% CI = −0.21 to 0.56; $p = 0.367$; Figure S5).

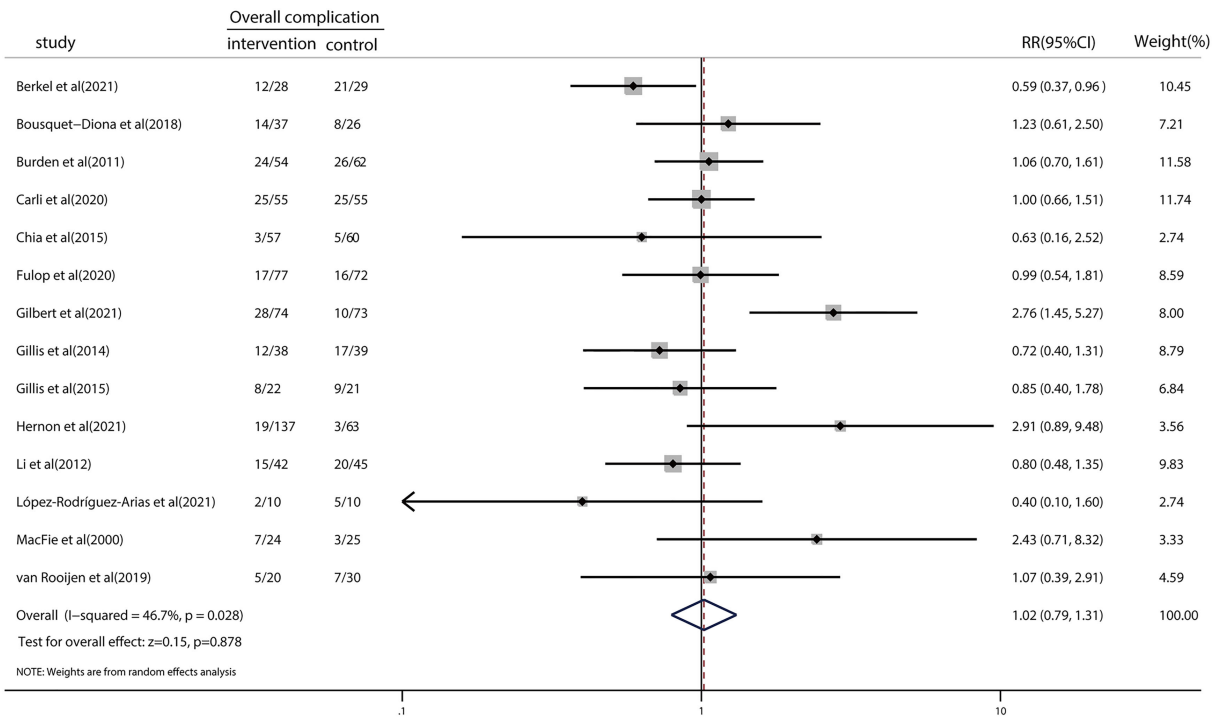


FIGURE 2
Forest plot for overall complications after colorectal surgery with or without preoperative prehabilitation strategies.

## Publication bias and sensitivity analysis

We performed Harbord's test to assess the publication bias of dichotomous data for the primary outcome. Egger's and Begg's tests were conducted to evaluate the publication bias of continuous data for the primary outcome. The result of Harbord's test was 0.291 for the overall complications. Regarding LOS, the results of Egger's test (0.375) and Begg's test (0.754) further revealed no publication bias. Visual inspection of the funnel plots did not raise concerns about publication bias (Figures S6, S7). The effect estimation of sensitivity analysis showed that the results were stable, regardless of pooled complications or pooled LOS (Figures S8, S9).

## Certainty of evidence

The certainty of evidence assessment of the primary outcomes is summarized in Table 2. The evidence was rated as moderate for overall complications, LOS, and 6MWT at 8 weeks postoperatively and high for 6MWT at 4 weeks postoperatively. The outcomes for overall complications and LOS were downgraded to one level for risk of bias. The 6MWT at 8 weeks postoperatively was also downgraded to one level owing to concerns regarding the risk of inconsistency.

# Discussion

This meta-analysis, which included 15 trials and 1,306 patients, compared prehabilitation intervention with standard care and rehabilitation in patients undergoing colorectal surgery. The main findings showed no significant differences in postoperative complications, LOS, and 6MWT. To analyze the postoperative complications, a meta-regression was performed based on the possible moderators (year of publication, age, type of control, and geographical location), with no statistic heterogeneity reported. The subgroup analysis of the intervention strategies used, including exercise, nutrition, and multimodal prehabilitation, was also conducted. Similarly, no significant differences were observed among the subgroups.

Poor prognosis after major surgery has been emphasized increasingly by clinicians. Major surgery is thus often compared to a marathon, i.e., only well-prepared patients can endure it (31, 32). Patients who undergo colorectal surgery are generally older and have multiple morbidities. They may also have a high risk of frailty,

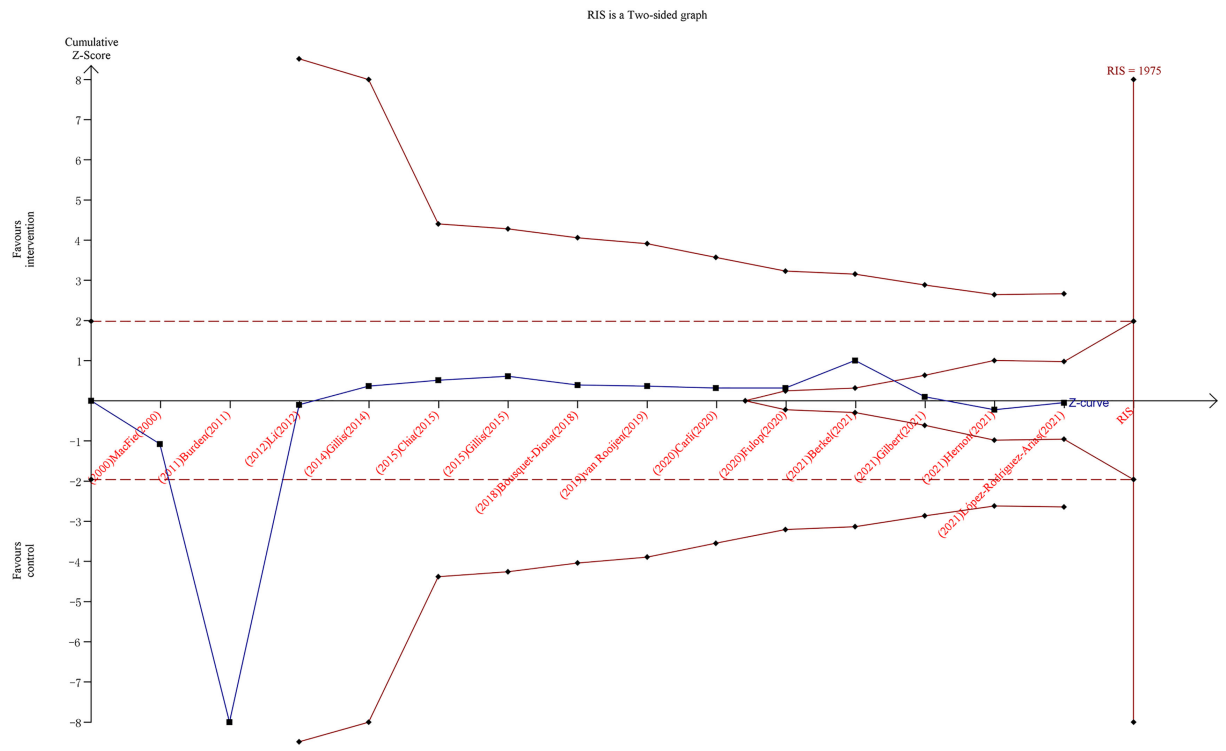


FIGURE 3
Trial sequential analysis for overall complications after colorectal surgery with or without preoperative prehabilitation strategies. The blue z-curve was drawn by applying a random-effects model.

with a decreased physiological reserve and anti-stress ability, which could trigger adverse outcomes and lower postoperative quality of life (33–35). Therefore, preoperative improvement is crucial for these patients. However, the current preoperative workup mainly focuses on identifying the risk factors, and less attention is paid to improving preoperative reserves (6).

Prehabilitation is an emerging strategy that aims to optimize patients for surgical procedures (36, 37). However, limited solid evidence proved the effects of prehabilitation in patients undergoing colorectal surgery, and detailed optimization strategies remain challenging. Previous systematic reviews had controversial conclusions regarding the effect of prehabilitation on patients undergoing colorectal surgery. In 2016, Bruns and colleagues reported that prehabilitation can improve the physical condition of patients for colorectal surgery, although no significant reduction in complications or LOS was observed (8). Moran et al. reported that prehabilitation appears to be beneficial in decreasing the incidence of postoperative complications after the intra-abdominal operation, only four of nine enrolled studies included patients undergoing colorectal surgery. The authors mainly focused on exercise programs and the methodologic quality of included studies was relatively low (38). In 2018, a meta-analysis by Gillis et al. documented that nutritional prehabilitation with or without exercise significantly reduced LOS by 2 days in patients undergoing colorectal surgery (9). Their results on LOS are inconsistent with ours. However, we included six studies published after 2018, thus making our current analysis much more comprehensive. In 2020, Lambert and others performed a meta-analysis on prehabilitation of patients undergoing hepatobiliary, colorectal, and upper gastrointestinal cancer surgeries (10). Their results demonstrated that prehabilitation was associated with a shorter LOS but had no effect on functional capacity, postoperative complications, or mortality. A recent Cochrane review, including three RCTs and a total of 250 patients, indicated that prehabilitation may improve functional capacity postoperatively and result in fewer complications, while no difference was reported regarding LOS (39). Our findings are partly in line with those of Lambert and others; however, our study provides a more comprehensive analysis of colorectal surgery as it included 1,306 patients from 14 RCTs and one prospective study. The certainty of evidence generated from our meta-analysis was also rated as moderate for the primary and secondary outcomes. In fact, the reasons for the lack of significant differences in postoperative complications, LOS, and 6MWT results are complex and multifactorial. It should be noted that most of the included studies were conducted after 2011 when the ERAS protocol was implemented. Studies have shown that ERAS alone significantly improved the short-term surgical outcomes of patients undergoing colorectal surgery (40); thus, the effect of prehabilitation

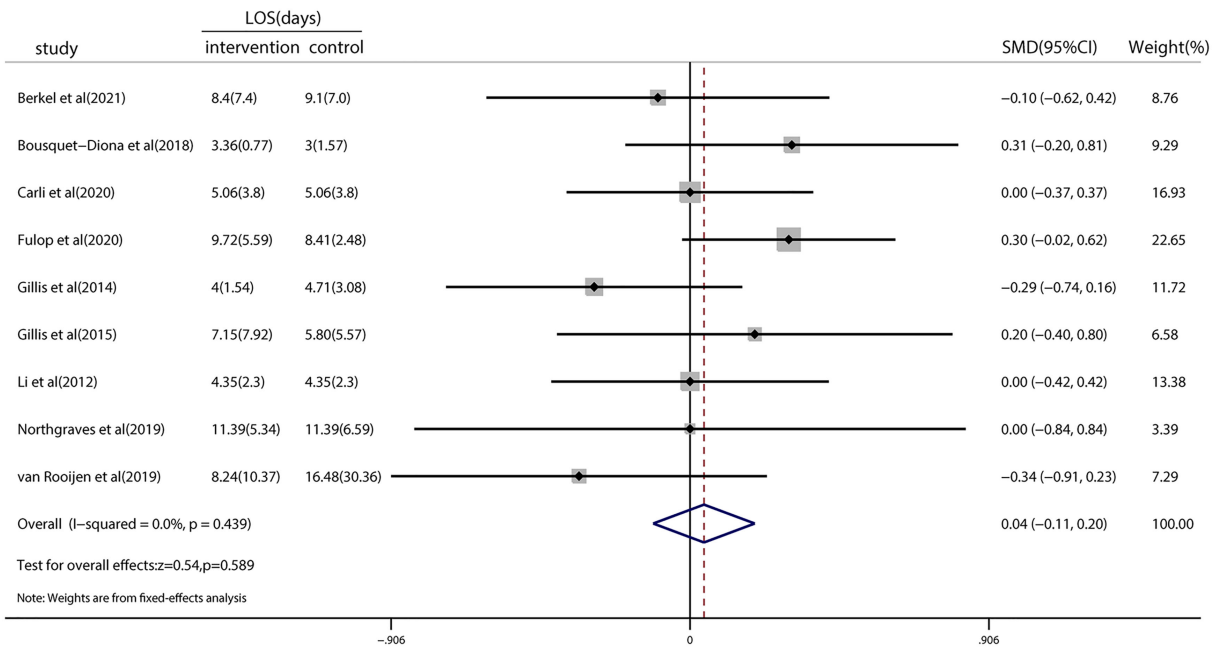


FIGURE 4
Forest plot for the length of hospital stay after colorectal surgery with or without preoperative prehabilitation strategies.

might be underestimated if assessed within an ERAS population. In our study, no prehabilitation strategy was found in the control group of the included trials. Thus, the reason why prehabilitation did not significantly affect the outcomes of patients undergoing colorectal surgery in our meta-analysis may not be attributed to any optimization in the control group.

In our study, we used TSA to further evaluate the endpoints of overall complications. Type I and II errors were set at 5% and 20%, respectively. The incidence of controls was 40% based on our enrolled data, and a 20% relative risk reduction was assigned to calculate the required information size. Following these settings, the optimal number of samples was 1,975, and 1,306 samples were included in this meta-analysis. The cumulative *z*-curve crossed the adjusted TSA boundary, favoring the intervention and control groups. This finding demonstrates that further trials to confirm this negative result are unnecessary. Thus, based on the current

TABLE 2 Summary of findings.

| Outcomes | Participants (studies) | Risk of bias | Inconsistency | Indirectness | Imprecision | Publication bias | RR/ SMD 95% CI | Overall quality of evidence (GRADE) |
|---|---|---|---|---|---|---|---|---|
| Overall complications | 1,285 (13 RCTs and one prospective study) | Serious[1] | No serious inconsistency | No serious indirectness | No serious imprecision | No serious publication bias | RR 1.02 95% CI (0.79, 1.31) | ⊕⊕⊕x̂ MODERATE[1] |
| LOS | 600 (9 RCTs) | Serious[1] | No serious inconsistency | No serious indirectness | No serious imprecision | No serious publication bias | SMD 0.04 95% CI (−0.11, 0.2) | ⊕⊕⊕x̂ MODERATE[1] |
| 6MWT at 4 weeks after surgery | 322 (3 RCTs) | No serious risk of bias | No serious inconsistency | No serious indirectness | No serious imprecision | No serious publication bias | SMD 0.16 95% CI (−0.06, 0.38) | ⊕⊕⊕⊕ HIGH |
| 6MWT at 8 weeks after surgery | 289 (3 RCTs) | No serious risk of bias | Serious[2] | No serious indirectness | No serious imprecision | No serious publication bias | SMD 0.18 95% CI (−0.21, 0.56) | ⊕⊕⊕x̂ MODERATE[2] |

[1]Risk of bias existed in two to three trials.
[2]$I^2$ > 50%, indicating that the inconsistency existed.

evidence, we can assume that prehabilitation has no advantages in terms of overall complications. We also searched the aforementioned database of clinical studies conducted worldwide, and we observed that many clinical trials on this theme are ongoing or complete. For example, over a dozen studies are registered at clinicaltrials.gov and at the stage of participant recruitment, to explore the effects of prehabilitation on patients undergoing colorectal surgery with various prehabilitation strategies. Unfortunately, no results are available currently, and whether these trials may change the conclusion of our current meta-analysis remains unknown.

This systematic review benefits from robust methods in keeping with the established guidelines (41), including a registered protocol. Three previous meta-analyses have shown that prehabilitation might be a promising intervention to improve certain adverse outcomes after surgery (e.g., lung resections, major abdominal surgery, and cardiac surgery) (42–44). Our study mainly focused on colorectal surgery and no significant benefits were observed. The results may only target patients undergoing colorectal surgery, and may not be applicable to other kinds of operations. Besides, our study had some limitations. First, we included one prospective study, which was bound to increase heterogeneity. Second, owing to insufficient information on mortality and confounders (e.g., age, tumor stage, radiotherapy, and chemotherapy) that may influence mortality, the effect of prehabilitation on postoperative mortality was not examined. Third, few studies on exercise and psychological prehabilitation have been conducted, making it difficult to fully analyze their effects. Fourth, the sample sizes of the included studies were small, reducing the confidence in the reported outcomes. Based on these limitations, more optimal and high-quality research is required in the near future. A recent umbrella review of 55 systematic reviews demonstrated that prehabilitation may yet improve postoperative outcomes with low certainty (45). The authors conducted the analysis with populations undergoing various surgical procedures, with cancer surgeries (22 of 55) being the most common focus of included reviews. However, including overlapping trials into the umbrella review can cause double counting of evidence, contributing a certain degree of limitation. Their work also highlights the optimization of trial execution to increase the certainty of the effectiveness of prehabilitation.

## Conclusions

In conclusion, this study demonstrated that prehabilitation of patients undergoing colorectal surgery does not significantly affect postoperative complications, LOS, and 6MWT. Thus, prehabilitation strategies may not be beneficial in colorectal surgery, and there is limited direct evidence supporting the recommendation of prehabilitation for patients undergoing colorectal surgery. Whether it is necessary to continue this program deserves further consideration. High-quality clinical trials for patients with a higher risk of postoperative complications are warranted, and targeted and intensive individualized prehabilitation plans are required to guide the best clinical practice.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

## Author contributions

LB, ZJ, and XZ designed the study. XZ, SW, WJ, HW, and KZ made contributions to the conduct of the study. XZ, SW, WJ, and ZJ made the data analysis. XZ and LB were the major contributors to writing the first draft of the manuscript. All authors listed contributed to reviewing and approving the final version of the manuscript.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher’s note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.958261/full#supplementary-material

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# Anemia tolerance versus blood transfusion on long-term outcomes after colorectal cancer surgery: A retrospective propensity-score-matched analysis

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**Background:** Perioperative anemia and transfusion are intertwined with each other, and both have adverse impacts on the survival of colorectal cancer (CRC) patients. But the treatment of anemia still relies on transfusion in several countries, which leads us to question the effects of anemia tolerance and transfusion on the long-term outcomes of CRC patients. We investigated the combined effect of preoperative anemia and postoperative anemia and of preoperative anemia and blood transfusion, which imposes a greater risk to survival, to compare the effects of anemia tolerance and transfusion on overall survival (OS) and disease-free survival (DFS) in patients undergoing CRC surgery.

**Methods:** A retrospective propensity-score-matched analysis included patients with CRC undergoing elective surgery between January 1, 2008, and December 31, 2014. After propensity-score matching, Kaplan–Meier survival analysis and univariable and multivariable Cox proportional hazards models were used to study the prognostic factors for survivals. In univariate and multivariate Cox regression analysis, two novel models were built.

**Results:** Of the 8,121 patients with CRC, 1,975 (24.3%) and 6,146 (75.7%) patients presented with and without preoperative anemia, respectively. After matching, 1,690 patients remained in each group. In the preoperative anemia and postoperative anemia model, preoperative anemia and postoperative anemia was independent risk factor for OS (HR, 1.202; 95% CI, 1.043–1.385;

P=0.011) and DFS (HR, 1.210; 95% CI, 1.050–1.395; P=0.008). In the preoperative anemia and transfusion model, preoperative anemia and transfused was the most dangerous independent prognostic factor for OS (HR, 1.791; 95% CI, 1.339–2.397; P<0.001) and DFS (HR, 1.857; 95% CI, 1.389–2.483; P<0.001). In patients with preoperative anemia, the OS and DFS of patients with transfusion were worse than those of patients without transfusion (P=0.026 in OS; P=0.037 in DFS).

**Conclusions:** Preoperative anemia and blood transfusion imposed a greater risk to OS and DFS in patients undergoing CRC surgery, indicating that the harm associated with blood transfusion was greater than that associated with postoperative anemia. These findings should encourage clinicians to be vigilant for the timely prevention and treatment of anemia, by appropriately promoting toleration of anemia and restricting the use of blood transfusion in patients with CRC.

KEYWORDS

**preoperative anemia and postoperative anemia, preoperative anemia and transfusion, long-term outcomes, colorectal cancer, propensity-score-matched analysis, anemia tolerance and blood transfusion**

## Introduction

Among all the types of cancers, colorectal cancer (CRC) has the third and second highest morbidity and mortality rates worldwide, respectively (1, 2). Although CRC is the fifth leading cause of cancer death among men and women in China, the death rate from CRC has been on the rise during the past few decades (3, 4). Currently, the most common treatment for CRC is radical resection; although progress has been made in diagnosis and treatment strategies, approximately half of the patients relapse within 3 years post-operation (5). Therefore, there is an urgent need to find prognostic factors capable of predicting patient prognosis in CRC, especially if it is possible to act on them and modify them accordingly.

A considerable number of patients with colon or rectal cancer suffer from anemia (38%–50% and 18%–50%, respectively) (6, 7). The possibility that anemia can affect the prognosis of cancer has aroused a widespread concern. Preoperative anemia in patients with cancer is usually the result of blood loss caused by advanced cancer progression or myelosuppression (8). Accumulating evidence has revealed that preoperative anemia is associated with worse outcomes in patients undergoing CRC surgery (6, 9–11). Surgical resection of tumors aggravates anemia (postoperative anemia), which is markedly common but is typically neglected after surgery (12–15). As pre- and postoperative anemia may be used as prognostic factors in patients with CRC, it is reasonable to further investigate which of the two is most influential, and whether their combined relationship could be informative for improving the prediction of patients' survival. However, this association has not been confirmed in a clinical study. Perioperative anemia and transfusion are always related; although anemia can be traditionally treated with transfusion, it is not a desirable treatment option. Indeed, transfusion may cause more harm than benefits to patients (14–16), which leads us to question the effects of anemia tolerance and transfusion on the long-term outcomes of cancer patients.

Currently, anesthesiologists and surgeons are paying increasing attention to both short- and long-term prognoses of cancer patients (17, 18). Enhanced recovery after surgery also focuses on perioperative anemia and its associated morbidity and mortality (19, 20). Therefore, we conducted this retrospective study to investigate the combined effect of preoperative and postoperative anemia, and preoperative anemia and blood transfusion, to determine which of these factors impose a greater risk to overall survival (OS) and disease-free survival (DFS) in patients undergoing colorectal surgery and to investigate the effects of anemia tolerance and transfusion on the long-term outcomes of CRC patients. Though two other studies investigated the combined effect of preoperative anemia and blood transfusion on complications and 30-day death rate in patients undergoing colectomy (21, 22), our study further evaluated the combined effect of preoperative anemia and blood transfusion on the long-term outcomes (longer median follow-up period) after CRC surgery. To the

best of our knowledge, the association between anemia tolerance and transfusion on the long-term outcomes of CRC patients has not been reported. First, we built two novel models to evaluate which of the two combined factors imposed a greater risk to OS and DFS in patients undergoing CRC surgery. Second, we aimed to guide physicians on treatment implementation and modification for anemia in this subset of patients.

# Materials and methods

## Study design

This retrospective study was performed at Shanghai Cancer Center, Fudan University, Shanghai, China and was approved by the appropriate ethics committee (IRB2105235-6). Informed consent was obtained from all subjects involved in the study. This study was conducted according to the Declaration of Helsinki and was consistent with the STROBE criteria.

## Study population and data sources

Among individuals (n = 13,721) who underwent CRC surgery at Shanghai Cancer Center from January 1, 2008, to December 31, 2014, 8121 were enrolled in this study. The inclusion criteria were as follows: histologically confirmed CRC, elective radical surgery for CRC, and older than 20 years of age. The exclusion criteria were as follows: incomplete data in medical records, benign tumor/carcinoma *in situ*, emergency operation and a previous history of cancer (Figure 1). Ultimately, 8,121 patients were included in this study. According to the diagnostic criteria in China (23), anemia is defined as serum hemoglobin (Hb) levels < 120 g/L for men or < 110 g/L for women, which is different from the criteria indicated by the World Health Organization criteria (24) (Hb < 130 g/L for men or Hb < 120 g/L for women); importantly, this biological reference interval is more suitable for Chinese individuals (25). Patients were divided into either the preoperative anemia group or not preoperative anemia group, according to their Hb levels before surgery.

## Variables and outcomes

The data were retrieved from Shanghai Cancer Center's electronic clinical information system. The patients' baseline characteristics included sex, age, American Society of Anesthesiology (ASA) score, preoperative Hb concentrations, preoperative hematocrit (HCT), preoperative adjuvant chemotherapy, tumor histology, tumor differentiation, vascular tumor thrombus, surgical margin positive, Pathologic Tumor Node Metastasis/Union for International Cancer Control (pTNM/UICC) stage, infiltrating lymph nodes > 12, number of cancer nodules > 1, and clinical conditions. Perioperative outcomes included intraoperative blood transfusion, intraoperative blood loss, postoperative Hb, postoperative anemia, reoperation within 30 days, duration of intensive care unit stay, and death.

The primary outcomes were OS and DFS. OS was defined as the time from the date of first treatment to the date of death due to any reason. DFS was defined as the time from the date of first treatment to the date of recurrence or metastasis or secondary primary tumor or death. The follow-up ended on December 31, 2019, ranging from 5 to 11 years (median: 69.6 months).

## Statistical analysis

SPSS (Version 25; IBM, Armonk, NY, USA) and R software (version 3.4.4, R Foundation for Statistical Computing, Austria) were used to analyze the data. Patients' baseline characteristics were presented as n and percent (%) for categorical variables and mean ± standard deviation (SD) for continuous variables. We compared the association between preoperative anemia and malignant clinicopathological features by *t*-test and Chi-squared test. Spearman analysis was used to assess the correlation between preoperative anemia and postoperative anemia.

We used propensity score matching to reduce any potential confounding factors related to baseline differences between the two groups. The key confounders including sex, age, preoperative adjuvant chemotherapy, tumor histology, pTNM/UICC stage, and lymph node invasion > 12 were matched. The nearest neighbor method was employed, and 0.05 SD was used as the caliper with 1:1 matching. The balanced distribution of matched patients in each group was evaluated by standardized mean difference (SMD). SMD < 0.10 meant a balanced distribution between the two groups. We used the R package "MatchIt" for propensity score matching. After matching, 1,690 patients remained in each group (Table 1).

In the propensity-matched cohort, Kaplan–Meier survival analysis was used to compare OS and DFS by log-rank test. We used the Cox proportional hazards model to study the prognostic factors for OS and DFS. The univariate Cox proportional hazards model was used to analyze all variables. Variables with a P-value < 0.05 were included in the multivariate analysis. Multivariate Cox proportional hazards model using the enter method was conducted to select variables. In univariate and multivariate Cox regression analysis, two new models were built. "Preoperative anemia and postoperative anemia" model included patients who were not anemic either before or after surgery, those who had preoperative anemia but not postoperative anemia, those with postoperative anemia but not preoperative anemia, and those with both pre- and postoperative anemia. "Preoperative anemia and transfusion" model included patients who were not anemic preoperatively and not transfused,

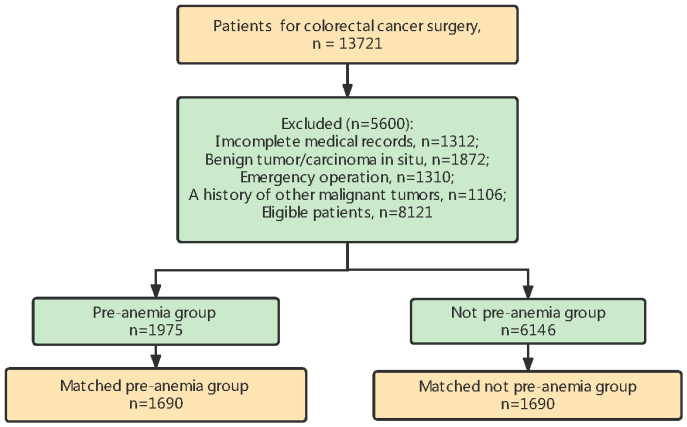



FIGURE 1
Flow chart of patient selection.

those who were not anemic before surgery but transfused, those with preoperative anemia only but not transfused, and those with preoperative anemia who were also transfused. The hazard ratio (HR) was not only compared in each model, but also compared between different models to investigate its prediction in cancer prognosis. Multivariate Cox analysis model 1 was designed to estimate preoperative anemia effect on survival. Multivariate Cox analysis model 2 was designed to estimate postoperative anemia effect on survival. Multivariate Cox analysis model 3 was designed to estimate the interaction of preoperative and postoperative anemia effect on survival. Multivariate Cox analysis model 4 was designed to estimate the interaction of preoperative anemia and transfusion effect on survival.

# Results

## Patient characteristics and outcome

Of the 8,121 patients who met our inclusion criteria, 1,975 (24.3%) patients presented with preoperative anemia and 6,146 (75.7%) did not show preoperative anemia (Figure 1). Patient characteristics are summarized in Table 1. Our median postoperative follow-up period was 69.4 (95% CI [confidence interval]: 68.7–70.0) months for all patients. Because there were significant differences in baseline characteristics that could influence cancer recurrence between the two groups, we used propensity score matching to reduce the imbalance. After matching, 1,690 patients remained in each group. SMD values were less than 0.1 for all characteristics except for surgical approach (Table 1). After matching, no significant differences were found for sex, age, preoperative adjuvant chemotherapy, tumor histology, tumor differentiation, pTNM/UICC stage, and number of infiltrating lymph nodes > 12, which were greatly different between the two groups before matching.

In the propensity-matched cohort, preoperative Hb was markedly higher in the not preoperative anemia group than in the preoperative anemia group (133 ± 12.1 g/L *vs.* 98 ± 14.2 g/L, P<0.001, n=1690 in each group, Table 1 and Figure 2A). A greater percentage of patients in the preoperative anemia group required blood transfusion (8.2% *vs.* 0.7%, P<0.001, Table 2) than that in the not preoperative anemia group. The postoperative Hb was markedly higher in the not preoperative anemia group than in the preoperative anemia group (124 ± 13.3 g/L *vs.* 99 ± 13.1 g/L, P<0.001, n=1690 in each group, Table 2 and Figure 2B). A higher percentage of patients in the preoperative anemia group (90.5% *vs.* 20.7%, P<0.001, Table 2) than that in the not preoperative anemia group exhibited postoperative anemia. Preoperative Hb values correlated positively with postoperative Hb concentrations ($r = 0.843$, $P < 0.001$, Figure 2C). The overall mortality rate was significantly higher in the preoperative anemia group (31.1% *vs.* 26.7%, P = 0.005) during the extended follow-up (+5 years). Summarizing this propensity-matched cohort, preoperative anemia was associated with more blood transfusion, more postoperative anemia, and higher mortality rate after CRC surgery.

## Kaplan–Meier survival and Cox regression proportional hazard survival for OS and DFS between preoperative anemia and non-preoperative anemia patients

In the propensity-matched cohort, patients who were not anemic preoperatively demonstrated better OS than those who were anemic before surgery (median survival time 130.9 months

TABLE 1 Patient baseline characteristics in the total study cohort and the Propensity score matched.

| Variables | Total study cohort | | | Propensity-matched cohort | | | SMD |
|---|---|---|---|---|---|---|---|
| | Pre-anemia (n = 1975) | Not pre-anemia (n = 6146) | P Value | Pre-anemia (n = 1690) | Not pre-anemia (n = 1690) | P Value | |
| **Sex, n(%)** | | | <0.001 | | | 0.890 | 0.005 |
| Female | 919 (46.5) | 2390 (38.9) | | 782 (46.3) | 786 (46.5) | | |
| Male | 1056 (53.5) | 3756 (61.1) | | 908 (53.7) | 904 (53.5) | | |
| **Age, n(%)** | | | <0.001 | | | 0.999 | 0.009 |
| ≤44 | 273 (13.8) | 795 (12.9) | | 225 (13.3) | 224 (13.3) | | |
| 45-54 | 369 (18.7) | 1264 (20.6) | | 317 (18.8) | 319 (18.9) | | |
| 55-64 | 576 (29.2) | 2339 (38.1) | | 480 (28.4) | 480 (28.4) | | |
| 65-74 | 473 (23.9) | 1273 (20.7) | | 419 (24.8) | 414 (24.5) | | |
| ≥75 | 284 (14.4) | 475 (7.7) | | 249 (14.7) | 253 (15.0) | | |
| **ASA score, n(%)** | | | 0.728 | | | 0.717 | 0.003 |
| I | 847 (42.9) | 2674 (43.5) | | 676 (40.0) | 680 (40.2) | | |
| II | 1090 (55.2) | 3368 (54.8) | | 930 (55.0) | 936 (55.4) | | |
| III | 38 (1.9) | 104 (1.7) | | 84 (5.0) | 74 (4.4) | | |
| **Preoperative Hb, (g/L)** | 97 ± 14.4 | 134 ± 12.7 | <0.001 | 98 ± 14.2 | 133 ± 12.1 | <0.001 | 2.628 |
| **Preoperative HCT, (%)** | 31 ± 3.6 | 40 ± 3.4 | <0.001 | 31 ± 3.6 | 40 ± 3.3 | <0.001 | 2.480 |
| **Preoperative adjuvant chemotherapy, n(%)** | | | <0.005 | | | 0.951 | 0.002 |
| Yes | 194 (9.8) | 480 (7.8) | | 143 (8.5) | 144 (8.5) | | |
| No | 1781 (90.2) | 5666 (92.2) | | 1547 (91.5) | 1546 (91.5) | | |
| **Surgical approach, n(%)** | | | 0.008 | | | 0.003 | 0.104 |
| Laparotomy | 1844 (93.4) | 5623 (91.5) | | 1587 (93.9) | 1541 (91.2) | | |
| Laparoscopy | 131 (6.6) | 523 (8.5) | | 103 (6.1) | 149 (8.8) | | |
| **Tumor histolog, n(%)** | | | <0.001 | | | 0.865 | 0.018 |
| adenocarcinoma | 1621 (82.1) | 5418 (88.2) | | 1412 (83.6) | 1407 (83.3) | | |
| mucoid adenocarcinoma | 328 (16.6) | 632 (10.3) | | 263 (15.6) | 265 (15.7) | | |
| signet-ring cell carcinoma | 26 (1.3) | 96 (1.6) | | 15 (0.9) | 18 (1.1) | | |
| **Tumor differentiation, n(%)** | | | <0.001 | | | 0.183 | 0.076 |
| Poor | 465 (23.5) | 1216 (19.8) | | 391 (23.1) | 367 (21.7) | | |
| Moderate | 1298 (65.7) | 4189 (68.2) | | 1127 (66.7) | 1149 (68.0) | | |
| Well | 22 (1.1) | 150 (2.4) | | 22 (1.3) | 36 (2.1) | | |
| Unknown | 190 (9.6) | 591 (9.6) | | 150 (8.9) | 138 (8.2) | | |
| **Vascular tumor thrombus, n (%)** | | | 0.159 | | | 0.837 | 0.007 |
| No | 1500 (75.9) | 4762 (77.5) | | 1312 (77.6) | 1307 (77.3) | | |
| Yes | 475 (24.1) | 1384 (22.5) | | 378 (22.4) | 383 (22.7) | | |
| **Surgical margin positive, n(%)** | | | 0.272 | | | 0.771 | 0.010 |
| No | 1937 (98.1) | 6050 (98.4) | | 1665 (98.5) | 1667 (98.6) | | |
| Yes | 38 (1.9) | 96 (1.6) | | 25 (1.5) | 23 (1.4) | | |
| **pTNM/UICC stage, n(%)** | | | <0.001 | | | 0.999 | 0.010 |
| 0-I | 175 (8.9) | 1269 (20.6) | | 170 (10.1) | 173 (10.2) | | |
| II | 632 (32.0) | 1611 (26.2) | | 615 (36.4) | 613 (36.3) | | |
| III | 782 (39.6) | 2370 (38.6) | | 758 (44.9) | 759 (44.9) | | |
| IV | 345 (17.5) | 724 (11.8) | | 114 (6.7) | 111 (6.6) | | |
| Unknown | 41 (2.1) | 172 (2.8) | | 33 (2.0) | 34 (2.0) | | |
| **Infiltrating lymph nodes>12, n (%)** | | | <0.001 | | | 0.961 | 0.002 |
| No | 309 (15.6) | 1487 (24.2) | | 247 (14.6) | 246 (14.6) | | |

*(Continued)*

TABLE 1 Continued

| Variables | Total study cohort | | | Propensity-matched cohort | | | SMD |
|---|---|---|---|---|---|---|---|
| | Pre-anemia (n = 1975) | Not pre-anemia (n = 6146) | P Value | Pre-anemia (n = 1690) | Not pre-anemia (n = 1690) | P Value | |
| Yes | 1666 (84.4) | 4659 (75.8) | | 1443 (85.4) | 1444 (85.4) | | |
| **Number of cancer nodule>1, n (%)** | | | 0.093 | | | 0.729 | 0.012 |
| No | 1646 (83.4) | 5223 (85.0) | | 1448 (85.7) | 1455 (86.1) | | |
| Yes | 327 (16.6) | 922 (15.0) | | 242 (14.3) | 235 (13.9) | | |
| **Clinical conditions** | | | | | | | |
| Diabetes | 285 (14.4) | 927 (15.1) | 0.479 | 185 (10.9) | 195 (11.5) | 0.584 | 0.008 |
| hypertension | 413 (21.9) | 1275 (20.7) | 0.874 | 334 (19.7) | 308 (18.2) | 0.254 | 0.032 |
| chronic respiratory insufficiency | 118 (5.97) | 328 (5.33) | 0.279 | 84 (4.97) | 91 (5.38) | 0.587 | 0.007 |

Data shown as mean±SD or n(%). ASA, American Association of Anesthesiologists; Hb, Hemoglobin; HCT, hematocrit; pTNM/UICC stage, Pathologic Tumor Node Metastasis / Union for International Cancer Control stage; SMD, standardized mean differences. Significance with P<0.05.

*vs.* 121.5 months; 5-year OS rate 75% *vs.* 71.5%, P=0.005; Figure 3A). Meanwhile, patients who were not anemic before surgery also exhibited better DFS than those who were anemic preoperatively (median survival time 134.6 months *vs.* 124.0 months; 5-year DFS rate 73.3% *vs.* 69.0%; P=0.003; Figure 3B).

After multivariate analysis, preoperative anemia remained an independent risk factor for decreased OS (HR, 1.144; 95% CI, 1.005–1.302; P=0.042, Table 3-Multivariate analysis 1) and DFS (HR, 1.166; 95% CI, 1.024–1.327; P=0.020, Table 4-Multivariate analysis 1). Altogether, a diagnosis of preoperative anemia was an independent predictor for worse OS and DFS after CRC surgery.

Similarly, after multivariate analysis, postoperative anemia was also an independent predictor for shorter OS (HR, 1.186; 95% CI, 1.042–1.350; P=0.010; Table 3-Multivariate analysis 2) and DFS (HR, 1.178; 95% CI, 1.035–1.341; P=0.013; Table 4-Multivariate analysis 2) of patients after CRC surgery.

## Kaplan–Meier Survival and Cox regression proportional hazard survival for OS and DFS in combined preoperative anemia and postoperative anemia

Patients who were not anemic either before or after surgery demonstrated the best OS when compared with those who had preoperative anemia but not postoperative anemia, those with postoperative anemia but not preoperative anemia, and those with both pre- and postoperative anemia (P=0.003, Figure 4A). Patients who did not show perioperative anemia also exhibited the best DFS of all groups of patients that were studied (P = 0.005, Figure 4B). However, patients with preoperative anemia had no difference in OS (P=0.886) and DFS (P=0.989), regardless of whether they presented with postoperative anemia or not.

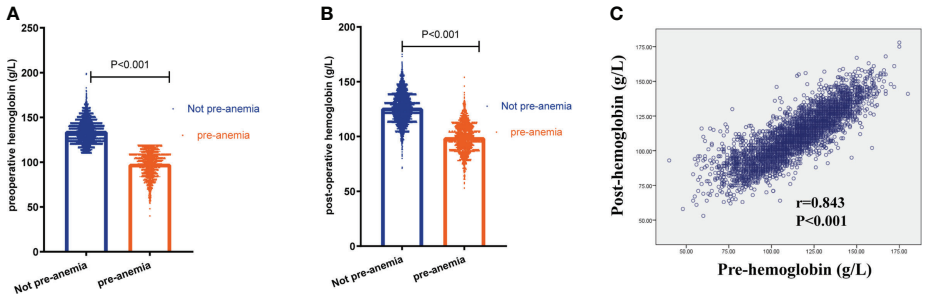



FIGURE 2
Preoperative anemia was associated with more postoperative anemia. **(A)** The level of preoperative hemoglobin in patients with and without preoperative anemia (pre-anemia) (133 ± 12.1 g/L *vs.* 98 ± 14.2 g/L, n=1690 in each group, P<0.001). **(B)** The level of postoperative hemoglobin in patients with or without pre-anemia (124 ± 13.3 g/L *vs.* 99 ± 13.1 g/L, P<0.001, n=1690 in each group). **(C)** The correlation between preoperative hemoglobin (pre-hemoglobin) and postoperative hemoglobin (post-hemoglobin) using Spearman analysis. Significance with P < 0.05.

TABLE 2 The outcome of patients in the total study cohort and the Propensity score matched cohort.

| Variables | Total study cohort | | | Propensity-matched cohort | | |
|---|---|---|---|---|---|---|
| | Pre-anemia (n = 1975) | Not pre-anemia (n = 6146) | P Value | Pre-anemia (n = 1690) | Not pre-anemia (n = 1690) | P Value |
| **Blood transfusion, n(%)** | | | <0.001 | | | <0.001 |
| No | 1812 (91.7) | 6098 (99.2) | | 1551 (91.8) | 1678 (99.3) | |
| Yes | 163 (8.3) | 48 (0.8) | | 139 (8.2) | 12 (0.7) | |
| **Amount of blood loss, n(%)** | | | 0.888 | | | 0.101 |
| <400ml | 1958 (99.1) | 6091 (99.1) | | 1674 (99.1) | 1682 (99.5) | |
| ≥400ml | 17 (0.9) | 55 (0.9) | | 16 (0.9) | 8 (0.5) | |
| **Postoperative Hb, (g/L)** | 99 ± 13.1 | 126 ± 13.6 | <0.001 | 99 ± 13.1 | 124 ± 13.3 | <0.001 |
| **Postoperative anemia, n(%)** | | | <0.001 | | | <0.001 |
| No | 202 (10.2) | 4990 (81.2) | | 160 (9.5) | 1341 (79.3) | |
| Yes | 1769 (89.8) | 1152 (18.8) | | 1530 (90.5) | 349 (20.7) | |
| **Reoperation within 30days, n(%)** | | | 0.626 | | | 1 |
| No | 1942 (98.3) | 6033 (98.2) | | 1661 (98.3) | 1661 (98.3) | |
| Yes | 33 (1.7) | 113 (1.8) | | 29 (1.7) | 29 (1.7) | |
| **Duration of Intensive Care Unit stay** | | | 0.426 | | | 0.481 |
| No | 1908 (96.6) | 5931 (96.2) | | 1619 (95.8) | 1627 (96.3) | |
| Yes | 67 (3.4) | 233 (3.8) | | 71 (4.2) | 63 (3.7) | |
| **Death, n(%)** | | | <0.001 | | | 0.005 |
| No | 1264 (64.0) | 4576 (74.5) | | 1165 (68.9) | 1239 (73.3) | |
| Yes | 711 (36.0) | 1570 (25.5) | | 525 (31.1) | 451 (26.7) | |

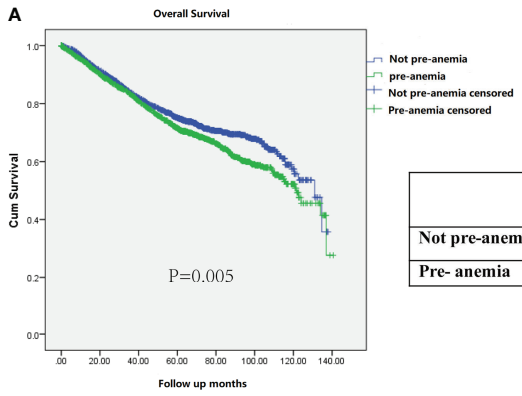
Data shown as mean±SD or n(%). Hb, Hemoglobin. Significance with P<0.05.

After the multivariable analysis, the presence of pre- and postoperative anemia remained an independent risk factor for shorter OS (HR, 1.202; 95% CI, 1.043–1.385; P=0.011, Table 3-Multivariate analysis 3) and worse DFS (HR, 1.210; 95% CI, 1.050–1.395; P=0.008, Table 4-Multivariate analysis 3). However, having preoperative but not postoperative anemia, and having postoperative but not preoperative anemia were not independent predictors for worse OS and DFS, indicating that appropriate prevention and treatment of anemia were required. In summary, both pre- and postoperative anemia was an independent predictor for negative OS and DFS of patients after CRC surgery, which experienced the highest mortality risk after CRC surgery in this model.

## Kaplan–Meier Survival and Cox regression proportional hazard survival for OS and DFS in combined preoperative anemia and transfusion

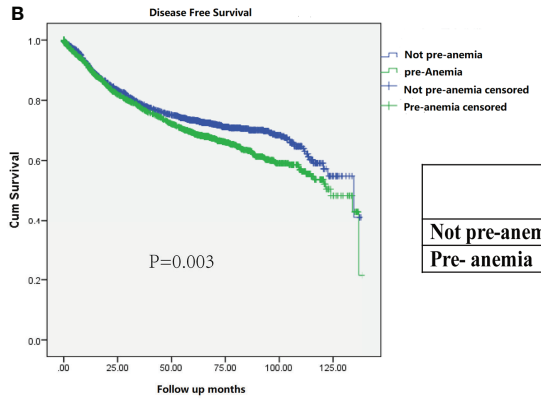
Patients who were not anemic preoperatively and not transfused showed the best OS when compared with those who were not anemic before surgery but transfused, those with preoperative anemia only but not transfused, and those with preoperative anemia who were also transfused (P=0.001, Figure 5A). Patients who were not preoperatively anemic and not transfused showed the best DFS of all studied groups of patients (P=0.001, Figure 5B). In patients with preoperative anemia, the OS and DFS of patients with transfusion were worse than those of patients without transfusion using Kaplan–Meier survival analysis (P=0.026 in OS; P=0.037 in DFS), indicating that the prognosis associated with intraoperative blood transfusion was worse than that associated with postoperative anemia.

After the multivariable analysis, preoperative anemia without or with transfusion were independent risk factors for OS (HR, 1.239; 95% CI, 1.079–1.423; P=0.002; HR, 1.791; 95% CI, 1.339–2.397; P<0.001, respectively; Table 3-Multivariate analysis 4) and DFS (HR, 1.246, 95% CI, 1.086–1.430; P=0.002; HR, 1.857; 95% CI, 1.389–2.483; P<0.001, respectively; Table 4-Multivariate analysis 4). Owing to the HRs of preoperative anemia with transfusion being higher than those of preoperative anemia without transfusion (HR 1.791 *vs.* 1.239 in OS in Table 3; HR 1.857 *vs.* 1.246 in DFS in Table 4), the risks of death and cancer progression in patients preoperatively anemic who were also transfused were the highest in this model. When comparing the most dangerous risk factors




| | Median survival time (months) |
|---|---|
| Not pre-anemia | 130.967 |
| Pre- anemia | 121.500 |

| | Follow up months | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 |
|---|---|---|---|---|---|---|---|---|---|
| Not pre-anemia | Number at risk | 1689 | 1452 | 1219 | 934 | 402 | 181 | 35 | 0 |
| | Survival rate | | 91% | 82% | 75% | 70% | 67.5% | 55.7% | |
| Pre-anemia | Number at risk | 1689 | 1398 | 1163 | 881 | 411 | 184 | 40 | 0 |
| | Survival rate | | 90.1% | 80.8% | 71.5% | 66.1% | 58.7% | 50.8% | |




| | Median survival time (months) |
|---|---|
| Not pre-anemia | 134.667 |
| Pre- anemia | 124.000 |

| | Follow up months | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 14 |
|---|---|---|---|---|---|---|---|---|---|
| Not pre-anemia | Number at risk | 1689 | 1322 | 1090 | 846 | 363 | 164 | 32 | 0 |
| | Survival rate | | 85.7% | 77.4% | 73.3% | 70.6% | 68.0% | 57.2% | |
| Pre-anemia | Number at risk | 1689 | 1265 | 1025 | 784 | 369 | 166 | 37 | 0 |
| | Survival rate | | 84.9% | 75.8% | 69.0% | 65.0% | 58.6% | 52.2% | |

FIGURE 3
**(A)** Kaplan–Meier survival curve for overall survival (OS) according to preoperative anemia (pre-anemia) in the propensity score-matched cohort. The OS rates, median survival time, and number at risk are shown. **(B)** Kaplan–Meier survival curve for disease-free survival (DFS) according to pre-anemia in the propensity score-matched cohort. The DFS rates, median survival time, and number at risk are shown. Significance with $P < 0.05$.

between two models, the HRs of preoperative anemia with transfusion were higher than those of preoperative and postoperative anemia (HR 1.791 *vs.* 1.202 in OS in Table 3; HR 1.857 *vs.* 1.210 in DFS in Table 4), indicating that the harm associated with blood transfusion was greater than that associated with postoperative anemia.

## Discussion

Our study demonstrated that preoperative anemia and postoperative anemia were independent risk factors for worse OS and DFS after colorectal surgery. Since preoperative anemia is highly associated with the presence of postoperative anemia

TABLE 3 Univariate analysis and multivariate Cox regression analysis for overall survival in the Propensity score matched cohort.

| Variables | Univariate analysis | | Multivariate analysis 1 | | Multivariate analysis 2 | | Multivariate analysis 3 | | Multivariate analysis 4 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value |
| **Pre-anemia** | | | | | | | | | | |
| No | 1 (reference) | | 1 (reference) | | | | | | | |
| Yes | 1.200 (1.058-1.360) | 0.005 | 1.144 (1.005-1.302) | 0.042 | | | | | | |
| **Post-anemia** | | | | | | | | | | |
| No | 1 (reference) | | | | 1 (reference) | | | | | |
| Yes | 1.248 (1.099-1.419) | 0.001 | | | 1.186 (1.042-1.350) | 0.010 | | | | |
| **Pre-Anemia and post-anemia** | | 0.003 | | | | | | 0.060 | | |
| Neither pre- nor post-anemia | 1 (reference) | | | | | | 1 (reference) | | | |
| Post-anemia but not pre-anemia | 1.316 (1.059-1.634) | 0.013 | | | | | 1.228 (0.987-1.528) | 0.066 | | |
| Pre-anemia but not post-anemia | 1.253 (0.931-1.686) | 0.136 | | | | | 1.147 (0.847-1.553) | 0.377 | | |
| Both pre- and post-anemia | 1.274 (1.109-1.464) | 0.001 | | | | | 1.202 (1.043-1.385) | 0.011 | | |
| **Pre-anemia and transfusion** | | 0.001 | | | | | | | | <0.001 |
| Not pre-anemia and not transfused | 1 (reference) | | | | | | | | 1 (reference) | |
| Not pre-anemia but transfused | 2.144 (0.888-5.178) | 0.090 | | | | | | | 1.735 (0.714-4.213) | 0.224 |
| Pre-anemia but not transfused | 1.173 (1.030-1.335) | 0.016 | | | | | | | 1.239 (1.079-1.423) | 0.002 |
| Pre-anemia and transfused | 1.606 (1.213-2.125) | 0.001 | | | | | | | 1.791 (1.339-2.397) | <0.001 |
| **Perioperative blood transfusion** | | | | | | | | | | |
| No | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | | |
| Yes | 1.516 (1.167-1.969) | 0.002 | 1.428 (1.092-1.868) | 0.009 | 1.443 (1.107-1.881) | 0.007 | 1.431 (1.094-1.871) | 0.009 | | |
| **Sex** | | | | | | | | | | |
| Male | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| Female | 0.833 (0.733-0.945) | 0.005 | 0.857 (0.754-0.975) | 0.019 | 0.854 (0.751-0.971) | 0.016 | 0.851 (0.748-0.968) | 0.014 | 0.857 (0.753-0.975) | 0.019 |
| **Age, years** | | <0.001 | | <0.001 | | <0.001 | | <0.001 | | <0.001 |
| ≤44 | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| 45-54 | 1.072 (0.825-1.393) | 0.602 | 1.180 (0.906-1.538) | 0.220 | 1.179 (0.905-1.537) | 0.222 | 1.181 (0.906-1.540) | 0.217 | 1.207 (0.925-1.573) | 0.165 |
| 55-64 | 1.230 (0.968-1.561) | 0.090 | 1.315 (1.032-1.676) | 0.027 | 1.313 (1.031-1.673) | 0.027 | 1.314 (1.031-1.674) | 0.027 | 1.343 (1.053-1.712) | 0.017 |
| 65-74 | 1.504 (1.186-1.907) | 0.001 | 1.699 (1.334-2.163) | <0.001 | 1.690 (1.327-2.152) | <0.001 | 1.688 (1.325-2.149) | <0.001 | 1.773 (1.390-2.261) | <0.001 |
| ≥75 | 2.337 (1.834-2.979) | <0.001 | 3.194 (2.489-4.098) | <0.001 | 3.163 (2.465-4.059) | <0.001 | 3.156 (2.459-4.050) | <0.001 | 3.287 (2.560-4.220) | <0.001 |
| **Preoperative adjuvant chemotherapy** | | | | | | | | | | |
| No | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| Yes | 1.256 (1.016-1.553) | 0.035 | 1.762 (1.372-2.261) | <0.001 | 1.756 (1.368-2.254) | <0.001 | 1.744 (1.358-2.239) | <0.001 | 1.554 (1.201-2.010) | 0.001 |

*(Continued)*

TABLE 3 Continued

| Variables | Univariate analysis | | Multivariate analysis 1 | | Multivariate analysis 2 | | Multivariate analysis 3 | | Multivariate analysis 4 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value |
| **Tumor histology** | | 0.002 | | 0.162 | | 0.185 | | 0.174 | | 0.167 |
| Adenocarcinoma | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| Mucoid adenocarcinoma | 0.976 (0.820-1.162) | 0.788 | 0.926 (0.766-1.120) | 0.430 | 0.929 (0.768-1.123) | 0.445 | 0.928 (0.767-1.122) | 0.440 | 0.927 (0.766-1.122) | 0.436 |
| Signet-ring cell carcinoma | 2.336 (1.444-3.778) | 0.001 | 1.518 (0.917-2.512) | 0.104 | 1.495 (0.903-2.475) | 0.118 | 1.506 (0.910-2.495) | 0.112 | 1.514 (0.914-2.506) | 0.107 |
| **Tumor differentiation** | | <0.001 | | 0.014 | | 0.012 | | 0.013 | | 0.008 |
| Well | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 | |
| Moderate | 1.888 (1.010-3.529) | 0.046 | 1.247 (0.663-2.343) | 0.494 | 1.238 (0.659-2.328) | 0.507 | 1.232 (0.655-2.316) | 0.518 | 1.206 (0.641-2.269) | 0.562 |
| Poor | 2.883 (1.533-5.423) | 0.001 | 1.570 (0.827-2.980) | 0.167 | 1.563 (0.824-2.966) | 0.172 | 1.553 (0.818-2.948) | 0.178 | 1.546 (0.814-2.936) | 0.183 |
| Unknown | 1.998 (1.032-3.866) | 0.040 | 1.501 (0.764-2.949) | 0.239 | 1.498 (0.763-2.944) | 0.241 | 1.487 (0.757-2.922) | 0.250 | 1.458 (0.741-2.867) | 0.275 |
| **Vascular cancer embolus** | | | | | | | | | | |
| No | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| Yes | 2.214 (1.939-2.527) | <0.001 | 1.490 (1.286-1.726) | <0.001 | 1.492 (1.288-1.729) | <0.001 | 1.489 (1.285-1.725) | <0.001 | 1.493 (1.287-1.730) | <0.001 |
| **Surgical margin positive** | | | | | | | | | | |
| No | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| Yes | 3.105 (2.158-4.467) | <0.001 | 1.541 (1.051-2.259) | 0.027 | 1.585 (1.083-2.322) | 0.018 | 1.554 (1.058-2.284) | 0.025 | 1.558 (1.063-2.285) | 0.023 |
| **pTNM/UICC stage** | | <0.001 | | <0.001 | | <0.001 | | <0.001 | | <0.001 |
| 0-I | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| II | 1.185 (0.885-1.587) | 0.255 | 1.205 (0.897-1.619) | 0.215 | 1.202 (0.895-1.614) | 0.222 | 1.201 (0.894-1.613) | 0.223 | 1.257 (0.935-1.689) | 0.130 |
| III | 2.480 (1.882-3.267) | <0.001 | 1.959 (1.468-2.614) | <0.001 | 1.947 (1.459-2.599) | <0.001 | 1.950 (1.461-2.602) | <0.001 | 2.022 (1.514-2.700) | <0.001 |
| IV | 8.982 (6.608-12.209) | <0.001 | 7.069 (5.097-9.804) | <0.001 | 7.020 (5.062-9.735) | <0.001 | 7.053 (5.085-9.783) | <0.001 | 7.718 (5.549-10.735) | <0.001 |
| Unkown | 0.766 (0.365-1.607) | 0.481 | 0.523 (0.239-1.142) | 0.104 | 0.518 (0.237-1.132) | 0.099 | 0.518 (0.237-1.132) | 0.099 | 0.528 (0.242-1.154) | 0.110 |
| **Infiltrating lymph nodes>12, n (%)** | | | | | | | | | | |
| No | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| Yes | 0.710 (0.605-0.833) | <0.001 | 0.839 (0.707-0.997) | 0.046 | 0.836 (0.704-0.993) | 0.042 | 0.836 (0.704-0.993) | 0.041 | 0.857 (0.721-1.019) | 0.080 |
| **Number of cancer nodule>1, n (%)** | | | | | | | | | | |
| No | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| Yes | 2.469 (2.288-3.066) | <0.001 | 1.469 (1.248-1.729) | <0.001 | 1.473 (1.252-1.734) | <0.001 | 1.468 (1.247-1.728) | <0.001 | 1.455 (1.236-1.713) | <0.001 |
| **Amount of blood loss** | | | | | | | | | | |
| <400ml | 1 (reference) | | | | | | | | | |
| ≥400ml | 1.781 (0.955-3.320) | 0.070 | | | | | | | | |
| **Reoperation within 30 days** | | | | | | | | | | |
| No | 1 (reference) | | | | | | | | | |
| Yes | 1.021 (0.631-1.649) | 0.934 | | | | | | | | |

Data shown as HR [hazard ratio] (95% CI [confidence interval]). pTNM/UICC stage, Pathologic Tumor Node Metastasis / Union for International Cancer Control stage; RBC, Red blood cell. Significance with $P < 0.05$.

TABLE 4 Univariate analysis and multivariate Cox regression analysis for disease-free survival in the Propensity score matched cohort.

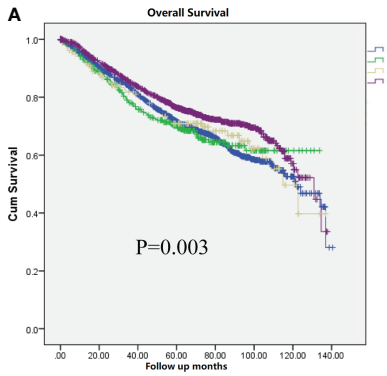
| Variables | Univariate analysis | | Multivariate analysis 1 | | Multivariate analysis 2 | | Multivariate analysis 3 | | Multivariate analysis 4 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value |
| **Pre-anemia** | | | | | | | | | | |
| No | 1 (reference) | | 1 (reference) | | | | | | | |
| Yes | 1.208 (1.065-1.370) | 0.003 | 1.166 (1.024-1.327) | 0.020 | | | | | | |
| **Post-anemia** | | | | | | | | | | |
| No | 1 (reference) | | | | 1 (reference) | | | | | |
| Yes | 1.236 (1.088-1.405) | 0.001 | | | 1.178 (1.035-1.341) | 0.013 | | | | |
| **Pre-anemia and post-anemia** | | 0.005 | | | | | | 0.066 | | |
| Neither pre- nor post-anemia | 1 (reference) | | | | | | 1 (reference) | | | |
| Post-anemia but not pre-anemia | 1.269 (1.022-1.577) | 0.031 | | | | | 1.164 (0.935-1.448) | 0.175 | | |
| Pre-anemia but not post-anemia | 1.274 (0.947-1.714) | 0.110 | | | | | 1.167 (0.861-1.582) | 0.321 | | |
| Both pre- and post- anemia | 1.272 (1.107-1.461) | 0.001 | | | | | 1.210 (1.050-1.395) | 0.008 | | |
| **Pre-anemia and transfusion** | | 0.001 | | | | | | | | <0.001 |
| Not pre-anemia and not transfused | 1 (reference) | | | | | | | | 1 (reference) | |
| Not pre-anemia but transfused | 2.215 (0.917-5.348) | 0.077 | | | | | | | 1.936 (0.796-4.707) | 0.145 |
| Pre-anemia but not transfused | 1.183 (1.039-1.347) | 0.011 | | | | | | | 1.246 (1.086-1.430) | 0.002 |
| Pre-anemia and transfused | 1.587 (1.199-2.101) | 0.001 | | | | | | | 1.857 (1.389-2.483) | <0.001 |
| **Perioperative blood transfusion** | | | | | | | | | | |
| No | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | | |
| Yes | 1.496 (1.152-1.943) | 0.003 | 1.493 (1.141-1.953) | 0.003 | 1.527 (1.171-1.990) | 0.002 | 1.495 (1.142-1.956) | 0.003 | | |
| **Sex** | | | | | | | | | | |
| Male | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| Female | 0.832 (0.733-0.945) | 0.005 | 0.839 (0.738-0.955) | 0.008 | 0.836 (0.734-0.951) | 0.006 | 0.834 (0.733-0.950) | 0.006 | 0.838 (0.736-0.954) | 0.007 |
| **Age, years** | | <0.001 | | <0.001 | | <0.001 | | <0.001 | | <0.001 |
| ≤44 | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| 45-54 | 1.113 (0.857-1.446) | 0.421 | 1.297 (0.996-1.690) | 0.054 | 1.298 (0.996-1.691) | 0.054 | 1.297 (0.996-1.690) | 0.054 | 1.326 (1.018-1.729) | 0.037 |
| 55-64 | 1.267 (0.997-1.608) | 0.053 | 1.359 (1.067-1.730) | 0.013 | 1.357 (1.066-1.729) | 0.013 | 1.356 (1.065-1.727) | 0.013 | 1.377 (1.081-1.755) | 0.010 |
| 65-74 | 1.537 (1.212-1.949) | <0.001 | 1.768 (1.389-2.250) | <0.001 | 1.755 (1.379-2.234) | <0.001 | 1.756 (1.379-2.235) | <0.001 | 1.818 (1.427-2.317) | <0.001 |
| ≥75 | 2.269 (1.780-2.892) | <0.001 | 3.000 (2.340-3.845) | <0.001 | 2.972 (2.318-3.809) | <0.001 | 2.968 (2.314-3.807) | <0.001 | 3.051 (2.379-3.913) | <0.001 |
| **Preoperative adjuvant chemotherapy** | | | | | | | | | | |
| No | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| Yes | 1.280 (1.035-1.582) | 0.023 | 1.891 (1.475-2.424) | <0.001 | 1.886 (1.472-2.418) | <0.001 | 1.881 (1.467-2.412) | <0.001 | 1.689 (1.306-2.182) | <0.001 |

*(Continued)*

TABLE 4 Continued

| Variables | Univariate analysis | | Multivariate analysis 1 | | Multivariate analysis 2 | | Multivariate analysis 3 | | Multivariate analysis 4 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value |
| **Tumor histology** | | 0.007 | | 0.181 | | 0.207 | | 0.195 | | 0.192 |
| Adenocarcinoma | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| Mucoid adenocarcinoma | 0.974 (0.818-1.159) | 0.765 | 0.931 (0.770-1.127) | 0.463 | 0.936 (0.774-1.133) | 0.499 | 0.934 (0.772-1.130) | 0.480 | 0.933 (0.771-1.128) | 0.473 |
| Signet-ring cell carcinoma | 2.155 (1.333-3.485) | 0.002 | 1.504 (0.911-2.484) | 0.111 | 1.485 (0.899-2.453) | 0.123 | 1.493 (0.904-2.468) | 0.118 | 1.495 (0.905-2.471) | 0.117 |
| **Tumor differentiation** | | <0.001 | | 0.074 | | 0.071 | | 0.076 | | 0.068 |
| Well | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| Moderate | 1.867 (0.999-3.490) | 0.050 | 1.268 (0.674-2.383) | 0.462 | 1.265 (0.673-2.379) | 0.465 | 1.258 (0.669-2.366) | 0.476 | 1.231 (0.654-2.316) | 0.519 |
| Poor | 2.803 (1.490-5.273) | 0.001 | 1.532 (0.807-2.909) | 0.193 | 1.531 (0.806-2.908) | 0.193 | 1.520 (0.800-2.887) | 0.201 | 1.497 (0.788-2.845) | 0.218 |
| Unknown | 1.932 (0.998-3.741) | 0.051 | 1.373 (0.698-2.701) | 0.359 | 1.377 (0.700-2.710) | 0.354 | 1.363 (0.693-2.684) | 0.369 | 1.332 (0.677-2.623) | 0.407 |
| **Vascular cancer embolus** | | | | | | | | | | |
| No | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| Yes | 2.246 (1.967-2.564) | <0.001 | 1.500 (1.294-1.738) | <0.001 | 1.503 (1.297-1.742) | <0.001 | 1.498 (1.293-1.737) | <0.001 | 1.494 (1.288-1.732) | <0.001 |
| **Surgical margin positive** | | | | | | | | | | |
| No | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| Yes | 3.464 (2.407-4.986) | <0.001 | 1.539 (1.047-2.261) | 0.028 | 1.597 (1.088-2.345) | 0.017 | 1.552 (1.052-2.289) | 0.027 | 1.555 (1.057-2.287) | 0.025 |
| **pTNM/UICC stage** | | <0.001 | | <0.001 | | <0.001 | | <0.001 | | <0.001 |
| 0-I | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| II | 1.157 (0.864-1.549) | 0.328 | 1.178 (0.877-1.582) | 0.277 | 1.175 (0.875-1.578) | 0.285 | 1.174 (0.874-1.578) | 0.286 | 1.225 (0.911-1.647) | 0.179 |
| III | 2.480 (1.883-3.267) | <0.001 | 1.943 (1.456-2.592) | <0.001 | 1.931 (1.447-2.576) | <0.001 | 1.936 (1.451-2.583) | <0.001 | 2.007 (1.504-2.680) | <0.001 |
| IV | 9.667 (7.111-13.143) | <0.001 | 7.332 (5.290-10.163) | <0.001 | 7.290 (5.260-10.104) | <0.001 | 7.313 (5.276-10.137) | <0.001 | 7.934 (5.708-11.027) | <0.001 |
| Unkown | 0.739 (0.352-1.551) | 0.424 | 0.476 (0.218-1.041) | 0.063 | 0.473 (0.216-1.033) | 0.060 | 0.472 (0.216-1.032) | 0.060 | 0.484 (0.221-1.057) | 0.069 |
| **Infiltrating lymph nodes>12, n (%)** | | | | | | | | | | |
| No | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| Yes | 0.683 (0.582-0.802) | <0.001 | 0.806 (0.680-0.956) | 0.013 | 0.806 (0.680-0.956) | 0.013 | 0.805 (0.678-0.954) | 0.012 | 0.820 (0.691-0.974) | 0.024 |
| **Number of cancer nodule>1, n (%)** | | | | | | | | | | |
| No | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | | 1 (reference) | |
| Yes | 2.679 (2.314-3.101) | <0.001 | 1.462 (1.240-1.723) | <0.001 | 1.467 (1.245-1.728) | <0.001 | 1.463 (1.241-1.724) | <0.001 | 1.453 (1.233-1.713) | <0.001 |
| **Amount of blood bloss** | | | | | | | | | | |
| <400ml | 1 (reference) | | | | | | | | | |
| ≥400ml | 1.703 (0.913-3.175) | 0.094 | | | | | | | | |
| **Reoperation within 30 days** | | | | | | | | | | |
| No | 1 (reference) | | | | | | | | | |
| Yes | 1.027 (0.636-1.661) | 0.912 | | | | | | | | |

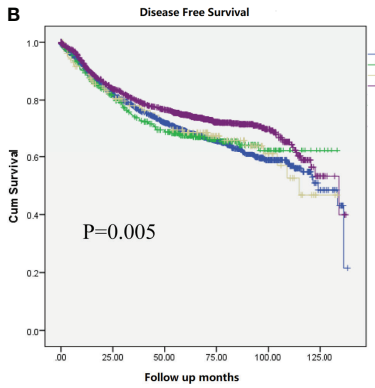
Data shown as HR [hazard ratio] (95% CI [confidence interval]). pTNM/UICC stage, Pathologic Tumor Node Metastasis / Union for International Cancer Control stage; RBC, Red blood cell. Significance with $P < 0.05$.




Neither pre- nor post-anemia vs. post-anemia but not pre-anemia, P=0.014
Neither pre- nor post-anemia vs. both pre- and post-anemia, P=0.001
Both pre- and post-anemia vs. Pre-anemia not post-anemia, P=0.886

| | Median survival time (months) |
|---|---|
| Both pre- and post-anemia | 121.500 |
| Post-anemia not pre-anemia | Not reach |
| Pre-anemia not post-anemia | 114.900 |
| Neither pre- nor post-anemia | 130.967 |

| | Follow up months | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 |
|---|---|---|---|---|---|---|---|---|---|
| Both pre- and post-anemia | Number at risk | 1529 | 1268 | 1059 | 796 | 361 | 163 | 33 | 0 |
| | Survival rate | | 90.3% | 80.8% | 71.5% | 65.8% | 58.2% | 50.9% | |
| Post-anemia not pre-anemia | Number at risk | 348 | 289 | 229 | 169 | 67 | 30 | 6 | 0 |
| | Survival rate | | 88.5% | 75.4% | 69.4% | 63.3% | 61.6% | 60% | |
| Pre-anemia not post-anemia | Number at risk | 159 | 129 | 103 | 84 | 49 | 20 | 6 | 0 |
| | Survival rate | | 87.6% | 79.5% | 70% | 66.8% | 58.9% | 39.7% | |
| Neither pre- nor post-anemia | Number at risk | 1340 | 1162 | 989 | 764 | 334 | 150 | 28 | 0 |
| | Survival rate | | 92.1% | 83.6% | 76.3% | 72% | 69.0% | 54.9% | |



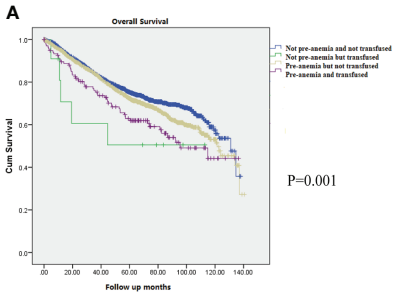

Neither pre- nor post-anemia vs. post-anemia but not pre-anemia, P=0.033
Neither pre- nor post-anemia vs. both pre- and post-anemia, P=0.001
Both pre- and post-anemia vs. pre-anemia not post-anemia, P=0.989

| | Median survival time (months) |
|---|---|
| Both pre- and post-anemia | 124.000 |
| Post-anemia not pre-anemia | Not reach |
| Pre-anemia not post-anemia | 114.900 |
| Neither pre- and post not anemia | 134.667 |

| | Follow up months | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 |
|---|---|---|---|---|---|---|---|---|---|
| Both pre- and post-anemia | Number at risk | 1529 | 1145 | 933 | 710 | 326 | 148 | 31 | 0 |
| | Survival rate | | 84.9% | 75.8% | 69.1% | 64.8% | 58.8% | 52.9% | |
| Post-anemia not pre-anemia | Number at risk | 348 | 265 | 212 | 153 | 63 | 27 | 6 | 0 |
| | Survival rate | | 83.3% | 72.1% | 67.8% | 65.7% | 62.5% | 60% | |
| Pre-anemia not post-anemia | Number at risk | 159 | 119 | 91 | 73 | 42 | 17 | 5 | 0 |
| | Survival rate | | 84.1% | 76.4% | 68.8% | 66.1% | 61.1% | 46.8% | |
| Neither pre-and post-anemia | Number at risk | 1340 | 1056 | 877 | 692 | 299 | 136 | 25 | 0 |
| | Survival rate | | 86.3% | 78.6% | 74.9% | 71.9% | 69.4% | 56.4% | |

FIGURE 4
**(A)** Kaplan–Meier survival curve for overall survival (OS) for both preoperative anemia (pre-anemia) and postoperative anemia (post-anemia), post-anemia but not pre-anemia, pre-anemia but not post-anemia, and neither pre- nor post-anemia in the propensity score-matched cohort. The OS rates, median survival time, and number at risk are shown. **(B)** Kaplan–Meier survival curve for disease-free survival (DFS) for both pre- and post-anemia, post-anemia but not pre-anemia, pre-anemia but not post-anemia, and neither pre- nor post-anemia in the propensity score-matched cohort. The DFS rates, median survival time, and number at risk are shown. The median survival time refers to the corresponding survival time when the survival rate is 50%. "not reach" means when a line is drawn vertically on the Y axis 0.5, it does not intersect with the survival curve. There is no corresponding survival time here. Significance with $P < 0.05$.

and the need for blood transfusions, we evaluated two new prognostic models involving these factors. In the preoperative anemia and postoperative anemia model, the presence of both preoperative anemia and postoperative anemia had the highest risk of worse OS and DFS. Patients with preoperative anemia had no difference in OS and DFS, regardless of whether they presented with postoperative anemia or not. In the preoperative anemia and transfusion model, preoperative anemia and

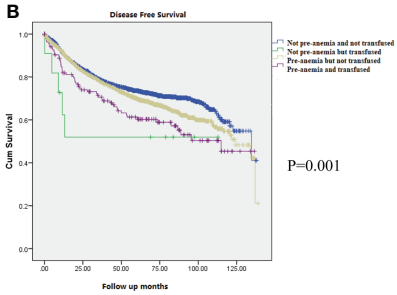



P=0.001

Not pre-anemia and not transfused vs. Pre-anemia but not transfused, P=0.016;
Not pre-anemia and not transfused vs. Pre-anemia and transfused, P=0.001;
Pre-anemia but not transfused vs. Pre-anemia and transfused, P=0.026

| | Median survival time (months) |
|---|---|
| Not pre-anemia and not transfused | 130.967 |
| Not Pre-anemia but transfused | Not reach |
| Pre-anemia but not transfused | 121.500 |
| Pre-anemia and transfused | 95.433 |

| | Follow up months | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 |
|---|---|---|---|---|---|---|---|---|---|
| Not pre-anemia and not transfused | Number at risk | 1677 | 1446 | 1213 | 920 | 366 | 173 | 33 | 0 |
| | Survival rate | | 91.6 | 82.1 | 75.1 | 70.5 | 67.6 | 55.8 | |
| Not Pre-anemia but transfused | Number at risk | 11 | 6 | 5 | | 3 | 1 | 0 | 0 |
| | Survival rate | | 60.6 | 50.5 | | | | | |
| Pre-anemia but not transfused | Number at risk | 1550 | 1289 | 1076 | 814 | 366 | 165 | 32 | 0 |
| | Survival rate | | 90.7 | 81.4 | 72.2 | 66.7 | 59.6 | 51.5 | |
| Pre-anemia and transfused | Number at risk | 138 | 107 | 85 | 66 | 37 | 22 | 9 | 0 |
| | Survival rate | | 82.6 | 72.8 | 61.9 | 57.6 | 51.7 | 44.2 | |




P=0.001

Not pre-anemia and not transfused vs. Pre-anemia but not transfused, P=0.011;
Not pre-anemia and not transfused vs. Pre-anemia and transfused, P=0.001;
Pre-anemia but not transfused vs. Pre-anemia and transfused, P=0.037.

| | Median survival time (months) |
|---|---|
| Not pre-anemia and not transfused | 134.667 |
| Not pre-anemia but transfused | Not reach |
| Pre-anemia but not transfused | 124.000 |
| Pre-anemia and transfused | 114.900 |

| | Follow up months | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 |
|---|---|---|---|---|---|---|---|---|---|
| Not pre-anemia and not transfused | Number at risk | 1677 | 1317 | 1085 | 824 | 339 | 157 | 30 | 0 |
| | Survival rate | | 86.0 | 77.5 | 73.4 | 70.7 | 68.1 | 57.2 | |
| Not Pre-anemia but transfused | Number at risk | 11 | 5 | | | 3 | 1 | 0 | 0 |
| | Survival rate | | | | | | | | |
| Pre-anemia but not transfused | Number at risk | 1550 | 1166 | 948 | 718 | 332 | 118 | 30 | 0 |
| | Survival rate | | 85.5 | 76.5 | 69.8 | 65.5 | 59.4 | 52.9 | |
| Pre-anemia and transfused | Number at risk | 138 | 98 | 75 | 60 | 34 | 19 | 9 | 0 |
| | Survival rate | | 77.2 | 67.9 | 60.3 | 57.2 | 50.4 | 45.3 | |

FIGURE 5
**(A)** Kaplan–Meier survival curve for overall survival (OS) for not preoperative anemia (pre-anemia) and not transfused, not pre-anemia but transfused, pre-anemia but not transfused, and pre-anemia and transfused in the propensity score-matched cohort. The OS rates, median survival time, and number at risk are shown. **(B)** Kaplan–Meier survival curve for disease-free survival (DFS) for not pre-anemia and not transfused, not pre-anemia but transfused, pre-anemia but not transfused, and pre-anemia and transfused in the propensity score-matched cohort. The DFS rates, median survival time, and number at risk are shown. The median survival time refers to the corresponding survival time when the survival rate is 50%. "not reach" means when a line is drawn vertically on the Y axis 0.5, it does not intersect with the survival curve. There is no corresponding survival time here. Significance with $P < 0.05$.

transfused was the most dangerous independent prognostic factor for OS and DFS. In patients with preoperative anemia, the OS and DFS of patients with transfusion were worse than those of patients without transfusion.

Our large study indicated that anemia before surgery was present in 24.3% of CRC patients and was strongly associated with worse OS and DFS. However, the mechanisms behind preoperative anemia and poor cancer outcomes were unclear, as some studies reported that low Hb indicates hypoxia, a decrease of oxygen-carrying function, and low tolerance to bleeding (26, 27). Hypoxia is the key initiating factor for tumors. Increasing evidence shows that anemia could lead to hypoxia in the tumor microenvironment, leading to up-regulation of hypoxia-inducible factor-1 α expression. Hypoxia-inducible factor-1 α could inhibit the effect of tumor infiltrating lymphocytes and promote immunosuppressive activity by activating tumor-associated macrophages; these factors further promoted tumor proliferation and revascularization (26, 27). Moreover,

preoperative anemia is also a sign of the severity of the underlying disease. In our study, after propensity score matching, SMD values for all characteristics were < 0.1 except for surgical approach. Preoperative anemia was associated with laparotomy. It would be interesting to explore whether laparotomy correlated with more bleeding and greater number of transfusions. There was no difference in bleeding between laparotomy and laparoscopy surgical approaches. A greater percentage of patients in the laparotomy group required blood transfusion (4.7% *vs*. 2.0%, P < 0.047, Supplementary Table 1). In our study, after matching, preoperative anemia was associated with laparotomy, which is related to immunomodulation as well as greater number of transfusions. This explains the association between preoperative anemia and poor prognosis in patients with CRC.

Furthermore, preoperative anemia correlated positively with postoperative anemia in our study. Single exposure to preoperative anemia or postoperative anemia was a risk factor for worse OS and DFS, yet postoperative anemia but not preoperative anemia and preoperative anemia but not postoperative anemia were no longer risk factors for OS and DFS in our study. This finding is very important for anesthesiologists and surgeons, as it indicates that treatment or intervention for preoperative anemia or postoperative anemia, which benefits cancer patients' outcomes, should be considered. Several studies concluded that blood management before surgery, according to preoperative anemia status, can effectively improve patients' safety and reduce medical expenditure, blood transfusion, hospital stay, complications, and mortality (28–32). The management of postoperative anemia includes erythropoiesis, blood loss prevention, and restricted blood transfusion strategies (14, 28, 31). Correct evaluation is crucial, with prevention being the best treatment (14). However, patients with preoperative anemia, regardless of whether they had postoperative anemia or not, presented no difference in OS and DFS. Therefore, anemia should warrant anesthesiologists and surgeons' attention, as they can use this combined assessment to identify particularly sensitive patients and implement effective strategies to improve their outcomes.

In our study, we showed that preoperative anemia was associated with a greater percentage of patients needing blood transfusions. Similarly, preoperative anemia is strongly correlated with perioperative blood transfusion and increased mortality in patients undergoing elective surgery (29, 33, 34). Anemia, blood loss, and transfusion can be considered "three evils" that adversely affect mortality (8), and are inextricably interrelated (35). One of the main purposes of this study was also to evaluate the interaction between preoperative anemia and intraoperative transfusions. In the preoperative anemia and transfusion model, we found that the combination of preoperative anemia with or without intraoperative blood transfusions were independent risk factors for OS and DFS after multivariate analysis. The HR of the combination of preoperative anemia and transfusion was much higher than the HR of preoperative anemia without transfusion, indicating that preoperatively anemic patients who were transfused had higher risks of death and cancer progression than those of patients who were not transfused. When comparing the most dangerous risk factors between the two models, the HR of the combination of preoperative anemia and transfusion was also higher than the HR of preoperative anemia and postoperative anemia. Our HRs with very narrow 95% CIs showed robust predictive values, indicating that the harm associated with blood transfusion was worse than that associated with postoperative anemia. Concurrently, in patients with preoperative anemia, the OS and DFS of patients with transfusion were significantly worse than those of patients without transfusion, suggesting that treating anemia with intraoperative blood transfusion should be considered carefully, and highlighting a need for strategies targeting anemia tolerance and for appropriately restricting the use of blood transfusion.

Now, is it better to tolerate anemia than to correct anemia with blood transfusion? The perioperative period is a critical window in the recovery of patients with an impaired immune response due to surgical trauma. Blood transfusion is thought to have immunomodulatory effects and may damage tumor immune surveillance and promote tumor growth and spread (36, 37). Moderate to severe anemia (first strike) and transfusion (second strike) may lead to elevated systemic inflammation and immunosuppression accompanied by endothelial dysfunction (6, 36, 38–40). Historically, treatment and management of patients with anemia mostly rely on blood transfusion. However, the fundamental purpose of medical treatment is not to treat "laboratory values," but to improve patients' conditions to achieve a better outcome (41). The indication of allogeneic blood transfusion should take into account the patient's underlying disease (42), laboratory test results, benefits and risks, and whether bleeding is present or absent (43–45). Patient blood management (PBM) has encouraged physicians to treat anemia, optimize hemostasis, minimize blood loss, promote toleration of anemia, and restrict transfusion where appropriate in order to improve patient prognosis (15, 43, 46–49). However, the actual implementations of Patient blood management (PBM) in many countries are not satisfactory (50, 51). Owing to barriers of application of PBM in many medical centers, consideration should be given to education and training to raise awareness of the clinical hazards of anemia and blood transfusion, and the need for alternatives to blood transfusion (31, 41, 49). Therefore, the results of our study may significantly aid health care providers in several countries.

Our study addressed an important topic. The advantage of our study was that our overall sample size (>8000) and allocation (>1000) in each group were much larger than those of previous studies, and the data were obtained from one of the largest cancer centers in China. Another advantage was that our median postoperative follow-up period was more than 5 years (median: 69.6 months), and we focused on CRC patients' long-term outcomes. Further, we were the first to build two novel models

to clarify the effect of anemia tolerance and transfusion on long-term survival after CRC surgery, which to the best of our knowledge, has not been reported previously. Our study adds to the growing body of literature regarding the efficacy of PBM on the identification of anemia, anemia tolerance, and restriction of transfusion use to lead to improved patient outcomes. Due to the poor application of PBM in many countries, our large sample cohort could provide more reference for physicians when they are considering anemia tolerance or blood transfusion for patients with CRC. Although these results contribute important information to the existing literature, our study also has several limitations. For example, this is a retrospective, and not a randomized, study from a singular institution, which cannot avoid the possibility of residual confounding factors.

Another limitation is that the indication for transfusion is unknown. This is inherent to the nature of this study (retrospective observational study). We can't get the information of the threshold levels for RBC transfusion of every patient. Hb thresholds of 7 to 8 g/dL are used for most hemodynamically stable medical and surgical patients to avoid unnecessary transfusions in our hospital. After matching for preoperative anemia, no significant differences were found for patient baseline characteristics between transfused and not transfused patients, except preoperative adjuvant chemotherapy (Supplementary Table 2).

## Conclusions

The combined prognostic value of preoperative anemia and blood transfusion imposed a greater risk to OS and DFS in patients undergoing CRC surgery. These findings should encourage clinicians to be vigilant for the timely prevention and treatment of anemia, by appropriately promoting toleration of anemia and restricting the use of blood transfusion in patients with CRC. Prospective randomized controlled trials are needed to explore perioperative risk and treatment opportunity in patients with CRC to improve their long-term prognosis.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

## Ethics statement

This retrospective study was performed at Shanghai Cancer Center, Fudan University, Shanghai, China and was approved by the appropriate ethics committee (IRB2105235-6). The patients/participants provided their written informed consent to participate in this study.

## Author contributions

Conceptualization, MW, and CM. Methodology, MW, MG, and TL. Investigation and formal analysis, MW, CZ, CS, QL, SC and YY. Writing-original draft preparation, MW, and DZ. Writing-Review and Editing, MW, DZ and XL. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.940428/full#supplementary-material

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# Intraoperative low-dose dopamine is associated with worse survival in patients with hepatocellular carcinoma: A propensity score matching analysis

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**Background:** Dopamine is widely used in patients during surgery. We evaluated the association between intraoperative low-dose dopamine administration and recurrence-free survival (RFS) and overall survival (OS) in patients with hepatocellular carcinoma (HCC).

**Methods:** Consecutive patients with nonmetastatic HCC who underwent radical hepatectomy were enrolled between 2008 and 2010. Univariate and multivariate logistic regression analyses were used to evaluate the prognostic factors for RFS and OS. Survival outcomes were evaluated using Kaplan–Meier analyses with the log-rank test. A one-to-one propensity score matching (PSM) analysis was performed to reduce confounding bias.

**Results:** A total of 805 HCC patients, including 699 patients who did not receive dopamine consumption and 106 patients who received low-dose dopamine during the operation, were retrospectively analyzed. The patients who were assigned low-dose dopamine had worse RFS ($p$ = 0.009) and OS ($p$ = 0.041) than those who did not receive dopamine. Multivariate regression analysis showed that the intraoperative administration of low-dose dopamine was an independent unfavorable predictor for RFS ($p$ = 0.004) but not for OS ($p$ = 0.059). After PSM, the low-dose dopamine-treated group still had significantly poorer RFS ($p$ = 0.003) and OS ($p$ = 0.002). When stratified by time of recurrence, patients with low-dose dopamine use had a significantly greater chance of recurrence within 2 years ($p$ = 0.007) but not after 2 years ($p$ = 0.186).

**Conclusions:** Intraoperative low-dose dopamine use has a negative impact on RFS and OS in HCC patients who have undergone radical hepatectomy. Further

prospective studies are required to assess the effects of low-dose dopamine on surgical outcomes in HCC patients.

KEYWORDS

hepatocellular carcinoma, hepatectomy, low-dose dopamine, recurrence, survival

## Introduction

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer death worldwide, accounting for 75-85% of primary liver cancer (1). Despite the improvement of surgical treatment and targeted therapy, the outcomes of HCC patients remain unfavorable because of the high rates of cancer recurrence and mortality (2–4).

Currently, hepatic resection is the mainstay of HCC treatment. However, intraoperative anesthetic management has a critical effect on HCC patients. During liver resection, maintaining low central venous pressure (CVP) is considered an important management aspect of hepatic parenchyma dissection to reduce intraoperative blood loss (5, 6). Low CVP can be achieved by restricting fluid input, increasing urine output, clamping the inferior vena cava and so on (7, 8). Nevertheless, lower CVP measures frequently lead to some complications, such as vital organ perfusion insufficiency and hemodynamic instability.

Dopamine, as an endogenous catecholamine, affects renal perfusion and cardiovascular control in a dose-dependent manner. Anesthesiologists usually adjust the dosage of dopamine due to the vital signs of patients during surgical operations. In clinical practice, low-dose dopamine, as a renal dose, is often applied less than 3 μg/kg/min to increase renal blood flow and urine volume (9), which is an anesthetic technique to maximize renal perfusion. This might be one of the reasons that low-dose dopamine use is widely administered in hepatic surgery.

The influence of anesthetic approaches on cancer patients is complex. Growing evidence from animal and human studies has revealed that the different types of anesthetic procedures can affect the tumor progression and survival outcomes in patients with malignancies (10–12). Our recent study found that dopamine promotes the proliferation, migration and invasion of HCC cell lines *in vitro* (13). However, few clinical trials have been performed to investigate the impact of dopamine use on survival outcomes in cancer patients due to the specific tumor microenvironment during surgery. Therefore, we conducted a retrospective cohort study of patients with HCC undergoing open hepatectomy to explore the association between intraoperative low-dose dopamine administration and the survival outcomes of the patients.

## Materials and methods

### Patients and study design

We retrospectively selected a total of 952 consecutive patients with newly-diagnosed nonmetastatic HCC at Sun Yat-sen University Cancer Center (SYSUCC) between January 2008 and December 2010. The inclusion criteria were as follows: (1) patients who underwent open radical hepatectomy; (2) patients who had postoperative tumor-free margins; and (3) patients who had complete clinicopathological and follow-up data. The exclusion criteria were as follows: (1) patients who had another primary malignancy before the diagnosis of HCC; (2) patients who received preoperative therapy; (3) patients who had severe preoperative physical conditions, such as Child–Pugh class C liver function, renal dysfunction and severe cardiovascular disease; and (4) patients who admitted surgical intensive care unit after surgery. The selected clinicopathological data were as follow: (1) patient characteristics before hepatectomy (sex, age, American Society of Anesthesiologists (ASA) physical status, HBsAg, cirrhosis, serum alpha-fetoprotein (AFP), gamma glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, albumin and creatinine); (2) malignant tumor factors (tumor size, tumor number, satellite nodules and vascular invasion); (3) administration of intraoperative low-dose dopamine (1-2 μg/kg/min); (4) clinical parameters during surgery (intraoperative fluid infusion, urine output, norepinephrine use, blood loss and duration of surgery) and within one week after operation (postoperative AFP, ALT, AST, bilirubin and creatinine) and (5) the time of tumor recurrence and death. This study conformed to the Declaration of Helsinki and was approved by the Institutional Ethics Committees of the SYSUCC (approval number: B2022-065-01). Owing to the nature of the retrospective study, the requirement for written informed consent was waived by the Institutional Review Board.

## Surgical treatment and follow-up

Open radical hepatectomy was performed or supervised by two consultant hepatic surgeons on the same treatment team. The surgical procedure was determined based on the patient tumor number, tumor size, liver function and physical status. All patients with HCC received regular follow-up every 3 months for the first 2 years after surgery and then every 6 months thereafter. Each follow-up consisted of blood tests and imaging examinations, including serum AFP, liver function tests, and at least one abdominal imaging scan, such as ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI). Annually, chest CT was screened as a standard procedure. Enrolled patients were followed up until tumor recurrence or death or until December 2021. HCC recurrence was classified into early or late recurrence by using 2 years as the cut-off (14–16) and was diagnosed by one of the following criteria: (a) liver tissue pathological diagnosis; and (b) typical lesion appearances in abdominal enhanced-contrast CT or MRI (hypervascularity enhanced on the arterial phase and washout on the portal venous phase) (2). The sites of recurrence included intrahepatic and extrahepatic recurrence. Recurrence-free survival (RFS) was measured as the interval between the date of initial hepatectomy and the date of recurrence, death from disease or the last follow-up. Overall survival (OS) was measured as the survival time from the date of initial hepatectomy to the date of death or the last follow-up.

## Statistical analysis

Categorical data were analyzed with the chi-square test or Fisher's exact test, as appropriate. Continuous data were analyzed with the *t*-test or Wilcoxon rank-sum test. RFS and OS in two cohorts comprising intraoperative low-dose dopamine and without low-dose dopamine were assessed by Kaplan–Meier analysis and were compared using a log-rank test. Univariate and multivariate logistic regression analyses were used to evaluate the prognostic factors for RFS and OS. To reduce selection bias and balance variables, a 1:1 matched cohort using propensity score matching (PSM) analysis was performed. All statistical tests were two-sided, and *P* value less than 0.05 were considered statistically significant. Except for the Kaplan-Meier curves, which were analyzed by the website statistical tool (http://www.bioinformatics.com.cn/), other statistical analyses were performed using SPSS software, version 22 (IBM Corporation, Armonk, NY, USA).

# Results

## Patient characteristics

From the initial group of 952 HCC patients, 147 were excluded according to the criteria. Ultimately, a total of 805 patients were enrolled in this study, including 699 patients who did not consume dopamine and 106 patients who received low-dose dopamine during radical hepatectomy (Figure 1). The baseline characteristics of the original cohort are summarized in Table 1. Before PSM, low-dose dopamine use was associated with a higher grade of ASA physical status ($p$ = 0.036), larger tumor size ($p$ = 0.032), lower AFP level ($p$ = 0.015) and lower ALT level ($p$ = 0.008). There were no significant differences between the two groups in terms of sex, age, HBsAg, cirrhosis, Child–Pugh classification, tumor number, satellite nodules, vascular invasion, AST level, total bilirubin level, direct bilirubin level, albumin level, or creatinine (all $p > 0.05$). In terms of intraoperative and postoperative clinical characteristics,

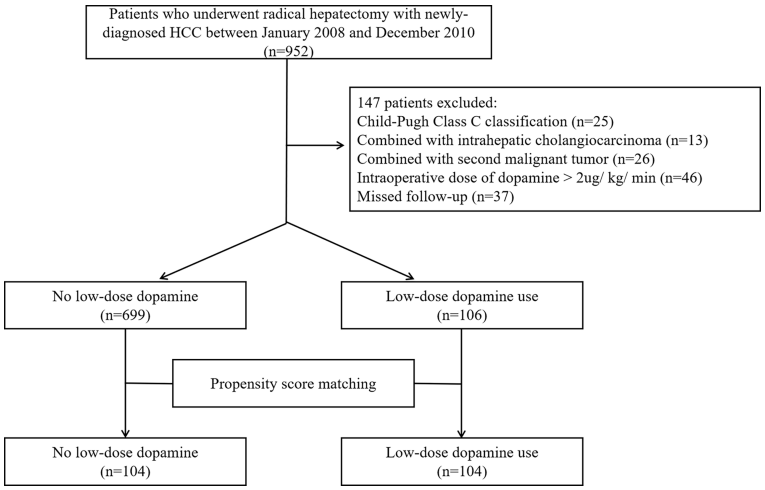


FIGURE 1
Flowchart of the patient selection.

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TABLE 1 Patient characteristics with low-dose dopamine administered before and after propensity score matching.

| Characteristics | Before matching | | | After matching | | |
|---|---|---|---|---|---|---|
| | Low-dose dopamine | | | Low-dose dopamine | | |
| | No (n=699) | Yes (n=106) | *P*-value | No (n=104) | Yes (n=104) | *P*-value |
| Sex | | | | | | |
| Female | 73 (10.4%) | 8 (7.5%) | | 11 (10.6%) | 8 (7.7%) | |
| Male | 626 (89.6%) | 98 (92.5%) | 0.356 | 93 (89.4%) | 96 (92.3%) | 0.470 |
| Age (years) | | | | | | |
| ≤ 50 | 370 (52.9%) | 48 (45.3%) | | 47 (45.2%) | 48 (46.2%) | |
| > 50 | 329 (47.1%) | 58 (54.7%) | 0.142 | 57 (54.8%) | 56 (53.8%) | 0.889 |
| ASA physical status | | | | | | |
| I | 30 (4.3%) | 0 (0.0%) | | 0 (0.0%) | 0 (0.0%) | |
| II | 653 (93.4%) | 101 (95.3%) | | 97 (93.3%) | 100 (96.2%) | |
| III | 16 (2.3%) | 5 (4.7%) | **0.036** | 7 (6.7%) | 4 (3.8%) | 0.353 |
| HBsAg | | | | | | |
| Negative | 28 (4.0%) | 5 (4.7%) | | 6 (5.8%) | 5 (4.8%) | |
| Positive | 671 (96.0%) | 101 (95.3%) | 0.731 | 98 (94.2%) | 99 (95.2%) | 0.757 |
| Cirrhosis | | | | | | |
| No | 217 (31.0%) | 28 (26.4%) | | 30 (28.8%) | 27 (26.0%) | |
| Yes | 482 (69.0%) | 78 (73.6%) | 0.334 | 74 (71.2%) | 77 (74.0%) | 0.641 |
| Child-Pugh classification | | | | | | |
| A | 695 (99.4%) | 106 (100.0%) | | 104 (100.0%) | 104 (100.0%) | |
| B | 4 (0.6%) | 0 (0.0%) | 0.435 | 0 (0.0%) | 0 (0.0%) | – |
| Tumor size (cm) | | | | | | |
| ≤ 5 | 342 (48.9%) | 40 (37.7%) | | 41 (39.4%) | 40 (38.5%) | |
| > 5 | 357 (51.1%) | 66 (62.3%) | **0.032** | 63 (60.6%) | 64 (61.5%) | 0.887 |
| Tumor number | | | | | | |
| 1 | 674 (96.4%) | 100 (94.3%) | | 100 (96.2%) | 98 (94.2%) | |
| > 1 | 25 (3.6%) | 6 (5.7%) | 0.299 | 4 (3.8%) | 6 (5.8%) | 0.517 |
| Satellite nodules | | | | | | |
| No | 557 (79.7%) | 82 (77.4%) | | 76 (73.1%) | 82 (78.8%) | |
| Yes | 142 (20.3%) | 24 (22.6%) | 0.581 | 28 (26.9%) | 22 (21.2%) | 0.330 |
| Vascular invasion | | | | | | |
| No | 642 (91.8%) | 93 (87.7%) | | 91 (87.5%) | 92 (88.5%) | |
| Yes | 57 (8.2%) | 13 (12.3%) | 0.162 | 13 (12.5%) | 12 (11.5%) | 0.831 |
| Preoperative AFP (ng/ml) | | | | | | |
| ≤ 20 | 291 (41.6%) | 60 (56.6%) | | 60 (57.7%) | 58 (55.8%) | |
| 20-400 | 170 (24.3%) | 19 (17.9%) | | 23 (22.1%) | 19 (18.3%) | |
| > 400 | 238 (34.0%) | 27 (25.5%) | **0.015** | 21 (20.2%) | 27 (26.0%) | 0.559 |
| Preoperative ALT (units/L) | | | | | | |
| ≤ 40 | 386 (55.2%) | 73 (68.9%) | | 69 (66.3%) | 71 (68.3%) | |
| > 40 | 313 (44.8%) | 33 (31.1%) | **0.008** | 35 (33.7%) | 33 (31.7%) | 0.768 |
| Preoperative AST (units/L) | | | | | | |
| ≤ 40 | 422 (60.4%) | 67 (63.2%) | | 66 (63.5%) | 65 (62.5%) | |
| > 40 | 277 (39.6%) | 39 (36.8%) | 0.577 | 38 (36.5%) | 39 (37.5%) | 0.886 |
| Preoperative total bilirubin (μmol/L) | | | | | | |
| ≤ 17.1 | 529 (75.7%) | 79 (74.5%) | | 81 (77.9%) | 77 (74.0%) | |
| > 17.1 | 170 (24.3%) | 27 (25.5%) | 0.797 | 23 (22.1%) | 27 (26.0%) | 0.516 |
| Preoperative direct bilirubin (μmol/L) | | | | | | |
| ≤ 6.9 | 582 (83.3%) | 92 (86.8%) | | 96 (92.3%) | 90 (86.5%) | |

*(Continued)*

TABLE 1 Continued

| Characteristics | Before matching | | | After matching | | |
|---|---|---|---|---|---|---|
| | Low-dose dopamine | | | Low-dose dopamine | | |
| | No (n=699) | Yes (n=106) | *P*-value | No (n=104) | Yes (n=104) | *P*-value |
| > 6.9 | 117 (16.7%) | 14 (13.2%) | 0.359 | 8 (7.7%) | 14 (13.5%) | 0.176 |
| Preoperative albumin (g/L) | | | | | | |
| ≤ 35 | 34 (4.9%) | 7 (6.6%) | | 6 (5.8%) | 7 (6.7%) | |
| > 35 | 665 (95.1%) | 99 (93.4%) | 0.448 | 98 (94.2%) | 97 (93.3%) | 0.775 |
| Preoperative creatinine (μmol/L) | | | | | | |
| ≤ 177 | 697 (99.7%) | 105 (99.1%) | | 104 (100.0%) | 103 (99.0%) | |
| > 177 | 2 (0.3%) | 1 (0.9%) | 0.301 | 0 (0.0%) | 1 (1.0%) | 0.316 |

P values of statistical significance are in bold.
ASA, American Society of Anesthesiologists; AFP, Alpha-fetoprotein; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase.

there were no significant differences in all variables between the two groups, including intraoperative fluid infusion, urine output, norepinephrine use, blood loss and duration of operation, postoperative AFP, ALT, AST, bilirubin and creatinine (all $p > 0.05$) (Supplementary Table 1).

## Outcomes in the overall cohort

For the cohort as a whole, the median RFS time was 39.47 months (interquartile range [IQR], 9.60-92.73 months), and the median OS time was 74.10 months (IQR, 26.65-139.02 months). During the follow-up, tumor recurrence was observed in 307 (43.9%) patients in without dopamine group and 56 (52.8%) patients in the low-dose dopamine group. The 1-, 3-, 5- and 10-year RFS rates in the without dopamine group and the low-dose dopamine group were 78.4%, 64.1%, 56.6%, 50.4% and 70.0%, 55.9%, 46.8%, 32.4%, respectively (Figure 2A). The Kaplan–Meier survival curves demonstrated that patients who received low-dose dopamine use had an unfavorable RFS compared with those who did not receive dopamine ($p = 0.009$, Figure 2A). Regarding OS, 511 (73.1%) patients in the without dopamine group and 79 (74.5%) patients in the low-dose dopamine group had died. The 1-, 3-, 5- and 10-year OS rates in the without dopamine group and the low-dose dopamine group were 89.0%, 70.8%, 59.1%, 31.2% and 83.8%, 51.4%, 41.9%, 26.7%, respectively (Figure 2B). The Kaplan–Meier survival curves showed that patients with low-dose dopamine consumption had worsened OS ($p = 0.041$, Figure 2B). When stratified by time of recurrence, early (≤ 2 years) recurrence and late recurrence (> 2 years) were observed in 240 patients and 123 patients, respectively. Patients with low-dose dopamine use had a significantly greater chance of recurrence within 2 years ($p = 0.025$) but not after 2 years ($p = 0.181$) (Table 2).

## Independent prognostic factors for RFS and OS

The predictors for RFS and OS in univariate and multivariate analyses are exhibited in Table 3. Univariable analysis indicated that tumor size, satellite nodules, AFP, ALT, AST, and low-dose dopamine use were associated with RFS (all $p < 0.05$), whereas Child–Pugh classification, tumor size, tumor number, satellite nodules, vascular invasion, AFP, ALT, AST, direct bilirubin, and low-dose dopamine use were associated

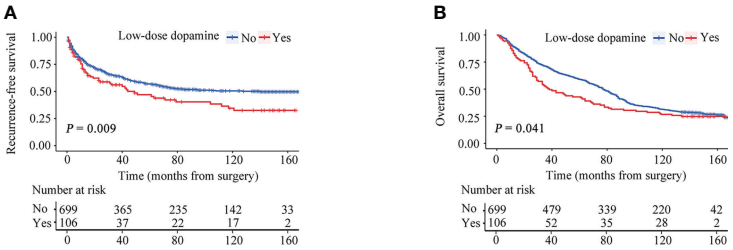


FIGURE 2
Recurrence-free survival (RFS) and overall survival (OS) curves stratified by dose of dopamine before propensity score matching. **(A)** RFS curves. **(B)** OS curves.

TABLE 2 Recurrence in both groups by time of recurrence before propensity score matching.

| | All patients | Low-dose dopamine | | Log - rank |
|---|---|---|---|---|
| | n = 805 | No (n = 699) | Yes (n = 106) | *P*-value |
| All recurrences, n (%) | 363 (45.1%) | 307 (43.9%) | 56 (52.8%) | **0.009** |
| Time of recurrence, n (%) | | | | |
| ≤ 2 years | 240 (29.8%) | 200 (28.6%) | 40 (37.7%) | **0.025** |
| > 2 years | 123 (15.3%) | 107 (15.3%) | 16 (15.1%) | 0.181 |

P values of statistical significance are in bold.

with OS (all $p < 0.05$). Multivariate analysis indicated that satellite nodules (hazard ration [HR]: 1.483; 95% confidence interval [CI]:1.150-1.921; $p = 0.002$), AFP (HR: 1.227; 95% CI: 1.088-1.384; $p = 0.001$), low-dose dopamine (HR: 1.527; 95% CI: 1.145-2.036; $p = 0.004$) were the significant prognostic factor for RFS, and Child–Pugh classification (HR: 2.846; 95% CI: 1.029-7.869; $p = 0.044$), tumor size (HR:1.338; 95% CI: 1.127-1.588; $p = 0.001$), tumor number (HR: 1.618; 95% CI: 1.089-2.405; $p = 0.017$), satellite nodules (HR: 1.441; 95% CI: 1.183-1.755; $p < 0.001$), vascular invasion (HR: 1.482; 95% CI: 1.125-1.954; $p = 0.005$), AFP (HR: 1.114; 95% CI: 1.015-1.224; $p = 0.023$), and AST (HR: 1.230; 95% CI: 1.009-1.499; $p = 0.040$) were independent prognostic factors for OS.

## Recurrence and prognosis after PSM

After PSM, the two groups were completely matched, including 208 patients. None of the baseline characteristics were significantly different (Table 1). Tumor recurrence was observed in 40 (38.5%) patients in the without dopamine group and 56 (53.8%) patients in the low-dose dopamine group. The 1-, 3-, 5- and 10-year RFS rates in the without dopamine group and the low-dose dopamine group were 82.4%, 70.6%, 62.9%, 59.0% and 69.5%, 55.5%, 46.4%, 34.2%, respectively (Figure 3A). In regard to OS, 64 (61.5%) patients in the without dopamine group and 77 (74.0%) patients in the low-dose dopamine group had died. The 1-, 3-, 5- and 10-year OS rates in the without dopamine group and the low-dose dopamine group were 93.3%, 79.8%, 71.2%, 42.3% and 84.5%, 53.4%, 43.7%, 27.2%, respectively (Figure 3B). Similar to the results before PSM, the low-dose dopamine-treated group still had significantly worse RFS ($p = 0.003$, Figure 3A) and OS ($p = 0.002$, Figure 3B) than the group without dopamine use in the matched cohort. In the stratified analyses, patients who infused low-dose dopamine had a significantly higher chance of recurrence within 2 years ($p = 0.007$) but not after 2 years ($p = 0.186$) (Table 4).

# Discussion

This study is the first to evaluate the impact of intraoperative dopamine dosage on the long-term survival outcomes in HCC patients. We found that low-dose dopamine is an independent unfavorable prognostic risk factor in patients who underwent open radical hepatectomy. Moreover, patients with low-dose dopamine use had unsatisfactory long-term RFS and OS. These findings suggested that the administration of low-dose dopamine during hepatic surgery might be an intraoperative medication for predicting prognosis in HCC patients.

Due to the complexity of open hepatic resection, most anesthesiologists control the CVP below 5 mmHg during hepatic parenchymal transection, which significantly reduces intraoperative bleeding and provides an optimal surgical visual field (17, 18). The underlying mechanism is that the controlled low CVP accelerates venous drainage from the hepatic vein and hepatic sinusoids, which results in less backflow from the liver transected surface and less blood loss during resection. However, a lower CVP could cause renal perfusion insufficiency and reduce the effective circulating volume. Based on the pharmacological properties of dopamine, this drug may diminish the probability of low arterial perfusion due to lower intraoperative CVP. Additionally, it has been suggested that low-dose dopamine use can augment renal blood flow *via* dopaminergic receptors located on the renal vasculature (19). Previous clinical trials demonstrated that low-dose dopamine increased the renal perfusion in patients with chronic renal impairment and renovascular disease, albeit to lesser extent than in healthy people (20, 21).

Despite potent renal vasodilatation, low-dose dopamine use could not affect the liver and kidney function of HCC patients based on our present study. However, several findings were illustrated that dopamine given resulted in some side effects, such as arrhythmia and delirium. Chiolero et al. demonstrated an association between low-dose dopamine infusion and unexpected ventricular arrhythmias (22). In our study, we did not discover any cardiac complications. This fact could be ascribed to several reasons. First, Chiolero's study selected patients with cardiac disease undergoing open-heart surgery, while our study did not enroll patients with severe cardiovascular disease. Second, hypothermia and cardioplegic solutions in cardiac surgery may have lowered the β-adrenergic stimulation threshold, which resulted in an increased incidence of arrhythmias in Chiolero's study (22). Furthermore, Yilmaz et al. provided evidence that dopamine administration could give rise to postoperative delirium in cardiac surgical patients (23). But we did not find similar complications in our study. This

TABLE 3 Univariate and multivariate analyses of recurrence-free survival and overall survival in patients before propensity score matching.

| Variables | RFS | | | OS | | |
|---|---|---|---|---|---|---|
| | Univariate Analysis | Multivariable Analysis | | Univariate Analysis | Multivariable Analysis | |
| | *P*-value | HR (95% CI) | *P*-value | *P*-value | HR (95% CI) | *P*-value |
| Sex | | | | | | |
| Female | | | | | | |
| Male | 0.176 | | | 0.569 | | |
| Age (years) | | | | | | |
| ≤ 50 | | | | | | |
| > 50 | 0.486 | | | 0.503 | | |
| ASA physical status | | | | | | |
| I | | | | | | |
| II | | | | | | |
| III | 0.991 | | | 0.405 | | |
| HBsAg | | | | | | |
| Negative | | | | | | |
| Positive | 0.579 | | | 0.548 | | |
| Cirrhosis | | | | | | |
| No | | | | | | |
| Yes | 0.051 | | | 0.438 | | |
| Child-Pugh classification | | | | | | |
| A | | | | | | |
| B | 0.281 | | | < 0.001 | 2.846 (1.029–7.869) | **0.044** |
| Tumor size (cm) | | | | | | |
| ≤ 5 | | | | | | |
| > 5 | **0.005** | 1.185 (0.957–1.467) | 0.120 | < 0.001 | 1.338 (1.127–1.588) | **0.001** |
| Tumor number | | | | | | |
| 1 | | | | | | |
| > 1 | 0.132 | | | **0.029** | 1.618 (1.089–2.405) | **0.017** |
| Satellite nodules | | | | | | |
| No | | | | | | |
| Yes | < **0.001** | 1.483 (1.150–1.912) | **0.002** | < 0.001 | 1.441 (1.183–1.755) | < 0.001 |
| Vascular invasion | | | | | | |
| No | | | | | | |
| Yes | 0.077 | | | < 0.001 | 1.482 (1.125–1.954) | **0.005** |
| Preoperative AFP (ng/ml) | | | | | | |
| ≤ 20 | | | | | | |
| 20-400 | | | | | | |
| > 400 | **0.006** | 1.227 (1.088–1.384) | **0.001** | **0.009** | 1.114 (1.015–1.224) | **0.023** |
| Preoperative ALT (units/L) | | | | | | |
| ≤ 40 | | | | | | |
| > 40 | **0.050** | 1.119 (0.878–1.425) | 0.364 | **0.046** | 1.049 (0.866–1.269) | 0.626 |
| Preoperative AST (units/L) | | | | | | |
| ≤ 40 | | | | | | |
| > 40 | < **0.001** | 1.269 (0.990–1.626) | 0.060 | < 0.001 | 1.230 (1.009–1.499) | **0.040** |
| Preoperative total bilirubin (μmol/L) | | | | | | |
| ≤ 17.1 | | | | | | |
| > 17.1 | 0.634 | | | 0.295 | | |
| Preoperative direct bilirubin (μmol/L) | | | | | | |

*(Continued)*

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TABLE 3 Continued

| Variables | RFS | | | OS | | |
|---|---|---|---|---|---|---|
| | Univariate Analysis | Multivariable Analysis | | Univariate Analysis | Multivariable Analysis | |
| | *P*-value | HR (95% CI) | *P*-value | *P*-value | HR (95% CI) | *P*-value |
| ≤ 6.9 | | | | | | |
| > 6.9 | 0.832 | | | **0.014** | 1.222 (0.985–1.516) | 0.069 |
| Preoperative Albumin (g/L) | | | | | | |
| ≤ 35 | | | | | | |
| > 35 | 0.652 | | | 0.456 | | |
| Preoperative Creatinine (μmol/L) | | | | | | |
| ≤ 177 | | | | | | |
| > 177 | 0.963 | | | 0.169 | | |
| Low-dose dopamine | | | | | | |
| No | | | | | | |
| Yes | **0.009** | 1.527 (1.145–2.036) | **0.004** | **0.041** | 1.260 (0.991–1.600) | 0.059 |

P values of statistical significance are in bold.
RFS, recurrence-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; ASA, American Society of Anesthesiologists; AFP, Alpha-fetoprotein; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase.

difference may be attributable to higher doses of dopamine and longer use duration in Yilmaz's study. In addition, delirium was very common after cardiac surgery (24). Therefore, further studies are required to evaluate the relationship between low-dose dopamine and clinical effects and its internal mechanism.

Despite the improvement of therapeutic strategies and surveillance plans, the postoperative recurrence rate of HCC is still high and strongly associated with poor prognosis. A series of studies have reported that AFP levels and satellite nodules are related to worse survival of liver cancer patients (25–27). These results were similar in our study. However, few studies have assessed the effect of the intraoperative dosage of dopamine on the prognosis of patients with liver cancer. In the present study, we found that the cohort assigned low-dose dopamine had worse long-term RFS and OS. These results remained similar after the use of PSM analysis to balance the confounding bias at the baseline characteristics. Several underlying molecular mechanisms by which dopamine affects prognosis may be considered. First, low-dose dopamine exerts its actions *via* the different dopamine receptor subtypes, grouped as D1-like receptors (DRD1 and DRD5) and D2-like receptors (DRD2, DRD3, and DRD4). Several studies have suggested that there is a close association between the expression of dopamine receptors and prognosis in cancer patients. We previously found that DRD1 was highly expressed in liver cancer tissues and the positive expression of DRD1 is associated with unfavorable RFS and OS in HCC patients (13). Similar results were obtained in another study, suggesting that DRD1 overexpression has a negative effect on prognosis in patients with advanced breast cancer (28). Furthermore, DRD2 agonist could suppress liver cancer cells proliferation, migration and invasion (29). To our knowledge, DRD2 has been reported higher expression in colorectal cancer and gastric cancer compared with non-tumor tissues, and DRD2 expression was related to a poor survival rate (30, 31). Second,

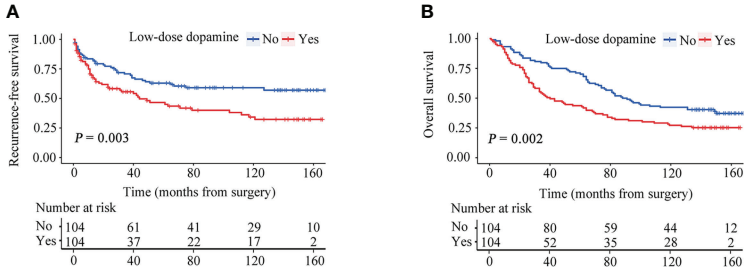


FIGURE 3
Recurrence-free survival (RFS) and overall survival (OS) curves stratified by dose of dopamine after propensity score matching. **(A)** RFS curves. **(B)** OS curves.

TABLE 4 Recurrence in both groups by time of recurrence after propensity score matching.

| | All patients | Low-dose dopamine | | Log - rank |
|---|---|---|---|---|
| | n = 208 | No (n = 104) | Yes (n = 104) | *P*-value |
| All recurrences, n (%) | 96 (46.2%) | 40 (38.5%) | 56 (53.8%) | **0.003** |
| Time of recurrence, n (%) | | | | |
| ≤ 2 years | 63 (30.3%) | 23 (22.1%) | 40 (38.5%) | **0.007** |
| > 2 years | 33 (15.9%) | 17 (16.3%) | 16 (15.4%) | 0.186 |

P values of statistical significance are in bold.

dopamine receptor ligands might influence the biology of tumor cells and alter the tumor microenvironment in a manner independent of their behaviors on neurotransmission, which affects the function on motivation, cognition and sensory (32). Hence, growing evidence emphasizes the importance of dopamine in cancer progression.

This retrospective study has several limitations. First, we do not have information on the other factors that could affect cancer recurrence, such as perioperative opioid consumption and postoperative complications (33–35). Second, the time interval from hepatectomy to recurrence is an important prognostic factor and somewhat controversial, ranging from 6 months to 2 years after surgery (36, 37). Although we defined early recurrence based on the majority of retrospective studies, the different cut-offs may contribute to the different long-term survival outcomes in HCC patients (38, 39). Third, this study was a single-institution study. Therefore, further prospective studies are needed to validate these findings.

In conclusion, compared with no use of dopamine, intraoperatively administered low-dose dopamine has a negative impact on RFS and OS in HCC patients who undergo radical hepatectomy. Dopamine consumption may be considered a potential predictor for the prognosis of patients with HCC. The underlying mechanisms of the association between the dosage of dopamine and the long-term prognosis in HCC patients should be further investigated.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

## Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Ethics Committees of Sun Yat-sen University Cancer Center. The ethics committee waived the requirement of written informed consent for participation.

## Author contributions

All authors contributed to the study conception and design. YW, RX and YY were involved in data analysis and interpretation. YW, RX, YY and DC were involved in manuscript writing, and all the listed authors revised the submitted manuscript and approved its final version before submission.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.947172/full#supplementary-material

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# Effect of dexmedetomidine on postoperative systemic inflammation and recovery in patients undergoing digest tract cancer surgery: A meta-analysis of randomized controlled trials

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Perioperative immune function, postoperative cognitive function and prognosis are momentous issues for patients undergoing digestive tract cancer surgery. Studies have investigated the efficacy of dexmedetomidine (DEX) administration on these issues, but the results are inconsistent. Therefore, this meta-analysis aimed to summarize all the existing evidence and draw a conclusion more accurately on these associations. Trials were located through electronic searches of the PubMed, Embase, the Cochrane Library and Web of Science databases sources (from the establishment date of databases to April 2022). Bibliographies of the retrieved articles were checked. A total of 17 RCTs involving 1619 patients were included. The results showed that DEX decreased the level of C-reactive protein (SMD = -4.26, 95%CI: -6.16, -2.36), TNF-α (SMD = -4.22, 95%CI: -5.91, -2.54) and IL-6 (SMD = -2.71, 95%CI: -4.46, -0.97), and increased the level of IL-10 (SMD = 1.74, 95%CI: 0.25, 3.24). DEX also increased CD4+ T cells (SMD = 0.55, 95%CI: 0.29, 0.82) and CD4+/CD8+ ratio (SMD = 0.62, 95%CI: 0.24, 1.01). Thus, DEX was associated with alleviation of postoperative systemic inflammatory response and immune dysfunction. Furthermore, DEX increased mini-mental state examination scores at 12h (SMD = 1.10, 95%CI: 0.74,1.45), 24h (SMD = 0.85, 95%CI: 0.59, 1.11), 48h (SMD = 0.89, 95%CI: 0.50, 1.28) and 72h (SMD = 0.75, 95%CI: 0.38, 1.11) after surgery. DEX decreased the occurrence of postoperative cognitive dysfunction (POCD) at 24h (OR = 0.22, 95%CI: 0.11, 0.46) and 72h (OR = 0.39, 95%CI: 0.22, 0.68) after surgery. DEX decreased first flatus time (SMD = -1.55, 95%CI: -2.82, -0.27) and hospital stay (SMD = -1.23, 95%CI: -1.88, -0.59).

Therefore, based on perioperative immune dysfunction alleviation, DEX attenuated POCD and potential neuroinflammation, improved postoperative recovery and clinical prognosis of patients undergoing digest tract cancer surgery. Further studies are necessary to elucidate the clinical application of DEX from an immunological perspective.

KEYWORDS

dexmedetomidine, immune function, postoperative cognitive dysfunction, digestive tract cancer, prognosis, meta-analysis

## Introduction

Systemic immune perturbations occur with cancer development (1). Tumor-burdened microenvironment affects the quantity and differentiation of T cells, neutrophils, and monocyte, especially for the elderly with underlying diseases and impaired immune function (2, 3). Radical surgery is the preferred treatment for most patients with early-stage cancer (4). However, the incidence of systemic inflammatory response syndrome (SIRS) increased during the perioperative period due to anesthesia, surgical trauma, and pre-existing comorbidities. The release of damage-associated molecular patterns (DAMPs) or alarmins following the surgical injury is the important involved mechanism (5, 6). DAMPs could activate immune cells including neutrophils and lymphocytes, and trigger the release of pro-inflammatory mediators including IL-6, IL-1, and TNF-α (7, 8). High mobility group box 1 protein (HMGB1) is a DAMP molecule. It affects the activation and differentiation of Treg and is associated with cancer recurrence and metastasis (9). Meanwhile, perioperative factors and peripheral inflammation are associated with central nervous system (CNS) neuroinflammation and pathologies (10, 11). Elevated inflammatory cytokines in the CNS are concentrated in the hippocampus, where the receptors of pro-inflammatory cytokines were highly expressed, leading to postoperative cognitive dysfunction (POCD), especially for the elderly with an impaired blood-brain barrier (BBB) (12, 13). Therefore, perioperative immune dysfunction and CNS neuroinflammation have momentous clinical implications in postoperative recovery, tumor recurrence, and metastasis, etc.

Dexmedetomidine (DEX) is a highly selective $\alpha_2$ adrenergic receptor agonist, especially for the $\alpha_{2A}$ adrenergic receptor located in the locus coeruleus nucleus (14). DEX has been frequently used in the perioperative period because of its sedative pharmacology. DEX could attenuate stress responses and emotional disorders, and create stable hemodynamic profiles during stressful events such as surgery or anesthetic induction (15). DEX could resemble natural sleep, increase physiological sleep-wake cycle for ICU patients, and reduce the risk of delirium (16). DEX could also reduce the level of postoperative inflammatory factors through PI3K-Akt signaling (17), and inhibit cancer development through the upregulation of miR-185 and inactivation of SOX9-Wnt-β-catenin signaling (18). Moreover, there is growing evidence that DEX has a potential role during perioperative period for the prevention and alleviation of inflammation and immune dysfunction (19).

Multiple RCTs have been conducted to determine whether perioperative intravenous DEX could alleviate postoperative SIRS and POCD in patients undergoing radical surgery (20, 21). However, due to the methodology and small sample size, interpretation of these studies has limitations and the results are inconsistent. Meanwhile, the mechanism by which DEX interferes with cellular and humoral immunity is still unclear. Therefore, this meta-analysis aimed to summarize all existing evidence and systematically review the impact of DEX on perioperative immune dysfunction, POCD, and postoperative recovery, to provide guidance for clinical treatment and prognosis.

## Methods

This meta-analysis was conducted based on the criteria of the Cochrane Handbook for Systematic Reviews of Interventions (version 6.2). The results were presented according to the preferred reporting items declared by Systematic Review and Meta-Analysis (PRISMA) 2020. Ethical approval was not required, as this study only included articles of published data in the public domain.

### Literature search

Two reviewers performed the literature search, systematically searching the PubMed, Embase, the Cochrane Library, and Web of Science databases sources until April 2022 for studies exploring the application of perioperative DEX in patients with digestive

tract cancer. The following search terms were used: (1) "Dexmedetomidine", "MPV-1440", "Precedex" or "Dexmedetomidine Hydrochloride", (2) "Esophageal Neoplasms", "Stomach Neoplasms", "Gastrointestinal Neoplasms", "Colorectal Neoplasms", "Colonic Neoplasms", "Rectal Neoplasms", "Intestinal Neoplasms", "Esophageal Cancer", "Gastric Cancer", "Intestinal Cancer", "Colorectal Cancer", "Colon Cancer", "Rectal Cancer", "Gastrointestinal Cancer" or "Digestive Tract Cancer". The above two categories of search terms were combined using the Boolean operator "and". The search strategies are shown in Table 1, and the detailed electronic search strategies for PubMed, Cochrane Library, EMBASE and Web of Science databases are shown in Supplementary Table 1. In addition, the reference lists of the retrieved articles and prior reviews were manually checked for additional eligible studies. We applied no linguistic restrictions in the literature search.

## Inclusion and exclusion criteria

RCTs conducted to compare DEX with placebo in patients undergoing digest tract tumor surgery were all enrolled. Included studies need to report at least one of the outcomes, including inflammatory factors, cellular immunity, cognitive function, and prognosis. Exclusion criteria were as follows: (1) reviews, letters, editorials, or observational (prospective or retrospective cohort) study; (2) comparisons of DEX with other sedatives(midazolam, fentanyl, propofol, etc.); (3) no intravenous administration; (4) no target outcomes; (5) data was unable to obtain or insufficient. If there were overlapping data among two or more studies, we included the one with the largest sample size.

## Study selection and data abstraction

Two reviewers independently screened the titles and abstracts of the retrieved studies from the electronic databases. Subsequently, eligible studies were selected after full-text screenings according to the pre-defined criteria. Disagreements were resolved by discussion between two reviewers or consultation with the corresponding authors. The following data of the included studies were abstracted: study characteristics (first author, year of publication, and study design), study population, baseline characteristics (age, sample size, interventions, and anesthesia method), outcomes (inflammatory factors, cellular immunity, cognitive function, and prognosis), and outcome data (sample size and the number of events between groups).

TABLE 1 The search strategies until February 2022.

| | Search terms | PubMed | Embase | Web of science | Cochrane |
|---|---|---|---|---|---|
| **#1** | Dexmedetomidine | 7555 | 15170 | 9929 | 6184 |
| **#2** | MPV-1440 | 7556 | 4 | 1 | 3 |
| **#3** | Precedex | 7558 | 523 | 54 | 82 |
| **#4** | Dexmedetomidine Hydrochloride | 7555 | 130 | 223 | 123 |
| **#5** | #1 OR #2 OR #3 OR #4 | 7559 | 15170 | 9931 | 6181 |
| **#6** | Esophageal Neoplasms | 63836 | 2205 | 3419 | 2395 |
| **#7** | Stomach Neoplasms | 119232 | 5731 | 5952 | 3896 |
| **#8** | Gastrointestinal Neoplasms | 443191 | 1149 | 12158 | 5394 |
| **#9** | Colorectal Neoplasms | 240215 | 5798 | 13841 | 8733 |
| **#10** | Colonic Neoplasms | 92777 | 1677 | 3429 | 4402 |
| **#11** | Rectal Neoplasms | 69204 | 1662 | 4772 | 3351 |
| **#12** | Intestinal Neoplasms | 270675 | 383 | 3813 | 2216 |
| **#13** | Esophageal Cancer | 73668 | 35427 | 53390 | 4858 |
| **#14** | Gastric Cancer | 152596 | 102860 | 128855 | 8623 |
| **#15** | Intestinal Cancer | 285551 | 1648 | 49274 | 4676 |
| **#16** | Colorectal Cancer | 271702 | 233478 | 251089 | 17298 |
| **#17** | Colon Cancer | 152047 | 113537 | 158616 | 8164 |
| **#18** | Rectal Cancer | 76673 | 42414 | 59070 | 6221 |
| **#19** | Gastrointestinal Cancer | 469120 | 18366 | 118013 | 11785 |
| **#20** | Digestive Tract Cancer | 159,200 | 516 | 30,874 | 621 |
| **#21** | #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 | 651,377 | 492021 | 964,761 | 56828 |
| **#22** | #5 AND #21 | 92 | 126 | 97 | 137 |

## Study quality assessment

The bias risks of RCTs were assessed using the revised Cochrane risk-of-bias tool for randomized trials (RoB2), which is more detailed and comprehensive than RoB1, and includes five domains: randomization process, deviations from the intended interventions, missing outcome data, measurement of the outcome, and selection of the reported results. The level of the bias risk in each domain and overall were scored as 'low risk', 'some concerns', or 'high risk'. We used the funnel plots to assess the publication bias, and there was no significant asymmetry in the funnel plots of the present data (Supplementary Figures 1–4). Thus, there was no significant publication bias. Supplementary Table 2 presents the GRADE analysis of the variables examined in this meta-analysis. The certainty was high for POCD and MMSE; moderate for CRP, IL-6, IL-10, lymphocyte subsets, first flatus time, hospital stay and extubation time, and low for TNF-α.

## Statistical analysis

Statistical analysis for this meta-analysis was This meta-analysis's statistical analyses were conducted using the Review Manager version 5.4 software (the Cochrane Collaboration 2014, Nordic Cochrane Centre Copenhagen, Denmark; https://community.cochrane.org/). The pooled effects were calculated and described by standard mean difference (SMD) with a 95% Confidence Interval (the Confidence Interval, 95% CI) and the risk ratio with 95% CI. The significant heterogeneity was indicated by a P-value of $< 0.10$ in the Cochrane Q test or an $I^2$ value of $> 50\%$, which led to the use of random-effects models and the exploration of a potential source of heterogeneity. Otherwise, the fixed-effects model was selected.

# Results

## Study selection outcome

The flow chart of literature retrieval is shown in Figure 1. Through searching the PubMed, Embase, Cochrane Library, and Web of Science databases, the initial search yielded 452 articles. We also identified the potential studies by searching the reference lists of published reviews, in this way, we found a relevant article from Chinese biological and medical database. Duplicate articles were removed. After screening the records, reviewing the title and abstract, and the full-text screenings with the pre-defined criteria to exclude 15 studies, a total of 17 studies were included in this meta-analysis.

## Study characteristics

17 RCTs involving 1619 patients undergoing surgical resection of digest tract tumors were finally included. Baseline characteristics and detailed administration of included studies are shown in Table 2. The mean age of patients ranged from 36.3 to 74.1 years, and the sample size was from 48 to 180. The detailed usage and dosage of DEX are shown in Table 2. DEX was administered intravenously in all studies. 14 studies administered a loading dose before induction and followed by continuous infusion. The other 3 studies only administered a loading dose before induction. The control group was injected intravenously with the same volume of normal saline.

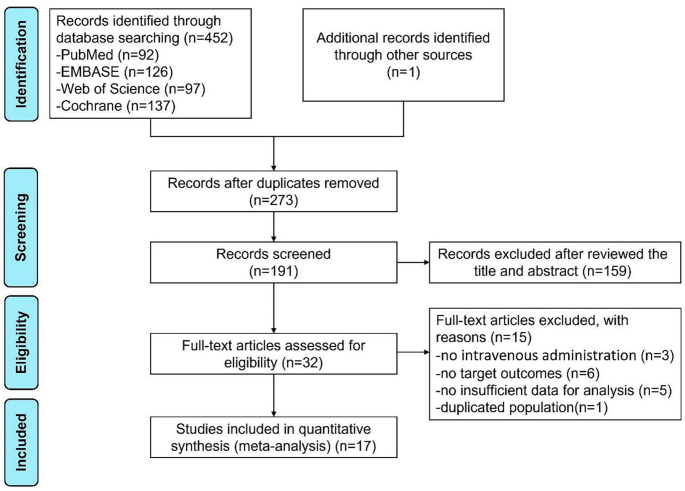


FIGURE 1
The flow chart of literature retrieval of this meta-analysis.

TABLE 2 Characteristics of included studies.

| Author -Year | Trial type | Simple size | | Mean Age (Years) | | Male ratio (%) | | Intervention study(DEX dose/ administration mode) | Neoplasm's type | Comparators | Anesthesia method |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CON | DEX | CON | DEX | CON | DEX | | | | |
| **Mao Y-2020** (22) | RCT | 29 | 29 | 63.5 | 65.2 | 82.8 | 79.3 | Bolus (0.5 ug/kg) before induction, and then continuous infusion (0.2-0.4 ug/kg/h) during operation | Esophageal Cancer | Saline | I.V. |
| **Dong W-2017** (23) | RCT | 37 | 37 | 38.7 | 36.3 | 54.1 | 62.2 | Bolus (1 ug/kg; 15 min) before induction, and then continuous infusion (0.2 ug/kg/h) during operation | Gastric Cancer | Saline | Combined |
| **Niu Y-2022** | RCT | 30 | 30 | 69.0 | 68.0 | 60.0 | 56.7 | Bolus (0.6 ug/kg; 15 min) before induction, and then continuous infusion (0.2 ug/kg/h) during operation | Gastric Cancer | Saline | I.V. |
| **Wang Z-2020** (17) | RCT | 50 | 60 | 68.3 | 68.4 | 60.0 | 63.3 | Bolus (0.5 ug/kg) before induction, and then continuous infusion (0.4 ug/kg/h) during operation | Gastric Cancer | Saline | Combined |
| **Zhu Z-2017** (24) | RCT | 45 | 45 | 51.5 | 51.8 | 55.6 | 48.9 | Bolus (0.6 ug/kg) before induction | Gastric Cancer | Saline | Combined |
| **Huai Q-2022** (25) | RCT | 40 | 40 | 63.5 | 61.7 | 65.0 | 60.0 | Bolus (0.5 ug/kg; 10 min) before induction, and then continuous infusion (0.4 ug/kg/h) during operation | Colon Cancer | Saline | Inhalation |
| **Wang K-2018** (26) | RCT | 69 | 72 | 45.3 | 42.5 | 47.8 | 50.0 | Bolus (1 ug/kg; 10-15 min) before induction, and then continuous infusion (1 ug/kg/h) during operation | Colon Cancer | Saline | Combined |
| **Zhao L-2020** (27) | RCT | 84 | 92 | 53.1 | 52.5 | 56.0 | 45.7 | Bolus (200ug) | Colon Cancer | Saline | I.V. |
| **Ben Z-2016** (28) | RCT | 44 | 44 | 68.6 | | 59.1 | | Bolus (0.5 ug/kg) before induction, and then continuous infusion (0.1 ug/kg/h) during operation | Rectal Cancer | Saline | I.V. |
| **Chen C-2016** (29) | RCT | 30 | 30 | 60.1 | 56.7 | 50.0 | 46.7 | Bolus (1 ug/kg; 10 min) before induction, and then continuous infusion (0.3 ug/kg/h) during operation | Colorectal Cancer | Saline | Inhalation |
| **Kong Y-2021** (30) | RCT | 60 | 120 | 65.0 | 65.0 | NA | NA | Bolus (0.5 ug/kg) before induction, and then continuous infusion (0.4/0.8 ug/kg/h) during operation | Colorectal Cancer | Saline | I.V. |
| **Liu X-2017** (31) | RCT | 48 | 48 | 69.1 | 68.4 | 54.2 | 52.1 | Bolus (1.5 ug/kg;30min) | Colorectal Cancer | Saline | I.V. |
| **Liu Y-2020** (32) | RCT | 24 | 24 | 68.6 | 69.6 | 54.2 | 62.5 | Bolus (0.5 ug/kg; 15 min) before induction, and then continuous infusion (0.6 ug/kg/h) during operation | Colorectal Cancer | Saline | I.V. |
| **Pan C-2016** (33) | RCT | 41 | 41 | 73.9 | 71.9 | NA | 48.8 | Bolus (0.5 ug/kg) before induction, and then continuous infusion (0.3 ug/kg/h) during operation | Colorectal Cancer | Saline | NA |
| **Sun W-2021** (34) | RCT | 28 | 28 | 59.0 | 60.0 | 60.7 | 67.9 | Bolus (1 ug/kg; 10 min) before induction, and then continuous infusion (0.5 ug/kg/h) during operation | Colorectal Cancer | Saline | Combined |
| **Zhang J-2019** (35) | RCT | 60 | 80 | 74.1 | 73.8 | 66.7 | 63.8 | Bolus (1 ug/kg; 15 min) before induction, and then continuous infusion (0.2-0.7 ug/kg/h) during operation | Colorectal Cancer | Saline | I.V. |
| **Zhang Y-2014** (36) | RCT | 20 | 60 | 71.5 | 72.0 | 55.2 | 60.0 | Bolus (0.5 ug/kg; 15 min) before induction, and then continuous infusion (0.2/0.5/0.8 ug/kg/h) during operation | Colorectal Cancer | Saline | I.V. |

## Risk of bias assessment

The risk of bias assessment for individual studies is shown in Figure 2. Of the included trials, nine studies did not give a specific randomization process, which was categorized as "some concerns". One study deviated from the intended intervention, and two studies were judged high risk in terms of the measurement of the outcomes. The remaining eight studies were identified as low risk.

## Synthesis of results

The different outcomes of 17 studies were synthesized. First, the changes of inflammatory mediators were concerned, as surgical and anesthetic factors can lead to systemic inflammatory responses that further exacerbate central inflammation. Then, T lymphocyte subsets were observed, which has been proven to be an important indicator of immune dysfunction, The systemic inflammatory response and immune dysfunction in the perioperative period will further aggravate the CNS neuroinflammation and cognitive dysfunction of patients, thus affecting postoperative recovery and clinical prognosis. Therefore, our study comprehensively considered the effects of DEX on inflammatory mediators, T lymphocytes, POCD, and postoperative recovery.

## Effects of DEX on postoperative inflammatory mediators

9 RCTs that reported the postoperative levels of inflammatory mediators in 889 patients with digest tract tumors were included (22–26, 28, 30, 31, 33). CRP has been used as a marker of acute inflammatory responses in a variety of psychiatric and physical conditions. As shown in Figure 3A, the pooled results based on the random-effects model indicated that the use of DEX was significantly associated with reduced CRP release (SMD = -4.26, 95%CI: -6.16, -2.36). Meanwhile, in order to illustrate the effects of DEX on inflammation, several inflammatory cytokines were investigated based on the involved studies. The results indicated that DEX decreased the release of TNF-α (SMD = -4.22, 95%CI: -5.91, -2.54, Figure 3B) and IL-6 (SMD = -2.71, 95%CI: -4.46, -0.97, Figure 3C), but increased the release of IL-10 (SMD = 1.74, 95%CI: 0.25, 3.24, Figure 3D). These release changes could improve cellular immunosuppression and attenuate the progress of digest tract cancer.

## Effects of DEX on postoperative T lymphocytes

T lymphocytes play roles in perioperative immune homeostasis and tumor resistance within the digestive tract; thus, the state and subsets of T lymphocytes were investigated. Three studies, including 391 patients, investigated the effects of DEX on T lymphocytes (23, 26, 27). T lymphocyte subsets included were CD3+, CD4+, and CD8+ T cells. At the same time, we calculated the CD4+/CD8+ ratio, which decreased and indicated impaired immune functions and poor prognosis. Separately, there was no significant difference in the counts of CD3+ T cells (SMD = 0.42, 95%CI: -0.57, 1.41) and CD8+ T cells (SMD = -0.02, 95%CI: -0.57, 0.54) between patients treated with and without DEX at 24h postoperatively. In contrast, CD4+ T cell counts (SMD = 0.55, 95%CI: 0.29, 0.82) and CD4+/CD8+ ratio (SMD = 0.62, 95%CI: 0.24, 1.01) increased in patients with DEX (Figure 4). Therefore, DEX attenuated the variation of cellular immune functions caused by surgical trauma, stress responses and other perioperative factors.

## Effects of DEX on cognitive function

Random-effects model and fixed-effect model were used to synthesize the MMSE scores and the incidence of POCD at different time points after surgery in 7 RCTs to consider the effect of DEX on postoperative cognitive function comprehensively (17, 28, 31–33, 35, 36). As shown in Figure 5, in digest tract tumor patients, DEX administration was associated with higher MMSE scores at 12h (SMD = 1.10, 95%CI: 0.74, 1.45), 24h (SMD = 0.85, 95%CI: 0.59, 1.11), 48h (SMD = 0.89, 95%CI: 0.50, 1.28) and 72h (SMD = 0.75, 95%CI: 0.38, 1.11) after surgery. DEX administration was also associated with a significant reduction in the occurrence of POCD at 24h (OR = 0.22, 95%CI: 0.11, 0.46) and 72h (OR = 0.39, 95%CI: 0.22, 0.68) after surgery. As previous studies indicated, the changes of cognitive function could be associated with perioperative immune function and neuroinflammation (37).

## Effects of DEX on postoperative recovery

The perioperative stress and inflammation could affect gastrointestinal motility, extubation time and hospital stay, which are considered as the indicators for patient recovery and clinical prognosis. Therefore, a random-effects model was used to synthesize the postoperative extubation time, first flatus time and hospital stay of 808 patients in 11 RCTs (22, 25, 27–34, 38). As shown in Figure 6, DEX administration decreased first flatus time (SMD = -1.55, 95% CI: -2.82, -0.27), and the length of hospital stay (SMD = -1.23, 95% CI: -1.88, -0.59). However, there was no significant difference in postoperative extubation time (SMD = -0.74, 95%CI: -2.08, 0.61).

# Discussion

This study investigated the effects of DEX administration on postoperative immune dysfunction, cognitive function, and


FIGURE 2
Methodological quality graph and summary of the included studies: Risk of bias summary **(A)**, Risk of bias graph **(B)**.

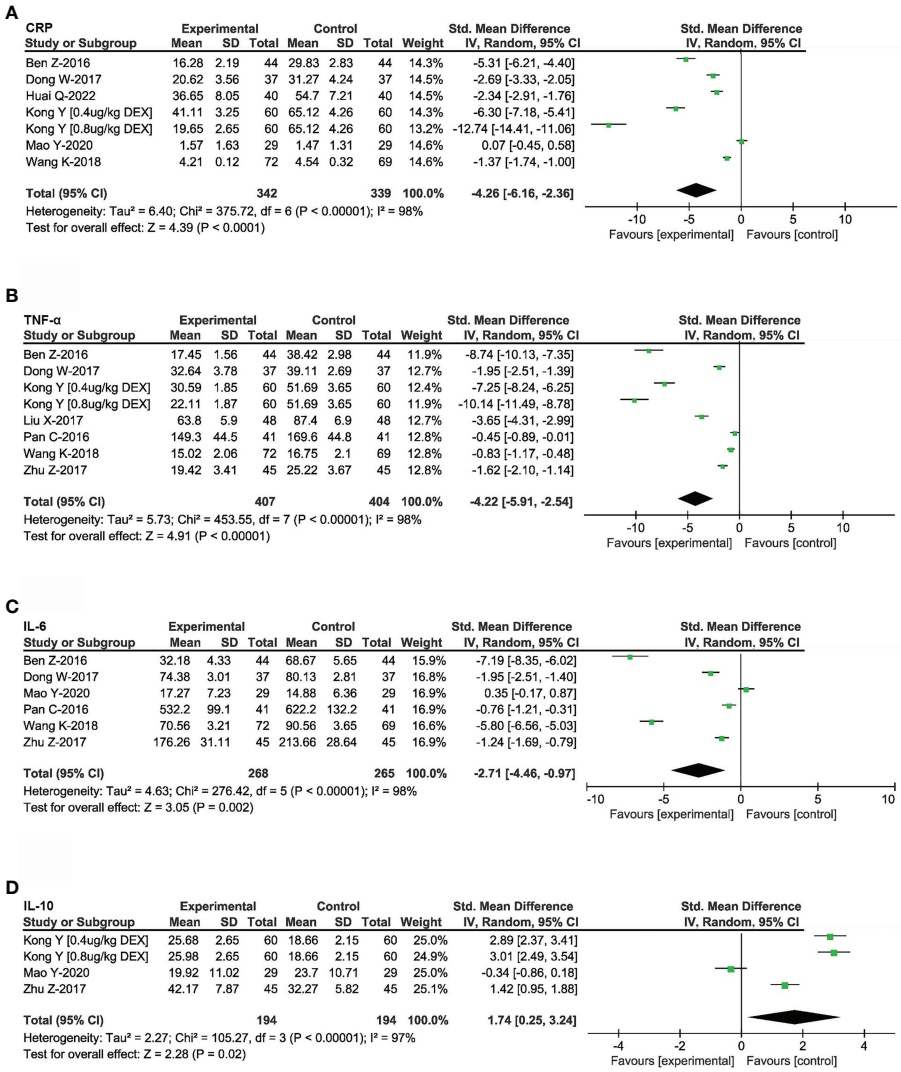


FIGURE 3
Effects of DEX on inflammatory mediators. Forest plot of odds ratio, analyzed by Mantel-Haenszel statistics in the random-effect model. Meta-analysis of the DEX effect on CRP **(A)**, TNF-a **(B)**, IL-6 **(C)** and IL-10 **(D)** respectively.

recovery. The results indicated that DEX decreased the level of CRP, TNF-α, and IL-6, and increased the level of IL-10. DEX increased CD4+ T cell counts and CD4+/CD8+ ratio. Furthermore, DEX led to higher MMSE scores during postoperative periods and a significant reduction in the occurrence of POCD. DEX decreased the first flatus time and the length of hospital stay, but not postoperative extubation time. Therefore, DEX administration attenuated postoperative systemic inflammatory response and immune dysfunction, improved cognitive function, and recovery in patients undergoing digest tract cancer surgery.

Previous studies showed that cancer could lead to systemic immune perturbations and affect responses to new immune challenges (1). Meanwhile, surgery could put body in a stress state, which leads to the imbalance of the neuro-endocrine-immune network, resulting in low cellular and humoral immune functions (39). The present results indicated that DEX could decrease postoperative levels of CRP, TNF-α and IL-6, and increase the level of IL-10. CRP is widely used as a marker of inflammation, infection, and tissue damage (40), and plays an important role in tumor development. The prognostic value of CRP has already been shown for digestive tract cancer (41). As a major cytokine in the acute phase, IL-6 is associated with the pathological progress of digest tract cancer (42). Low serum IL-6 level has been shown to be an independent prognostic factor for disease-free survival of patients with hepatitis B virus-related

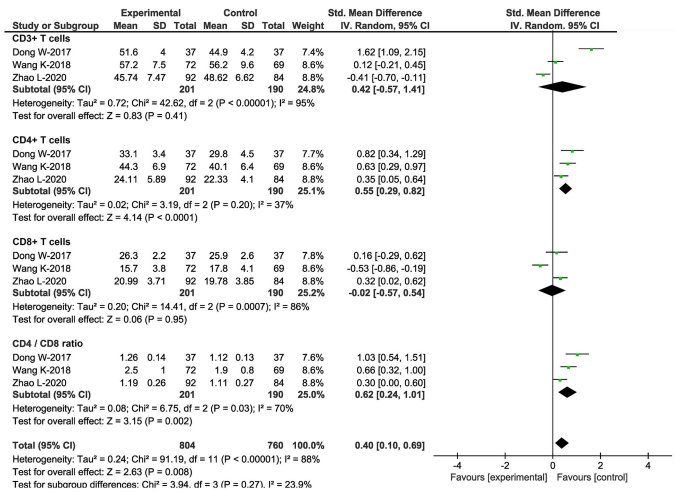


FIGURE 4
Effects of DEX on T lymphocytes. Forest plot of odds ratio, analyzed by Mantel-Haenszel statistics in the random -effect model. Meta-analysis of the DEX effect on CD3+ T cells, CD4+ T cells, CD8+ T cells and CD4+ /CD8+ ratio respectively.

hepatocellular carcinoma who underwent hepatic resection (43). IL-10 is an anti-inflammatory mediator and plays a dual role in immune modulation, depending on cell type and environment (44). The immunosuppressive role of IL-10 has led to the general view that its presence during the development of cancer would facilitate tumor immune escape (45). However, A recent study showed that IL-10 potentiated the antitumor activity of CD8+ T cells by increasing its tissue infiltration, inducing IFN-γ, and favoring effective T cell memory responses (46, 47). Previous studies showed that surgery triggered a central response *via* afferent nerves to activate the sympathetic-adrenal-medullary (SAM) axis, and increased blood leukocyte counts (48, 49). Dexmedetomidine could affect SAM, reduce the release of epinephrine and norepinephrine, and decrease inflammatory factors (50, 51). DEX could also reverse HMGB1 related systemic and hippocampal inflammatory responses through the following mechanisms: 1) stimulation of the vagus nerve, 2) elimination of DAMP molecules and damaged mitochondria through PINK1-mediated mitophagy, 3) promotion the resolution of inflammation through TGF-31 secreted by F4/80Ly6G (52–54). Therefore, DEX could attenuate the negative effects of DAMP and inflammatory responses during digest tract cancer surgery.

T-lymphocyte could maintain the homeostasis within digestive tract mucosa and play an important role in anti-tumor immunity. The present results indicated that DEX could increase postoperative CD4+ T cells and CD4+/CD8+ ratio. CD4+ T cells play an important role in anti-tumor response. Recent studies have shown that CD4+ T cells not only enhanced the tumoricidal activity of other anti-tumor effector cells, but also blocked tumor growth through directly affecting the progression of tumor cell cycle (55). All mature peripheral T-lymphocytes, labeled by CD3+, represent the general level of immunity, and reduction of CD4+/CD8+ ratio indicates decreased immune function and poor prognosis (56, 57). Therefore, DEX could attenuate perioperative cellular immune function suppression, and further alleviate inflammatory response.

The patients with cancer are more vulnerable to postoperative systemic immune dysfunction and the peripheral environment is connected with CNS (58). Pro-inflammatory signals from peripheral immune system could enter CNS and cause neurotoxic symptoms (59). During radical surgery, intracellular substances released from damaged tissues and organs will be recognized by immune cells (60). Immune cells affect the expression of inflammatory factors, which can trigger the CNS response and amplify neuroinflammation through vagal afferents or BBB (61, 62). The inflammatory cells in CNS release more inflammatory cytokines which concentrate in specific brain regions, leading to the occurrence of POCD (37, 63, 64) and resulting in the development of neurodegenerative diseases (65). The present results indicated that DEX decreased the occurrence of POCD, which could be related to the effect of DEX on perioperative immune function and potential neuroinflammation.

Surgery is a common treatment for gastrointestinal tumors. The digestive tract is an important immune organ, and surgery could cause irreversible damage to it. Resection of digest tract cancer leads to anatomical abnormality and deficient intestinal function, and pneumoperitoneum induces ischemia and hypoxia in intestinal mucosa, which impair intestinal mucosa barrier

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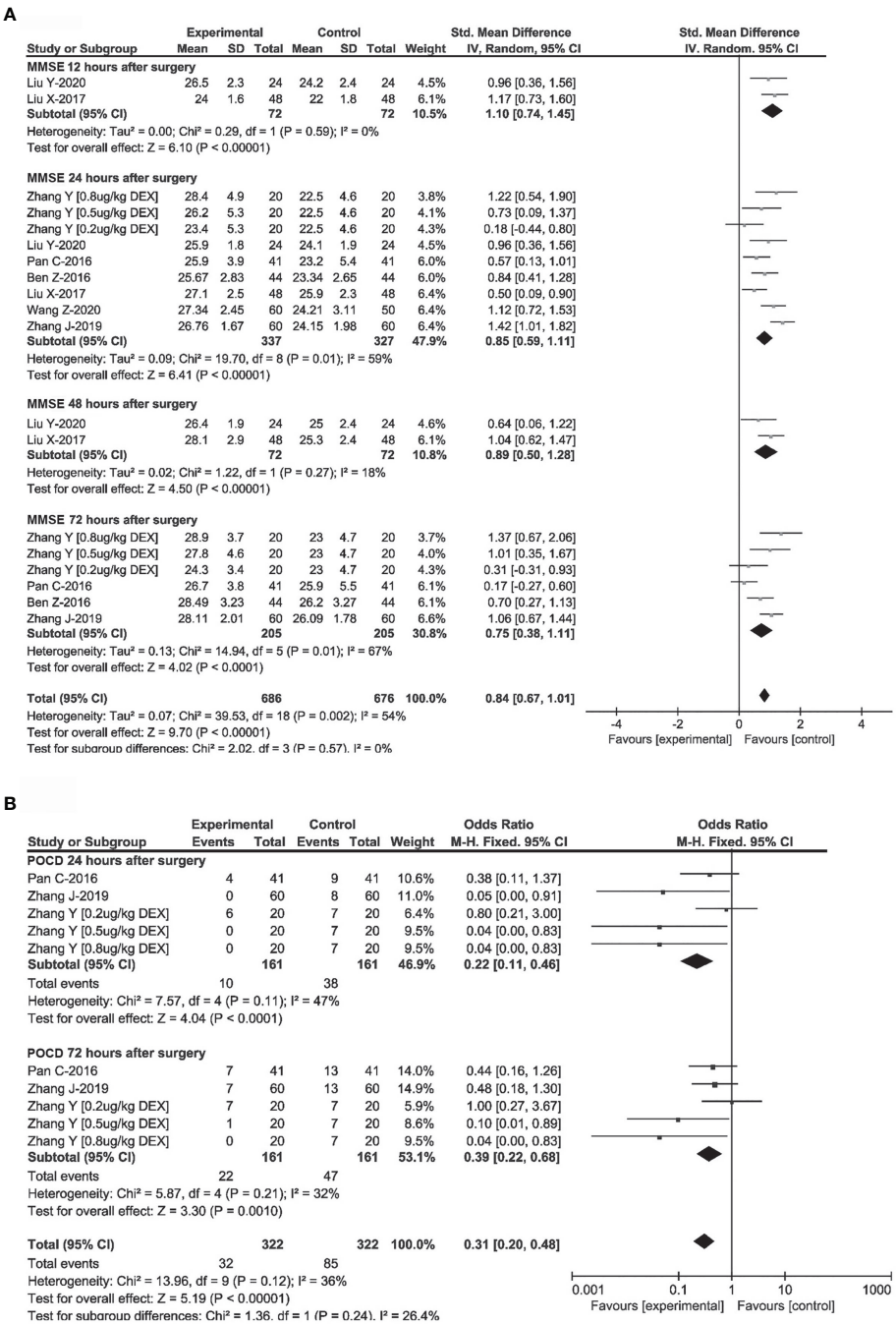


FIGURE 5
Effects of DEX on postoperative cognitive function. Forest plot of odds ratio, analyzed by Mantel-Haenszel statistics in the random-effect model. Meta-analysis of the DEX effect on MMSE at 24h, 48h, 72h after surgery **(A)**, and the occurrence of POCD at 24h, 72h after surgery **(B)** respectively.

function and result in intestinal bacterial translocation and inflammatory responses (56, 66). Meanwhile, advanced age, malnutrition, co-morbidities, and the occurrence of POCD could also affect the recovery of gastrointestinal motility, length of hospital stay and even the prognosis of patients. The present result indicated that DEX could decrease first flatus time and the length of hospital stay. The potential mechanisms for flatus time reduction include: 1) DEX reduces surgical stress and pain, then improves hemodynamics stability and intestinal microcirculation alteration (67), 2) DEX improves cognitive

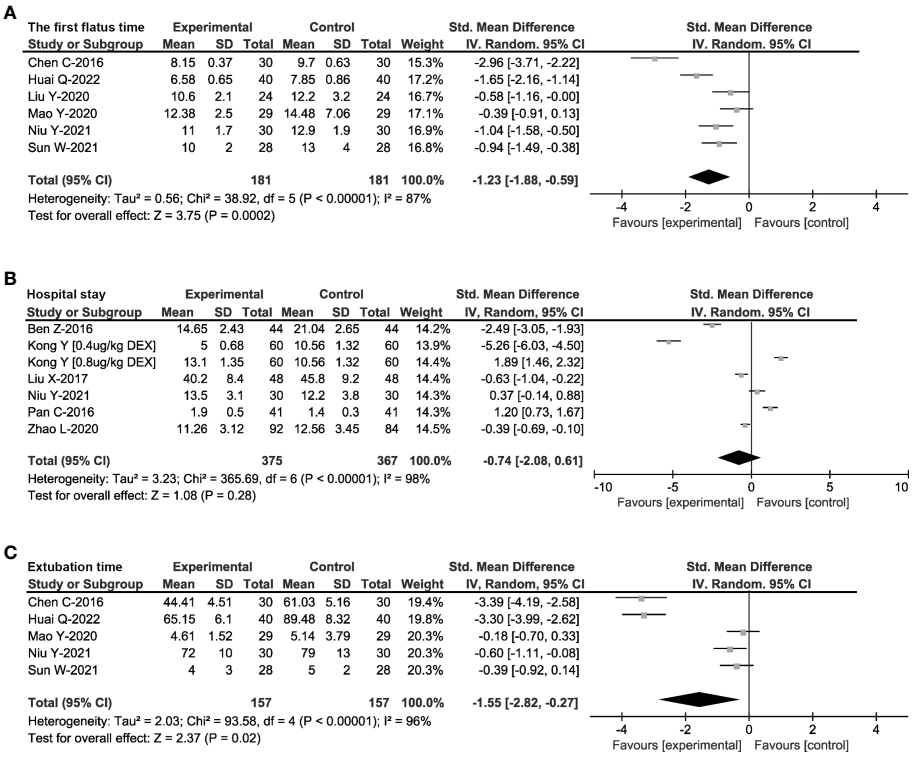


FIGURE 6
Effects of DEX on prognosis. Forest plot of odds ratio, analyzed by Mantel-Haenszel statistics in the random-effect model. Meta-analysis of the effect on the first flatus time **(A)**, hospital stay **(B)** and postoperative extubation time **(C)** respectively.

dysfunction and early postoperative activity; 3) DEX accelerates intestinal wound healing through increasing intestinal epithelial cell proliferation (68). Postoperative systemic immune dysfunction, POCD and gastrointestinal dysfunction lead to an array of symptoms, and other physiological/psychological diseases. They could affect the length of hospital stay, and the standard of living after discharge. Therefore, DEX administration could be valuable strategy for the patients with tumors to improve postoperative gastrointestinal function and prognosis.

This meta-analysis describes the effect of dexmedetomidine on postoperative systemic inflammation and recovery from the levels of immunomodulators, cellular immunity, cognitive function and prognosis. Hence, the coverage is more comprehensive. Meanwhile, the practical and precise strategies used for comprehensive searches of four databases, inclusion and exclusion criteria, and consideration of study quality indicated the stability and robustness of the present meta-analysis. At the same time, the meta-analysis has some limitations. Firstly, variations in the types and duration of surgery, inconsistent baseline data, concentration and duration of DEX administration may contribute the heterogeneity among studies. However, the funnel plots showed no significant asymmetry, indicating acceptable heterogeneity, as well as the stability and robustness of this meta-analysis. Secondly, the two included studies didn't describe the detailed blinding process in the methods, leading to the suspicion that patients and investigators were aware of the experimental groups. Then the two studies were identified as high risk in the bias assessment. Thirdly, the RCTs included in this meta-analysis covered a long-time span, in which the surgical methods and equipment may have changed. All these factors may lead to instability in the present analysis. Finally, this meta-analysis has not been pre-registered in a protocol (eg. in PROSPERO), which may result in potential bias. Thus, more prospective studies with larger samples sizes and standardized protocols are required in the future to accurately determine the effects of DEX in postoperative systemic inflammation.

In conclusion, the present study found that DEX administration attenuated postoperative systemic inflammatory response and immune dysfunction. Then, DEX decreased the occurrence of POCD, the first flatus time and length of hospital stay of the patients undergoing digest tract cancer surgery. These results provided a potential therapeutic strategy to improve perioperative immune function, CNS function and clinical prognosis of digest

tract cancer. Further studies are necessary to elucidate the clinical application of DEX from an immunological perspective.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

## Author contributions

WX and YuZ collected and performed the data extraction, and wrote the manuscript. ZS and SL contributed to data analysis. KF and YeZ contributed to study design and data analysis. YW, CL, and MZ coordinated data collection and manuscript revision. HZ and ZZ contributed to study design and manuscript revision. CN conceptualized and designed the study, supervised data collection, drafted and revised the manuscript. All authors approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.970557/full#supplementary-material

SUPPLEMENTARY TABLE 1
GRADE Summary of findings for inflammatory mediators, T lymphocytes, cognitive function and prognosis. a. Most information from studies at low or moderate risk of bias (one study at high risk of bias). 1. rate down for inconsistency due to high heterogeneity. 2. rate down for imprecision due to wide CIs.

SUPPLEMENTARY TABLE 2
Detailed search strategy terms for PubMed, Cochrane Library, EMBASE and Web of Science databases.

SUPPLEMENTARY FIGURE 1
The funnel plots were used to assess the publication bias for the STD Mean Difference of the DEX effects on CRP **(A)**, TNF-a **(B)**, IL-6 **(C)** and IL-10 **(D)**.

SUPPLEMENTARY FIGURE 2
The funnel plots were used to assess the publication bias for the STD Mean Difference of the DEX effects on CD3+ T cells, CD4+ T cells, CD8+ T cells and CD4/CD8 ratio respectively.

SUPPLEMENTARY FIGURE 3
The funnel plots were used to assess the publication bias for the STD Mean Difference of the DEX effects on MMSE at 24h, 48h, 72h after surgery **(A)**, and the occurrence of POCD at 24h, 72h after surgery **(B)**.

SUPPLEMENTARY FIGURE 4
The funnel plots were used to assess the publication bias for the STD Mean Difference of the DEX effects on the first flatus time **(A)**, hospital stay **(B)** and postoperative extubation time **(C)**.

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# Risk factors for postoperative pulmonary complications in elderly patients receiving elective colorectal surgery: A retrospective study

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**Study objective:** Postoperative pulmonary complications (PPCs) are common and associated with adverse outcomes impairing long-term survival and quality of recovery. This single-centered retrospective study aimed to examine factors associated with PPCs in patients receiving elective colorectal surgery aged ≥60 years.

**Methods:** Between January 2019 and December 2019, 638 patients at the Shanghai Changhai Hospital who had received elective surgery for colorectal cancer were enrolled in this study. Patients were divided into the PPC group (n=38) and non-PPC group (n=600). Neutrophil–lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR), red blood cell distribution width (RDW), and systemic inflammatory index (SII) were selected and caculated to indicate preoperative and postoperative inflammatory status. Receiver operating characteristic curve and bivariate correlation analyses were performed to evaluate the identified risk factors.

**Main results:** The overall incidence of PPCs was approximately 5.96%. Multivariate regression analysis identified age (OR = 1.094, 95%CI 1.038–1.153, $P$ = 0.001), preoperative RDW (OR = 1.159, 95%CI 1.025–1.309, $P$ = 0.018), and preoperative SII (OR = 1.001, 95%CI 1.000–1.003, $P$ = 0.035) as independent risk factors for PPCs. The cut-off values of age, preoperative RDW, and preoperative SII for predicting PPCs were 69.5 (sensitivity 0.658, specificity 0.653), 13.2 (sensitivity 0.789, specificity 0.552) and 556.1 (sensitivity 0.579, specificity 0.672), respectively.

**Conclusions:** Age, preoperative RDW, and preoperative SII were identified as independent risk factors for PPC occurrence in elderly patients receiving elective colorectal surgery. Further studies are warranted to evaluate whether normalization of preoperative RDW and SII, as modifiable risk factors, are associated with improved surgical outcomes.

KEYWORDS

colorectal surgery, advanced age, retrospective analysis, risk factors, postoperative pulmonary complications

# Introduction

Colorectal cancer ranks third in terms of global cancer incidence and is the second leading cause of cancer-related mortality according to the Global Cancer Statistics 2020 (1). Surgery is the primary curative treatment for colorectal cancer. Perioperative complications after major surgery remain a considerable healthcare burden and are associated with increased mortality and morbidity (2). Postoperative pulmonary complications (PPCs) are common, with an incidence of 2% to 40%, and are associated with adverse outcomes impairing long-term survival and quality of recovery (3). Several studies have been performed to explore and determine the perioperative risk factors for PPCs (4).

Red blood cell distribution width (RDW) is a simple measure of the broadness of erythrocyte size distribution, conventionally called anisocytosis. Increases in RDW observed in patients are generally associated with chronic inflammation or poor nutritional status, and RDW has been suggested as a long-term inflammatory biomarker (5). A recent retrospective study involving 21,842 patients receiving non-cardiac surgery indicated that increased preoperative RDW is associated with increased long-term mortality (6). The systemic immune-inflammation index (SII) is a derivative and new inflammatory biomarker, derived from neutrophils (NEUT), lymphocytes, and platelet counts, and has been used to evaluate the outcome of patients with solid cancers and coronary heart disease (7). Our recent prospective study, with a total of 76 patients aged >65 years receiving elective orthopedic surgery, indicated that postoperative cognitive decline (POCD) in such patients was associated with a significantly high level of SII admission (8). SII was independently associated with the occurrence of POCD in the study cohort. However, studies that investigated the relationship between routine blood test results and PPCs are few.

This single-centered study aimed to examine factors associated with PPCs in patients with colorectal cancer aged ≥60 years. We acknowledge that factors associated with PPCs after colorectal surgery procedures are multifactorial. The current study was conducted to elucidate the relationship of routine blood test results with PPCs.

**Abbreviations:** PPC, postoperative pulmonary complications; POCD, postoperative cognitive decline; NLR, neutrophil–lymphocyte ratio; PLR, platelet–lymphocyte ratio; RDW, red blood cell distribution width; SII, systemic inflammatory index; WBC, white blood cell count; NEUT, neutrophil count; GRA, neutrophilic granulocyte percentage; HGB, hemoglobin; ALB, albumin; RDW, red blood cell distribution width; PLT, platelet count; MONO, monocyte count; LY, lymphocyte count.

# Materials and methods

## Study design and data source

Ethical approval for this retrospective study was provided by the Ethics Committee of Changhai Hospital (CHE 2020-148), and the requirement for obtaining informed consent was waived. This study adhered to the ethical standards set by the National Health Commission of the People's Republic of China.

DoCare Anesthesia Clinical Information System (Medical System,V3.1.0 Build153; Suzhou, China) and electronic case system of Shanghai Changhai Hospital were used for study data retrieval. Patients were systematically identified with the keywords "colorectal cancer", "radical resection", and "general anesthesia". Patients who received radical surgery for colorectal cancer for the first time from January 2019 to December 2019 were selected, and patients aged >60 years with American Society of Anesthesiologists (ASA) physical status I–III, and complete clinical data were screened and selected for this retrospective analysis. Patients who met any of the following criteria were excluded: serious intraoperativecardiovascular and cerebrovascular events (such as cardiac arrest, acute myocardial infarction, and acute cerebral infarction); patients with preoperative severe respiratory diseases (such as severe asthma, an acute exacerbation of chronic obstructive pulmonary disease, history of pulmonary tissue resection leading to significant loss of pulmonary function, pulmonary hypertension with any cause, and respiratory insufficiency or failure); patients with preoperatively existing tumor metastasis or receiving long-term chemotherapy; lack of clinical data related to the study; general anesthesia without endotracheal intubation; and no radical surgical treatment was performed.

## Data collection

For eligible patients in this study, the following relevant information was retrieved: demographic data (sex, age, height, weight, and smoking status; history of hypertension, diabetes, coronary heart disease, stroke; pulmonary imaging changes; pulmonary underlying diseases; and immune system diseases); surgical information (surgical site: rectum, use of laparoscopy,

enterostomy, intestinal adhesion, combined viscerectomy, anesthesia time, operation time, blood loss, urine volume, crystal volume, colloid volume, red blood cell suspension, plasma, and perioperative sufentanil usage); results of preoperative and postoperative blood tests (white blood cell count, neutrophil count, neutrophil percentage, hemoglobin (HGB), albumin (ALB), RDW, platelet count, monocyte count, and lymphocyte count); and prognosis during hospitalization (PPC, recovery and discharge, death, and total length of stay).

Neutrophil–lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR), and SII were selected and calculated to indicate preoperative and postoperative inflammatory status. NLR is defined as the ratio of neutrophil count to lymphocyte count; PLR is defined as the ratio of platelet count to lymphocyte count; and SII is defined as the ratio of neutrophil count multiplied by platelet count to lymphocyte count (9).

We also compared and analyzed the changes in routine blood test results, including NLRs, PLRs, and SII, preoperatively and postoperatively in both groups. Among them, HGB, ALB, platelet count, and lymphocyte count demonstrated a downward trend postoperatively; thus, the preoperative value minus the postoperative value was considered the change value (Δ) for comparison; the other remaining indicators were compared with the postoperative value minus the preoperative value.

## PPCs

PPCs are defined as new-onset events of respiratory complications during postoperative hospitalization, mainly including pulmonary infection, atelectasis, pleural effusion, and acute respiratory failure. PPC was measured according to the Melbourne Group Scale (MGS) scoring criteria. The MGS scoring criteria included: body temperature > 38 °C; white blood cell count increased to $>11.2\times10^9$/L; postoperative atelectasis or chest X-ray findings; new cough or/and purulent sputum; positive sputum pathogen culture; postoperative clinical diagnosis of new pneumonia; blood oxygen saturation <90% when breathing air; and prolonged hospitalization. PPC was diagnosed when the patient meets four or more of the criteria.

## Statistical analysis and data management

Normality of continuous data was tested by the Kolmogorov–Smirnov method. According to data distribution, variance analysis and post-hoc verification were used for continuous variables conforming to normal distribution, and the results are expressed as mean ± standard deviation (X ± S). The Mann–Whitney U test was performed on non-normally distributed data, and the results are presented as median and quartile spacing M (Q25, Q75). The chi-square test was used to assess differences between groups, and the results are expressed as number of cases or percentage (%). Multivariate logistic regression analyses were performed incorporating all factors with $P < 0.1$ on bivariate analyses and a prevalence of at least 1%, as well as other variables with potentially clinical importance, using the backward stepwise selection technique and accepting statistical significance at $P < 0.05$. *A* bivariate correlation analysis was performed to verify the linear relationship of PPCs with diagnostic conditions and associated risk factors in order to clarify the positive and negative correlations between variables. PPCs, dyspnea, pneumonia, pleural effusion, atelectasis, acute respiratory failure, T > 38 °C within 7 days postoperatively, $SpO_2$< 90%, new cough sputum were considered "variables". The test of significance option was set to "two-tailed test". Correlation coefficients were set to the "Pearson" option. In the calculation results, a negative value of the correlation coefficient indicates a negative correlation between the two variables, and a positive value indicates a positive correlation between the two variables. Results are expressed with OR values and 95%CI. $P$ values < 0.05 were considered significant. All analyses were performed using IBM SPSS® Statistics V22 (IBM Corporation, NY, USA).

# Results

## General characteristics and postoperative outcomes

In total, 2,164 patients with colorectal cancer received surgery during the study period, including 1,652 patients who underwent radical resection for the first time (Figure 1). Of the patients, 735 patients aged >60 years with complete data were selected. After applying the exclusion criteria, 638 patients were finally included in the study. The mean age of the patients was 68.5 ± 6.0 years, and 65.5% (n = 418) of the patients were men. The 30-day all-cause mortality was approximately 0.47% (3/638) (Figure 1; Table 1). According to the presence or absence of PPCs postoperatively, the patients were divided into two groups: the PPC group (n = 38) and non-PPC group (n = 600). The overall incidence of PPCs was approximately 5.96%. The characteristics and postoperative in-hospital outcomes of patients are presented in Table 1. The mean age was higher in the PPC group (72.8 ± 7.4 vs. 68.3 ± 5.8., $P < 0.001$) than that in the non-PPC group. The median length of hospital stay of the PPC group was significantly longer than that of the non-PPC group (13.7 ± 5.9 vs. 11.3 ± 3.9, $P < 0.001$).

## Risk factors for PPCs

In order to investigate the risk factors for PPCs in elderly patients receiving elective colorectal surgery, surgical and anesthesia characteristics of the patients were compared

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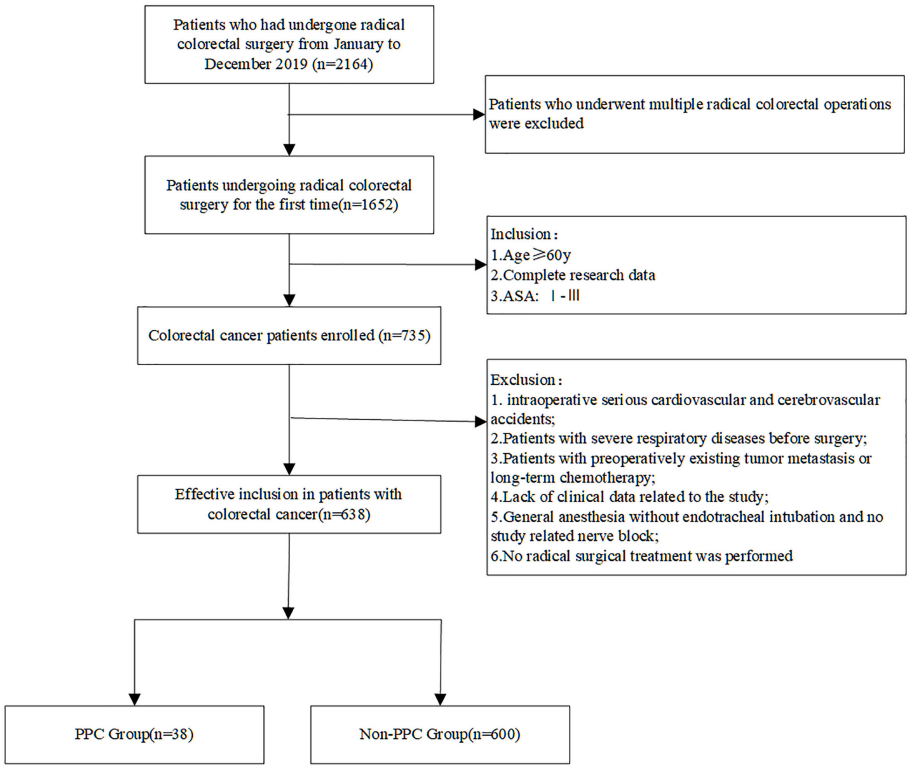


FIGURE 1
Flow diagram of patient inclusion.

TABLE 1 General characteristics and postoperative outcomes of patients.

| Parameter | PPCs (n = 38) | Non-PPCs (n = 600) | *P* |
|---|---|---|---|
| Sex, n (male%) | 26(68.4) | 392 (65.3) | 0.698 |
| Age, y | 72.8 ± 7.4 | 68.3 ± 5.8 | 0.000* |
| BMI, kg/m2 | 22.7 ± 3.6 | 23.1 ± 3.3 | 0.407 |
| ASA, n | | | 0.786 |
| I, n | 15(39.5) | 247(41.2) | |
| II, n | 16(42.1) | 267(44.5) | |
| III, n | 7(18.4) | 86(14.3) | |
| Smoking history, n (%) | 7(18.4) | 132(22.0) | 0.604 |
| Hypertension, n (%) | 18(47.4) | 255(42.5) | 0.556 |
| Diabetes mellitus, n (%) | 6(15.8) | 85(14.2) | 0.781 |
| Coronary heart disease, n (%) | 3(7.9) | 31(5.2) | 0.468 |
| Stroke, n (%) | 0(0.0) | 18(3.0) | 0.279 |
| Perioperative changes in lung CT or X-ray, n (%) | 22(57.9) | 287(47.8) | 0.229 |
| Non-acute (severe) pulmonary disease, n (%) | 1(2.6) | 30(5.0) | 0.510 |
| Autoimmune diseases, n (%) | 0(0.0) | 4(0.7) | 0.614 |
| Hospital deaths, n (%) | 2(5.26) | 1(0.01) | 0.000* |
| Length of hospital stay, days | 13.7 ± 5.9 | 11.3 ± 3.9 | 0.000* |

PPCs, postoperative pulmonary complications; BMI, body mass index; ASA, American Society of Anesthesiologists; CT, computed tomography.
*P value < 0.05, with statistical significance.

TABLE 2 Surgical and anesthesia characteristics of patients.

| Variable | PPCs(n = 38) | Non-PPCs(n = 600) | *P* |
|---|---|---|---|
| Surgical site | | | 0.070 |
| Rectum | 16(42.1) | 343(57.2) | |
| Colon | 22(57.9) | 257(42.8) | |
| Laparoscopy assistance, n (%) | 14(36.8) | 264(44.0) | 0.388 |
| Enterostomy performed, n (%) | 8(21.1) | 196(32.7) | 0.137 |
| Adhesiolysis performed, n (%) | 8(21.1) | 91(15.2) | 0.331 |
| Combined viscerectomy, n (%) | 3(7.9) | 82(13.7) | 0.310 |
| Anesthesia time, hour | 3.9 ± 1.6 | 3.7 ± 1.5 | 0.498 |
| Operation time, hour | 3.3 ± 1.6 | 3.1 ± 1.5 | 0.315 |
| Intraoperative sufentanil use, μg | 43.0 ± 10.0 | 45.3 ± 10.9 | 0.206 |
| postoperative sufentanil use, μg | 55(0,80) | 58(0,80) | 0.651 |
| PCA with sufentanil, n(%) | 27(71.1) | 391(65.2) | 0.459 |

PPCs, postoperative pulmonary complications; PCA, patient-controlled analgesia.

(Table 2). Interestingly, no significant differences in tumor surgical site, use of laparoscopy, enterostomy, or combined visceral resection were identified between the groups. The operation time and duration of anesthesia were also comparable between the groups. The amount of opioids used perioperatively was generally considered a risk factor for PPCs; however, no significant differences in intraoperative and postoperative use of sufentanil were observed between the groups (Table 2). Additionally, we examined whether perioperative fluid and blood managements were associated with PPC occurrence in the patients. As presented in Table 3, no significant differences in the amount of urine output, fluid infusion, or the ratio of colloid liquid to crystal liquid were observed between the two groups; however, the amount of intraoperative blood loss in the PPC group was higher than that in the non-PPC group ($P = 0.044$). Meanwhile, a significant increase in the proportion of patients receiving blood transfusion was also noted in the PPC group (21.1% vs 7.5%, $P = 0.003$) (Table 3).

Considering that this retrospective study aimed to explore risk factors for PPCs based on results of perioperative blood tests, we then analyzed relevant variables obtained or calculated. Table 4 presents the blood test results between the groups preoperatively and postoperatively. The PPC group had significantly lower preoperative plasma HGB (P = 0.001) and ALB (P < 0.001) levels but significantly higher preoperative RDW ($P < 0.001$) and NEUT ($P=0.021$) compared with those in the non-PPC group. In terms of inflammation related indices, the NLRs ($P = 0.022$), PLRs ($P = 0.014$), and SII ($P = 0.007$) in the PPC group were significantly higher than those in the non-PPC group. Postoperatively, the PPC group had significantly higher blood NEUT ($P = 0.029$) and RDW ($P < 0.001$) but significantly lower HGB (P = 0.018) and ALB (P = 0.003) levels than the non-PPC group. However, when comparing the inflammatory related indices, only the patients in the PPC group had a significantly higher SII ($P = 0.042$) (Table 4). The changes in the routine blood test results preoperatively and postoperatively in both groups were compared, and only the change in platelet count was significantly different between both groups ($P = 0.009$) (Table 5).

Variables with significant differences were then selected for the univariate logistic regression analysis, and age, preoperative NEUT, preoperative HGB, preoperative ALB, preoperative RDW, preoperative NLRs, preoperative PLRs, preoperative SII, and intraoperative blood transfusion were identified as risk factors (Table 6). In the subsequent multivariate regression

TABLE 3 Perioperative fluid and blood management in patients.

| Variable | PPCs(n = 38) | Non-PPCs(n = 600) | *P* |
|---|---|---|---|
| Blood loss, L | 0.2(0.1,0.3) | 0.2(0.1,0.2) | 0.044* |
| Urine output, L | 0.6(0.4,1.0) | 0.6(0.5,1.0) | 0.600 |
| Crystal liquid infusion, L | 1.7(1.5,2.1) | 1.8(1.3,2.2) | 0.740 |
| Colloid liquid infusion, L | 0.5(0.5,1.0) | 0.5(0.5,1.0) | 0.168 |
| Ratio of colloid liquid to crystal liquid | 0.3(0.2,0.6) | 0.3(0.2,0.5) | 0.244 |
| Total liquid infusion volume, L | 2.4(2.1,2.8) | 2.3(2.1,2.8) | 0.776 |
| Patients with intraoperative blood transfusion, n (%) | 8(21.1) | 45(7.5) | 0.003* |

*P value < 0.05, with statistical significance.

TABLE 4 Results of preoperative and postoperative blood tests in patients.

| Variable | PPCs (n=38) | Non-PPCs (n=600) | *P* |
|---|---|---|---|
| **Preoperative** | | | |
| WBC, $10^9$/L | 6.0(5.2,7.9) | 5.8(4.8,6.8) | 0.197 |
| NEUT, $10^9$/L | 3.8(3.0,4.9) | 3.4(2.7,4.3) | 0.021* |
| GRA,% | 61.0 ± 9.1 | 59.2 ± 9.6 | 0.254 |
| HGB, g/L | 115.5 ± 25.8 | 127.4 ± 21.8 | 0.001* |
| ALB, g/L | 37.8 ± 5.1 | 40.5 ± 3.9 | 0.000* |
| RDW, % | 13.9(13.2,17.1) | 13.1(12.4,14.0) | 0.000* |
| PLT, $10^9$/L | 244.7 ± 90.1 | 221.1 ± 71.1 | 0.052 |
| MONO, $10^9$/L | 0.5(0.4,0.7) | 0.5(0.4,0.6) | 0.175 |
| LY, $10^9$/L | 1.6(1.1,2.1) | 1.6(1.3,2.0) | 0.429 |
| NLRs | 2.5(1.7,3.7) | 2.1(1.6,2.8) | 0.022* |
| PLRs | 168.7(117.4,206.2) | 131.6(102.9,172.8) | 0.014* |
| SII | 602.5(347.4,932.0) | 420.4(316.8,645.0) | 0.007* |
| **Postoperative** | | | |
| WBC, $10^9$/L | 10.7 ± 3.1 | 10.2 ± 3.4 | 0.454 |
| NEUT, $10^9$/L | 9.5 ± 3.4 | 8.3 ± 3.1 | 0.029* |
| GRA, % | 82.7 ± 5.9 | 80.7 ± 7.9 | 0.131 |
| HGB, g/L | 108.5 ± 18.3 | 115.9 ± 18.8 | 0.018* |
| ALB, g/L | 32.3 ± 4.6 | 34.3 ± 3.8 | 0.003* |
| RDW, % | 14.4(13.0,17.2) | 13.0(12.4,14.1) | 0.000* |
| PLT, $10^9$/L | 208.5 ± 65.1 | 199.7 ± 64.7 | 0.413 |
| MONO, $10^9$/L | 0.8(0.6,1.0) | 0.8(0.6,1.0) | 0.659 |
| LY, $10^9$/L | 0.8(0.7,1.1) | 0.9(0.6,1.2) | 0.500 |
| NLRs | 10.1(7.4,15.9) | 8.9(6.1,13.4) | 0.064 |
| PLRs | 234.1(187.4,324.2) | 225.0(160.7,308.5) | 0.278 |
| SII | 2023.9(1457.4,3522.2) | 1734.1(1099.4,2717.7) | 0.042* |

WBC, white blood cell count; NEUT, neutrophils count; GRA, neutrophilic granulocyte percentage; HGB, hemoglobin; ALB, albumin; RDW, red blood cell distribution width; PLT, platelet count; MONO, monocyte count; LY, lymphocyte count; NLRs, neutrophil to lymphocyte ratios; PLRs, platelet to lymphocyte ratios; SII, systemic immune-inflammation index.
*P value < 0.05, with statistical significance.

TABLE 5 Value changes in blood test results preoperatively and postoperatively.

| Variable | PPCs (n = 38) | Non-PPCs (n = 600) | *P* |
|---|---|---|---|
| Δ WBC, $10^9$/L | 4.2 ± 3.5 | 4.2 ± 3.3 | 0.901 |
| Δ NEUT, $10^9$/L | 5.2 ± 3.5 | 4.7 ± 3.1 | 0.379 |
| Δ GRA,% | 21.7 ± 9.4 | 21.5 ± 10.2 | 0.931 |
| Δ HGB, g/L | 7.1 ± 21.7 | 11.5 ± 15.6 | 0.095 |
| Δ ALB, g/L | 5.5 ± 5.8 | 6.2 ± 4.7 | 0.379 |
| Δ RDW, % | 0.1 ± 0.6 | 0.0 ± 0.7 | 0.331 |
| Δ PLT, $10^9$/L | 36.2 ± 58.6 | 21.4 ± 31.3 | 0.009* |
| Δ MONO, $10^9$/L | 0.3(0.1,0.5) | 0.3(0.1,0.5) | 0.931 |
| Δ LY, $10^9$/L | 0.7(0.3,1.0) | 0.7(0.4,1.1) | 0.660 |
| Δ NLRs | 8.3(4.7,12.6) | 6.6(4.0,11.0) | 0.254 |
| Δ PLRs | 73.2(38.5,134.1) | 81.6(33.9,154.4) | 0.548 |
| Δ SII | 1330.2(778.2,2496.2) | 1244.7(689.1,2126.1) | 0.397 |

Δ, difference between preoperative and postoperative blood indices; WBC, white blood cell count; NEUT, neutrophil count; GRA, neutrophilic granulocyte percentage; HGB, hemoglobin; ALB, albumin; RDW, red blood cell distribution width; PLT, platelet count; MONO, monocyte count; LY, lymphocyte count; NLRs, neutrophil to lymphocyte ratios; PLRs, platelet to lymphocyte ratios; SII, systemic immune-inflammation index.
*P value < 0.05, with statistical significance.

TABLE 6 Logistics analysis of risk factors related to postoperative pulmonary complications.

| Variable | Univariate analysis | | | | Multivariate analysis | | | |
|---|---|---|---|---|---|---|---|---|
| | β value | OR | 95%CI | *P* value | β value | OR | 95%CI | *P* value |
| Age | 0.104 | 1.109 | 1.058-1.163 | 0.000* | 0.090 | 1.095 | 1.037-1.155 | 0.001* |
| Preoperative NEUT | 0.218 | 1.243 | 1.057-1.463 | 0.008* | -0.031 | 0.970 | 0.922-1.020 | 0.234 |
| Preoperative HGB | -0.022 | 0.979 | 0.966-0.992 | 0.002* | -0.015 | 0.985 | 0.954-1.018 | 0.381 |
| Preoperative ALB | -0.148 | 0.862 | 0.799-0.930 | 0.000* | -0.085 | 0.919 | 0.834-1.012 | 0.087 |
| Preoperative RDW | 0.162 | 1.176 | 1.078-1.282 | 0.000* | 0.174 | 1.190 | 1.048-1.351 | 0.007* |
| Preoperative NLRs | 0.176 | 1.193 | 1.036-1.373 | 0.014* | -0.034 | 0.967 | 0.670-1.395 | 0.856 |
| Preoperative PLRs | 0.004 | 1.004 | 1.000-1.008 | 0.031* | -0.005 | 0.995 | 0.988-1.002 | 0.204 |
| Preoperative SII | 0.001 | 1.001 | 1.000-1.001 | 0.002* | 0.002 | 1.002 | 1.000-1.003 | 0.022* |
| Bleeding | 1.079 | 2.941 | 0.649-13.328 | 0.162 | 0.755 | 2.127 | 0.367-12.327 | 0.400 |
| Intraoperative blood transfusion | 1.191 | 3.289 | 1.424-7.595 | 0.005* | 0.325 | 1.384 | 0.434-4.417 | 0.583 |

NEUT, neutrophil count; HGB, hemoglobin; ALB, albumin; RDW, red blood cell distribution width; NLRs, neutrophil to lymphocyte ratios; PLRs, platelet to lymphocyte ratios; SII, systemic immune-inflammation index.
*P value < 0.05, with statistical significance.

analysis, age (OR = 1.094, 95%CI 1.038–1.153, $P$ = 0.001), preoperative RDW (OR = 1.159, 95%CI 1.025–1.309, $P$ = 0.018), and preoperative SII (OR = 1.001, 95%CI 1.000–1.003, $P$ = 0.035) were identified as independent risk factors for PPCs (Table 6).

A receiver operating characteristic curve (ROC) analysis of the risk factors age, preoperative RDW, and preoperative SII, was performed to predict the occurrence of PPCs, with area under the curve of 0.683 (95%CI 0.586–0.779, $P$ = 0.000), 0.683 (95%CI 0.590–0.775, $P$ = 0.000) and 0.629 (95%CI 0.532–0.727; $P$ = 0.007), respectively (Table 7; Figure 2). The cut-off values of age, preoperative RDW, and preoperative SII for predicting PPCs were 69.5 (sensitivity 0.658, specificity 0.653), 13.2 (sensitivity 0.789, specificity 0.552), and 556.1 (sensitivity 0.579, specificity 0.672), respectively. A risk factor prediction model with the abovementioned three independent risk factors for the occurrence of PPCs was also established, and the ROC curve analysis was performed. The area under the ROC curve was 0.744 (with the sensitivity 0.684, and the specificity 0.753), and the Youden index was 0.437 (Figure 3).

Finally, a bivariate correlation analysis was performed using the independent risk factors for PPCs and PPCs. The results revealed that age, preoperative RDW, and preoperative SII were positively correlated with PPCs and its subtypes. Additionally, with the increase of values in age, preoperative RDW, and preoperative SII, the probability of occurrence of PPCs demonstrated an upward trend (Table 8).

## Discussion

This study demonstrated that age, preoperative RDW, and preoperative SII were significant predictors of PPCs in elderly patients receiving elective colorectal surgery. We also constructed the ROC curve of cut-off values of age, preoperative RDW, and preoperative SII for predicting PPCs. To the best of our knowledge, this is the first study to evaluate the role of perioperative RDW and SII for predicting PPCs in elderly patients receiving elective colorectal surgery.

Surgical trauma and the influence of the tumor itself can produce inflammatory reactions in the body (10). Among all of the inflammatory cells, neutrophils play an important role in the inflammatory processes, while lymphocytes are involved in the regulation of the immune system. In this study, NLR, PLR, and SII were used to analyze the preoperative and postoperative inflammatory states. NLR is the ratio of neutrophil to lymphocyte count, and is considered a hematological marker of systemic inflammation. In the recent years, NLR and PLR have been used as inflammatory indices for inflammation and severity of diseases. It is widely recognized that the systemic

TABLE 7 Cut-off value for age, preoperative RDW, and preoperative SII for predicting postoperative pulmonary complications.

| Parameter | Cut-off value | Specificity | Sensitivity | AUC | Youden index | 95% CI | *P* value |
|---|---|---|---|---|---|---|---|
| Age | 69.5 | 0.653 | 0.658 | 0.683 | 0.311 | 0.586-0.779 | 0.000* |
| Preoperative RDW | 13.2 | 0.552 | 0.789 | 0.683 | 0.341 | 0.590-0.775 | 0.000* |
| Preoperative SII | 556.1 | 0.672 | 0.579 | 0.629 | 0.251 | 0.532-0.727 | 0.007* |

RDW, red blood cell distribution width; SII, systemic immune-inflammation index.
*P value < 0.05, with statistical significance.

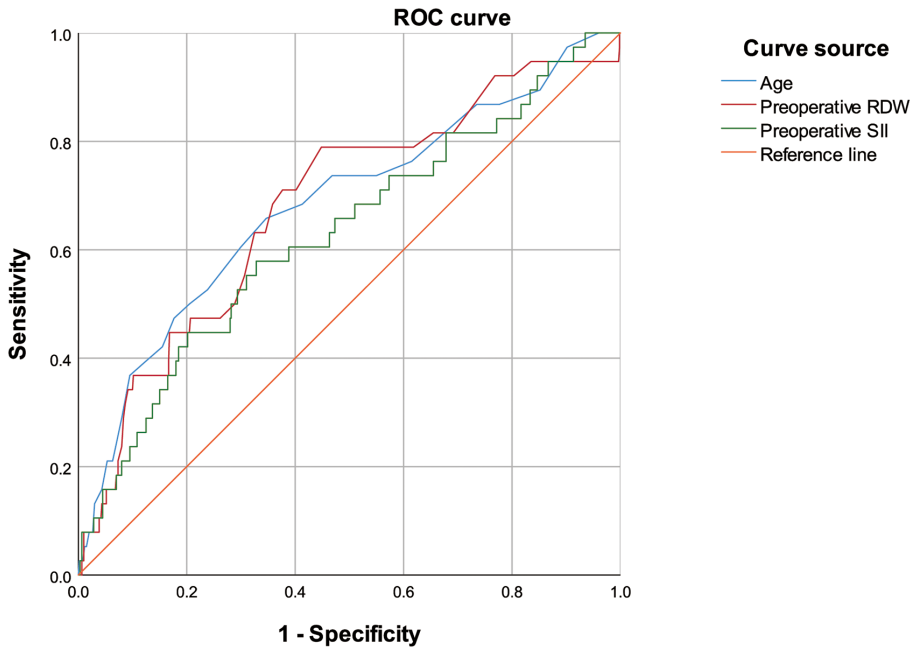


FIGURE 2
Receiver operating characteristic curve of age, preoperative red blood cell distribution width, and preoperative systemic inflammatory index for predicting postoperative pulmonary complications.

inflammatory response accompanies the development of cancer, and thus providing us with new insights and methods for evaluating patients' systematic inflammation status and outcomes (11). Some studies have reported that NLR can reflect early postoperative complications in order to achieve early diagnosis and treatment (12). NLR is also considered a predictive marker for patients with colorectal cancer, which is of great significance for predicting preoperative metastasis and evaluating postoperative prognosis (13). The association of PLRs with tumor survival and progression has been reported, and a PLR>150 was identified as an independent predictor of tumor recurrence in patients with hepatocellular carcinoma (14, 15). SII is a new inflammatory biomarker derived in recent years, defined as (platelet*neutrophil)/lymphocyte count. Based on the relationship between neutrophils, lymphocytes, and platelet count, SII is often used for the clinical evaluation of different disease states and has demonstrated a substantial predictive effect on the progression and treatment prognosis of patients with tumors (7,16). A very recent study, including 548 patients with stage I–II gastric cancer after receiving radical surgery, has suggested that preoperative low SII (<508.3) was associated with a significantly higher 5-year survival rate (17). Meanwhile, our study discovered that an SII value > 556.1 was associated with a higher incidence of PPCs.

In our study, the preoperative NLR, PLR, and SII in the PPC group were significantly higher than those in the non-PPC group, suggesting that these variables have potential for predicting PCC occurrence. Since these variables can be easily obtained by routine blood testing, their roles deserve further evaluation. Perioperative anemia has long been considered a risk factor for perioperative complications in patients receiving surgery. Our study also indicated that the pre- and postoperative anemia, indicated by a low threshold of HGB level, was associated with PPC occurrence. However, HGB was not identified as an independent risk factor for PPC. The underlying reasons may be the relatively low number of PPC occurrences, or that HGB is less sensitive than the RDW.

The observed association of RDW and SII with PPCs deserves further exploration, considering that chronic inflammation might exist preoperatively and that both may be associated with unfavorable outcomes. RDW was again commonly used in the assessment of anemia, while being recently regarded as an indicator of long-term inflammation (18). The release of inflammatory factors, oxidative stress, and poor nutritional status are perceived as potential causes of RDW elevation, leading to retention of abnormally sized red cells (19). Increased values in RDW have been considered a negative predictor of survival in several types of malignancies (20). A recent retrospective study including 591 patients with colorectal cancer has reported that only patients with early-stage colorectal cancer may have a worse survival when presenting with an elevated RDW (6). Due to incomplete data collection, information on the depth of tumor invasion, node involvement metastatic disease, TNM

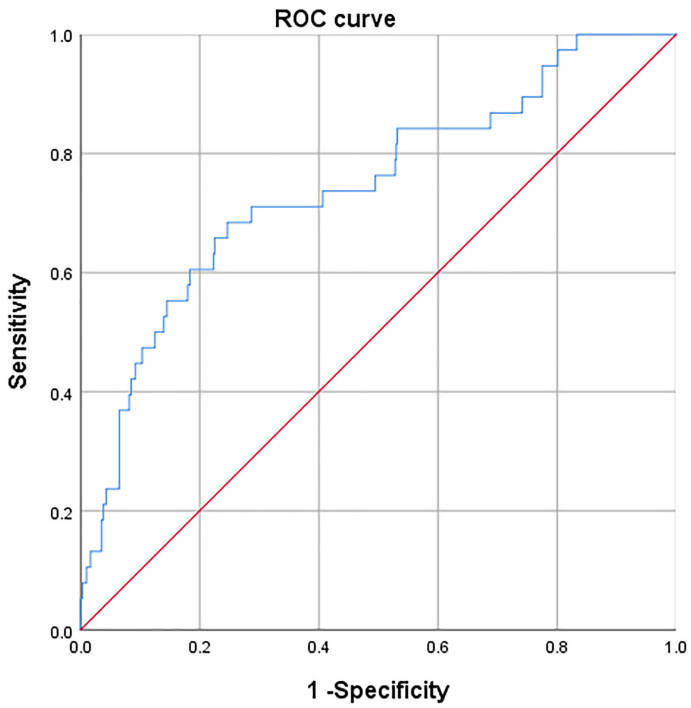


FIGURE 3
Receiver operating characteristic curve of three independent risk factors for predicting postoperative pulmonary complications.

stage, and tumor grading for each patient was not retrieved, and thus were not analyzed for their association with PPCs. A recent retrospective cohort study has reported that RDW values between 14.8% and 15.8%, and >15.8% were associated with increased long-term mortality after noncardiac surgery (21). Our study discovered that an RDW value > 13.2% was associated with a significant increase in PPC occurrence. Studies exploring whether normalization of the RDW benefits perioperative outcomes including PPCs in patients receiving surgery will be of interest. Since RDW is a modifiable variable, whether it can be used for risk prediction or stratification for PPCs deserves further research. Moreover, patients with high RDW and SII values may be more prone to perioperative hemodynamic instability, amplified inflammatory response, and mediated postoperative adverse outcomes including PPCs.

Advanced age is a certain risk factor for perioperative complications of patients receiving abdominal surgery due to a decline in physical function and increased comorbidities (22). Our

TABLE 8 Bivariate correlation analysis of preoperative related factors and postoperative pulmonary complications.

| | Correlation | PPCs | Dyspnea | Pneumonia | Pleural effusion | Atelectasis | Acute respiratory failure | T > 38 °C within 7 days postoperatively | $SpO_2$ < 90% | New cough and/or sputum |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | Correlation Coefficient | 0.179 | 0.131 | 0.111 | 0.077 | 0.034 | 0.060 | -0.020 | 0.043 | 0.154 |
| | *P* values | 0.000* | 0.001* | 0.005* | 0.052 | 0.387 | 0.130 | 0.609 | 0.276 | 0.000* |
| Preoperative RDW | Correlation Coefficient | 0.156 | 0.008 | 0.035 | 0.035 | 0.010 | 0.052 | 0.099 | 0.070 | 0.155 |
| | *P* values | 0.000* | 0.847 | 0.382 | 0.384 | 0.795 | 0.191 | 0.012* | 0.077 | 0.000* |
| Preoperative SII | Correlation Coefficient | 0.137 | 0.022 | 0.087 | 0.039 | 0.085 | 0.201 | 0.044 | 0.165 | 0.051 |
| | *P* values | 0.001* | 0.571 | 0.027* | 0.321 | 0.031* | 0.000* | 0.262 | 0.000* | 0.198 |

HGB, hemoglobin; ALB, albumin; RDW, red blood cell distribution width; NLRs, neutrophil to lymphocyte ratios; PLRs, platelet to lymphocyte ratios; SII, systemic immune-inflammation index; PPCs, pulmonary complications.
*P value < 0.05, with statistical significance.

study also confirmed that age is risk factor for PPCs in this population. Interestingly, limited studies have explored the effect of aging on perioperative inflammatory response. One recent retrospective cohort study, with a total of 25,095 patients who received cardiac surgeries, has reported that age was strongly associated with a reduced prevalence of postoperative systemic inflammatory response syndrome (23). This inverse association indicated that an overall reduced postoperative immune response in aging population, also known as immunosenescence, may influence perioperative medication strategies and deserves further research.

Moreover, the incidence of PPCs in our cohort is relatively lower than that of previous studies by approximately 5% to 33% (24, 25). This might be caused by differences among institutional guidance or experiences. Thus, the results can only represent the correlation of related factors included in this study, rather than absolute causality, and may not apply to other abdominal surgical populations. The sensitivity and specificity of the ROC curves were not particularly excellent, which may be mainly caused by the low incidence of PPCs in our study population. Therefore, the value of preoperative RDW and SII for predicting PPCs deserves further exploration.

This study also had some limitations. Firstly, this was a small-sample single-center retrospective study, and the patients who had received colorectal surgery were aged >60 years, which may have resulted in selection bias and confounding factors. Secondly, we did not examine the percentage of patients receiving blood transfusion preoperatively, which might skew the value of RDW. Thirdly, in order to determine whether the present risk factors have high predictive power, multi-center clinical large samples and observational studies need to be conducted. Lastly, other inflammatory markers, such as hs-CRP, procalcitonin, and hematologic parameters such as mean platelet volume, and immature and fragmented platelet forms were not evaluated in the current study, which deserve further investigation. Pneumoperitoneum could affect the lung mechanics in several ways; thus, the application and duration of intraoperative pneumoperitoneum are considered risk factors for PPCs in patients receiving abdominal surgeries. However, our data revealed that the proportion of patients undergoing laparoscopy assistance for colorectal surgery was comparable between the two groups, and no significant difference in the incidence of PPCs was observed between both groups. Moreover, intraoperative ventilation strategies, management of perioperative use of muscle relaxants, and several reported risk factors for PPCs were not examined thoroughly, and deserve further investigation.

In conclusion, by analyzing the perioperative related factors in elderly patients with colorectal cancer, this study identified age, preoperative RDW, and preoperative SII as independent risk factors for PPC occurrence. Further studies comprehensively evaluating the potential risk factors for colorectal surgery and related PPCs are necessary in the future. Future studies should also clarify whether normalization of preoperative RDW and SII, as modifiable risk factors, may improve surgical outcomes.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

## Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Changhai Hospital. The ethics committee waived the requirement of written informed consent for participation.

## Author contributions

LB, YD, GS, and JB designed the study. GS, CW, HH, HW, YZ, YS, and JH contributed to the conduct of the study. YD, GS, HH, and HW performed the data analysis. LB and YD were the major contributors to writing the first draft of the manuscript. All authors listed reviewed and approved the final version of the manuscript.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher’s note

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# Long-term effect of hospital volume on the postoperative prognosis of 158,618 patients with esophageal squamous cell carcinoma in China

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**Background:** The impact of hospital volume on the long-term survival of esophageal squamous cell carcinoma (ESCC) has not been well assessed in China, especially for stage I–III stage ESCC. We performed a large sample size study to assess the relationships between hospital volume and the effectiveness of ESCC treatment and the hospital volume value at the lowest risk of all-cause mortality after esophagectomy in China.

**Aim:** To investigate the prognostic value of hospital volume for assessing postoperative long-term survival of ESCC patients in China.

**Methods:** The date of 158,618 patients with ESCC were collected from a database (1973–2020) established by the State Key Laboratory for Esophageal Cancer Prevention and Treatment, the database includes 500,000 patients with detailed clinical information of pathological diagnosis and staging, treatment approaches and survival follow-up for esophageal and gastric cardia cancers. Intergroup comparisons of patient and treatment characteristics were conducted with the $X^2$ test and analysis of variance. The Kaplan-Meier method with the log-rank test was used to draw the survival

curves for the variables tested. A Multivariate Cox proportional hazards regression model was used to analyze the independent prognostic factors for overall survival. The relationship between hospital volume and all-cause mortality was assessed using restricted cubic splines from Cox proportional hazards models. The primary outcome was all-cause mortality.

**Results:** In both 1973-1996 and 1997-2020, patients with stage I-III stage ESCC who underwent surgery in high volume hospitals had better survival than those who underwent surgery in low volume hospitals (both P<0.05). And high volume hospital was an independent factor for better prognosis in ESCC patients. The relationship between hospital volume and the risk of all-cause mortality was half-U-shaped, but overall, hospital volume was a protective factor for esophageal cancer patients after surgery (HR<1). The concentration of hospital volume associated with the lowest risk of all-cause mortality was 1027 cases/year in the overall enrolled patients.

**Conclusion:** Hospital volume can be used as an indicator to predict the postoperative survival of ESCC patients. Our results suggest that the centralized management of esophageal cancer surgery is meaningful to improve the survival of ESCC patients in China, but the hospital volume should preferably not be higher than 1027 cases/year.

**Core tip:** Hospital volume is considered to be a prognostic factor for many complex diseases. However, the impact of hospital volume on long-term survival after esophagectomy has not been well evaluated in China. Based on a large sample size of 158,618 ESCC patients in China spanning 47 years (1973-2020), We found that hospital volume can be used as a predictor of postoperative survival in patients with ESCC, and identified hospital volume thresholds with the lowest risk of death from all causes. This may provide an important basis for patients to choose hospitals and have a significant impact on the centralized management of hospital surgery.

KEYWORDS

**hospital volume, esophageal squamous cell carcinoma, esophagectomy, postoperative survival, retrospective analysis**

## Introduction

Esophageal cancer is the seventh most common malignant tumor (604,100 new cases in 2020) and the sixth deadliest tumor (544,000 deaths in 2020) in the world (1, 2). With the development of the economy and the increase in people's health consciousness, most patients with esophageal cancer prefer to choose medium volume or high volume hospitals instead of low volume hospitals in China. For hospitals, doctors and patients, hospital volume has been recognized as an important determinant of patient survival (3, 4). Halm et al. found that admission to higher-volume hospitals was associated with a reduction in mortality for many surgical conditions and medical procedures (5). Several studies have also showed that patients with esophageal cancer who received treatment in higher volume hospitals had significantly better long-term survival rates than patients treated at lower volume hospitals (4, 6–8). However, several other studies found that the hospital volume is not an important predictor of survival in esophageal cancer, nor should it be used as an alternative measure of surgical quality (9, 10). To better understand the relationship between hospital volume and the effectiveness of treatment in China, we analyzed the mortality and survival of 158,618 stage I–III patients with ESCC who underwent esophagectomy at different volume hospitals.

# Materials and methods

## Patients

A total of 158,618 patients who diagnosed as ESCC between 1973 and 2020 from the 500,000 esophageal and gastric cardia carcinoma databases (1973–2020), established by The State Key Laboratory for Esophageal Cancer Prevention and Treatment, were enrolled in this retrospective study (11–14). Patients were selected according to the following criteria: (1) Patients were diagnosed with ESCC by gastroscopy biopsy or postoperative histopathology. (2) Patients had no other malignant tumors except for ESCC. (3) Patients had a clear diagnosis time and underwent surgery only (patients with minimally invasive resection and preoperative and postoperative chemoradiotherapy were excluded). (4) Patients have complete clinical records. All medical records were reviewed for consistency and completeness.

## Hospital volume

Hospital volume was defined as the annual average number of esophagectomy procedures per hospital. To determine hospital volume groups, we created a multivariate Cox proportional hazards model with restricted cubic splines (RCS, Figure 1). The covariates in the model included sex, age, region, urban/rural residence, smoking history, drinking history, cancer family history, incisal edge residue, tumor location, differentiation and pathological stage. The RCS can explain the

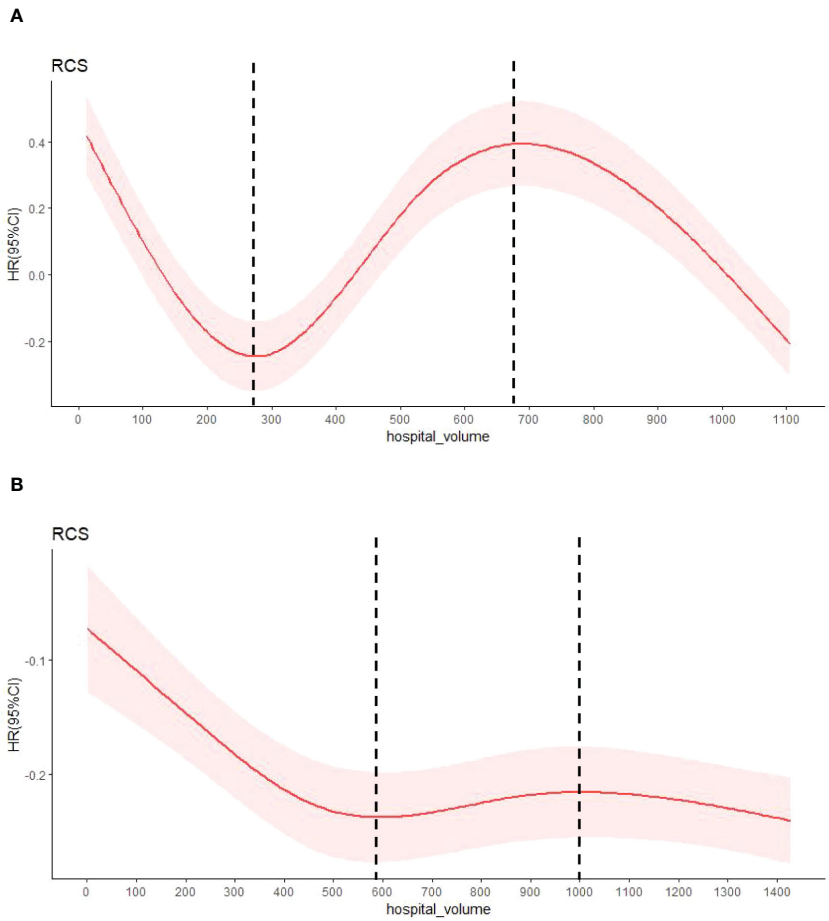


FIGURE 1
The HR by annual average hospital volume with restricted cubic spline fit. **(A)** shows patients with esophageal cancer from 1973 to 1996, and **(B)** from 1997 to 2020.The relationship between known covariable-adjusted risk of death and annual hospital volume. The solid red line represents a restricted cubic spline (RCS) fit and the light red shadow represents a 95% confidence interval for the RCS fit. The vertical dashed lines are the extremes of the curve.

nonlinear relationship between the average annual hospital volume and survival rate, combined with the change in hazard ratio (HR), and the two extreme points of the curve are finally determined (in 1973-1996: 276.638 and 688.573; in 1997-2020: 596.181 and 1004.919). All hospitals were divided into low volume (1-277 cases/years and 1-596 cases/years), medium volume (278-689 cases/years and 597-1004 cases/years) and high volume (690-1106 cases/yearsand 1005-1428 cases/years) groups according to two integer extreme points.

## High/Low incidence area

Based on the epidemiological findings of esophageal cancer, the age and mortality rate were adjusted to include ESCC mortality rates of more than 60 per 100,000 is recognized as an ESCC high incidence area, while the others are a low incidence area. Zoning reference to 《esophageal cancer》.

## Urban/Rural residence

Those living in county level and above were classified as urban residents, while the rest were classified as rural residents.

## Smoking and alcohol consumption history

Smoking consumption history refers to smoking more than 1 cigarette per day, continuous or cumulative smoking for more than 6 months in a lifetime.

Alcohol consumption history refers to according to the record of excessive drinking, more than 4 standard cups (A standard cup is a drink containing 18 milliliters of alcohol.) per day and drinking more than 3 times a week.

## Family history of cancer

A positive family history of cancer is 2 or more cancer patients in the same family within consecutive 3 generations.

A negative family history of cancer means that only one patient with cancer in the same family within consecutive 3 generations.

## Treatment

Refer to the NCCN guidelines for the 1st edition of esophageal cancer in 2015 (15), and this study only included patients undergoing surgery of ESCC. The surgical methods mainly include Sweet procedure, Ivor-Lewis procedure, Mckeown procedure and transhiatal esophagectomy. Because transhiatal esophagectomy is rarely used in China, only Sweet procedure, Ivor-Lewis procedure and Mckeown procedure were considered in the surgical approach analysis in this study.

## Tumor staging

The time span of diagnosis of ESCC patients in this study was large, pathological staging of esophageal cancer has been updated in different editions (the sixth edition in 2002, the seventh edition in 2009, and the eighth edition in 2017).In order to reduce the error, the TNM staging of esophageal and esophagogastric junction cancer, the sixth edition jointly published by the International Union Against Cancer (UICC) and the American Cancer Federation (AJCC), was uniformly used in this study (16).

## Follow-up

The study follow-up was mainly carried out by correspondence, telephone calls, home visits and direct contact between village doctors and patients or their families or through systems such as the new cooperative medical database, the Medical Security Administration database and the registration and management of citizen death information. In 2 years after discharge, the patients were followed up every 3 months. Once every six months for 3-5 years. Then, follow-up was conducted once a year and until death, emigration, or the end of the study period (January 2021), whichever occurred first. Of the 158,618 ESCC patients 103,252 patients (65.1%) were followed-up successfully.

## Statistical analysis

Statistical analysis was performed using SPSS(Windows version 21.0) and R. The t test and chi-square test were used to compare the differences in categorical and continuous variables, respectively, between different ESCC groups. The survival outcome was estimated by the Kaplan-Meier method and the multivariate Cox proportional hazards regression model. Multivariate analysis adjusted for sex, age, region, urban/rural residence, smoking history, drinking history, cancer family history, incisal edge residue, tumor location, differentiation, pathological stage and diagnosis time. A value of $P< 0.05$ was considered statistically significant.

The association between hospital volume and all-cause mortality was assessed on a continuous scale using restricted cubic splines based on the Cox proportional hazards model. To balance best-fit and overfitting on the main splines of

mortality, the Akaike information criterion was used to selects the number of knots between 3 and 7 as the lowest value, but if the number of different knots is within two, the lowest number was chosen. The hospital volume associated with the lowest risk of death was the value of the lowest hazard ratio on the spline curve.

# Results

## Patient eligibility

A total of 258,647 patients with ESCC were evaluated for eligibility. Of these 63,421 patients were excluded due to nonsurgical reasons. In addition, 31,453 patients with unclear staging records and 5,155 patients with stage 0 and IV were excluded. A total of 158,618 patients with ESCC were included, including 24,060 cases were diagnosed between 1973 and 1996, and 134,558 cases between 1997 and 2020. The 24,060 patients were from 38 hospitals, including 28 low volume hospitals, 7 medium volume hospitals and 3 high volume hospitals, and the 134,558 patients were from 101 hospitals, including 73 low volume hospitals, 21 medium volume hospitals and 7 high volume hospitals (Figure 2).

## Population demographics

From the archived clinical records, we retrieved the clinicopathological features of ESCC patients in this study during two time periods (Tables 1, 2). In both time periods, the patients were mainly male (1973-1996:59.8%, 1997-2020:65.9%), 50-70 years old (1973-1996:67.4%, 1997-2020:75.2%), high incidence area (1973-1996:81.1%, 1997-2020:57.4%) and rural residents (1973-1996:89.5%, 1997-2020:88.5%). Nearly half of the patients in two groups had a positive family history of cancer (1973-1996:49.0%, 1997-2020:41.0%). In addition, almost all female patients had no cigarette smoking and alcohol consumption. In contrast, nearly 60 percent of male patients had a history of cigarette smoking (1973-1996:63.9%, 1997-2020:66.2%) and 40 percent had a history of alcohol consumption(1973-1996:36.6%, 1997-2020:45.7%). Almost two-thirds of the patients were diagnosed with stage III, 6.3% and 9.7% of patients with stage I underwent surgery at two time periods, respectively. The stage III patients from 1997 to 2020 were significantly higher than those from 1973 to 1996 (72.1% *vs.* 66.6%), and the positive rate of incisal edge residue was significantly lower than those from 1973 to 2020 (3.6% *vs.* 6.2%). In all patients with clear surgical approach records, left thoracotomy was the main method in both time periods(1973-1996:93.9%, 1997-2020:80.1%). Two-thirds of the tumor was located in the middle chest and were moderate differentiation. There were 5,631 postoperative complications, the most common of which were pulmonary complications(22.0%), anastomotic leak (20.3%) and incision infection(18.5%), the next were cardiovascular complications(10.1%), chylothorax(2.1%), recurrent laryngeal nerve injury(1.8%), etc., while surgical death (0.9%) and hoarseness(0.6%) were rare. Relapse was recorded in 2,673 patients, most of whom received radiotherapy or chemotherapy. The 1-year, 3-year, 5-year and 7-year survival rates of patients at both time periods were 83.4%, 69.8%, 59.3%, 11.4%(1973-1996) and 76.3%, 58.4%, 47.4%, 24.5(1997-2020), respectively.

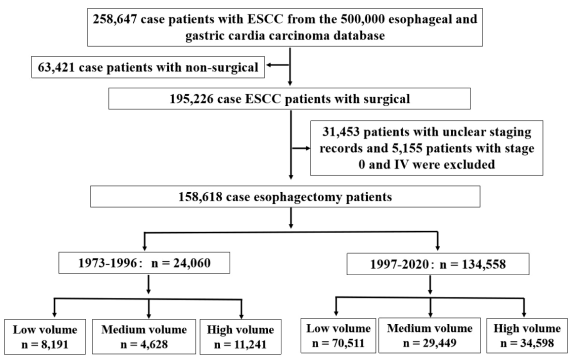


FIGURE 2
Study population selection criteria from database. The study population was selected form the 500,000 esophageal and gastric cardia carcinoma database (1973-2020) and included patients who were diagnosed ESCC from 1973 to 2020. The final cohort sample size was 158,618.

## Univariate intergroup analysis by hospital volume

From 1973 to 1996, there were 14,390 male patients with a mean age of 54.5 ± 9.3 years and 9,670 female patients with a mean age of 54.8 ± 9.0 years. Individuals presenting to high volume hospitals were mostly from high incidence areas, more likely to be older at diagnosis and a positive family history of cancer, and had more stage III patients than the other two subgroups. The percentage of positive incisal edge residue was lowest in medium volume hospitals (1.2%), but the operative death and in-hospital death were both higher. A total of 426 ESCC patients underwent right thoracotomy, of which 413 (96.9%) were in high volume hospitals, 6 case(1.4%) were in low volume hospitals, and 7 case(1.6%) were in medium volume hospitals (Table 1).

From 1997 to 2020, there were 88,674 male patients with a mean age of 60.0 ± 8.5 years and 45,884 female patients with a mean age of 60.8 ± 8.4 years. There was no significant difference in operative death and in-hospital death among hospitals with different volume. Individuals presenting to high volume

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TABLE 1 Relationship between the clinicopathological features of ESCC patients and hospital volume during 1973-1996, n(%).

| Characteristics | No. of The Patients Examined | | Hospital Volume | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Low | | Medium | | High | | P |
| Sex | | | | | | | | | 0.026 |
| Male | 14390 | (59.8) | 4836 | (59.0) | 2844 | (61.5) | 6710 | (59.7) | |
| Female | 9670 | (40.2) | 3355 | (41.0) | 1784 | (38.5) | 4531 | (40.3) | |
| Total | 24060 | (100.0) | 8191 | (100.0) | 4628 | (100.0) | 11241 | (100.0) | |
| Age | | | | | | | | | 0.000 |
| <40 | 1275 | (5.3) | 496 | (6.1) | 284 | (6.1) | 495 | (4.4) | |
| 40- | 5572 | (23.2) | 2143 | (26.2) | 1142 | (24.7) | 2287 | (20.3) | |
| 50- | 9380 | (39.0) | 3329 | (40.6) | 1910 | (41.3) | 4141 | (36.8) | |
| 60- | 6840 | (28.4) | 2032 | (24.8) | 1177 | (25.4) | 3631 | (32.3) | |
| 70- | 993 | (4.1) | 191 | (2.3) | 115 | (2.5) | 687 | (6.1) | |
| Total | 24060 | (100.0) | 8191 | (100.0) | 4628 | (100.0) | 11241 | (100.0) | |
| Regions | | | | | | | | | 0.000 |
| HIA | 19514 | (81.1) | 6768 | (82.6) | 3056 | (66.0) | 9690 | (86.2) | |
| LIA | 4546 | (18.9) | 1423 | (17.4) | 1572 | (34.0) | 1551 | (13.8) | |
| Total | 24060 | (100.0) | 8191 | (100.0) | 4628 | (100.0) | 11241 | (100.0) | |
| Urban/Rural Residence | | | | | | | | | 0.000 |
| Urban | 2524 | (10.5) | 965 | (11.8) | 422 | (9.1) | 1137 | (10.1) | |
| Rural | 21536 | (89.5) | 7226 | (88.2) | 4206 | (90.9) | 10104 | (89.9) | |
| Total | 24060 | (100.0) | 8191 | (100.0) | 4628 | (100.0) | 11241 | (100.0) | |
| Cigarette Smoking | | | | | | | | | 0.000 |
| Yes | 9626 | (40.0) | 3218 | (39.3) | 1979 | (42.8) | 4429 | (39.4) | |
| No | 14434 | (60.0) | 4973 | (60.7) | 2649 | (57.2) | 6812 | (60.6) | |
| Total | 24060 | (100.0) | 8191 | (100.0) | 4628 | (100.0) | 11241 | (100.0) | |
| Alcohol Consumption | | | | | | | | | 0.000 |
| Yes | 5722 | (23.8) | 2237 | (27.3) | 676 | (14.6) | 2809 | (25.0) | |
| No | 18338 | (76.2) | 5954 | (72.7) | 3952 | (85.4) | 8432 | (75.0) | |
| Total | 24060 | (100.0) | 8191 | (100.0) | 4628 | (100.0) | 11241 | (100.0) | |
| Cancer Family History | | | | | | | | | 0.000 |
| Positive | 11790 | (49.0) | 2481 | (30.3) | 1645 | (35.5) | 7664 | (68.2) | |
| Negative | 12270 | (51.0) | 5710 | (69.7) | 2983 | (64.5) | 3577 | (31.8) | |
| Total | 24060 | (100.0) | 8191 | (100.0) | 4628 | (100.0) | 11241 | (100.0) | |
| Tumor Location# | | | | | | | | | 0.000 |
| Upper | 4039 | (16.9) | 1042 | (12.7) | 1497 | (32.5) | 1500 | (13.5) | |
| Middle | 14429 | (60.3) | 5109 | (62.4) | 2186 | (47.4) | 7134 | (64.1) | |
| Lower | 5461 | (22.8) | 2039 | (24.9) | 930 | (20.2) | 2492 | (22.4) | |
| Total | 23929 | (100.0) | 8190 | (100.0) | 4613 | (100.0) | 11126 | (100.0) | |

*(Continued)*

TABLE 1 Continued

| Characteristics | No. of The Patients Examined | | Hospital Volume | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Low | | Medium | | High | | P |
| Differentiation | | | | | | | | | 0.000 |
| Well | 5353 | (26.1) | 2364 | (34.2) | 672 | (16.3) | 2317 | (24.4) | |
| Moderate | 11223 | (54.7) | 3383 | (49.0) | 2751 | (66.7) | 5089 | (53.6) | |
| Poor | 3949 | (19.2) | 1158 | (16.8) | 700 | (17.0) | 2091 | (22.0) | |
| Total | 20525 | (100.0) | 6905 | (100.0) | 4123 | (100.0) | 9497 | (100.0) | |
| Incisal Edge Residue | | | | | | | | | 0.000 |
| Negative | 13772 | (93.8) | 4270 | (93.0) | 3428 | (98.8) | 6074 | (91.7) | |
| Positive | 913 | (6.2) | 321 | (7.0) | 43 | (1.2) | 549 | (8.3) | |
| Total | 14685 | (100.0) | 4591 | (100.0) | 3471 | (100.0) | 6623 | (100.0) | |
| Lymph Node Metastasis | | | | | | | | | 0.000 |
| Negative | 14081 | (58.5) | 4544 | (55.5) | 2826 | (61.1) | 6711 | (59.7) | |
| Positive | 9979 | (41.5) | 3647 | (44.5) | 1802 | (38.9) | 4530 | (40.3) | |
| Total | 24060 | (100.0) | 8191 | (100.0) | 4628 | (100.0) | 11241 | (100.0) | |
| Pathological Stage | | | | | | | | | 0.000 |
| I | 1523 | (6.3) | 911 | (11.1) | 199 | (4.3) | 413 | (3.7) | |
| II | 6516 | (27.1) | 1500 | (18.3) | 1930 | (41.7) | 3086 | (27.5) | |
| III | 16021 | (66.6) | 5780 | (70.6) | 2499 | (54.0) | 7742 | (68.9) | |
| Total | 24060 | (100.0) | 8191 | (100.0) | 4628 | (100.0) | 11241 | (100.0) | |
| Surgical Approaches$ | | | | | | | | | 0.000 |
| Left | 6601 | (93.9) | 1725 | (99.7) | 553 | (98.8) | 4323 | (91.3) | |
| Right | 426 | (6.1) | 6 | (0.3) | 7 | (1.2) | 413 | (8.7) | |
| Total | 7027 | (100.0) | 1731 | (100.0) | 560 | (100.0) | 4736 | (100.0) | |
| Operative Deaths* | | | | | | | | | 0.000 |
| Yes | 29 | (0.1) | 7 | (0.1) | 15 | (0.3) | 7 | (0.1) | |
| No | 24031 | (99.9) | 8184 | (99.9) | 4613 | (99.7) | 11234 | (99.9) | |
| Total | 24060 | (100.0) | 8191 | (100.0) | 4628 | (100.0) | 11241 | (100.0) | |
| Death in Hospital& | | | | | | | | | 0.000 |
| Yes | 89 | (0.4) | 23 | (0.3) | 35 | (0.8) | 31 | (0.3) | |
| No | 23971 | (99.6) | 8168 | (99.7) | 4593 | (99.2) | 11210 | (99.7) | |
| Total | 24060 | (100.0) | 8191 | (100.0) | 4628 | (100.0) | 11241 | (100.0) | |

HIA, high incidence area; LIA, low incidence area.
#:Because of the small number, cervical esophageal cancer was divided into the upper segment.
$:Left, Sweet procedure. Right: Ivor-Lewis procedure+Mckeown procedure.
*: Operative Death: Death within 14 days of esophagectomy or death during the hospitalization in which the primary procedure was performed.
&: Death in Hospital: Death within the same hospital admission or within 30 days.

hospitals were from high incidence areas, more likely to have a positive family history of cancer. But the percentage of positive incisal edge residue was highest in high volume hospitals (6.2%) (Table 2).

Long-term survival analysis for 1973-1996 patients and the Kaplan-Meier curve for overall survival demonstrated a survival benefit for treatment at high volume hospitals (log-rank $P$ = 0.000). Specifically, patients at medium and high

TABLE 2 Relationship between the clinicopathological features of ESCC patients and hospital volume during 1997-2020, n(%).

| Characteristics | No. of The Patients Examined | | Hospital Volume | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Patients Examined | | Low | | Medium | | High | | P |
| Sex | | | | | | | | | 0.000 |
| Male | 88674 | (65.9) | 47692 | (67.6) | 20027 | (68.0) | 20955 | (60.6) | |
| Female | 45884 | (34.1) | 22819 | (32.4) | 9422 | (32.0) | 13643 | (39.4) | |
| Total | 134558 | (100.0) | 70511 | (100.0) | 29449 | (100.0) | 34598 | (100.0) | |
| Age | | | | | | | | | 0.000 |
| <40 | 1244 | (0.9) | 761 | (1.1) | 233 | (0.8) | 250 | (0.7) | |
| 40- | 12703 | (9.4) | 7146 | (10.1) | 2614 | (8.9) | 2943 | (8.5) | |
| 50- | 46388 | (34.5) | 24920 | (35.3) | 10136 | (34.4) | 11332 | (32.8) | |
| 60- | 54821 | (40.7) | 27972 | (39.7) | 12473 | (42.4) | 14376 | (41.6) | |
| 70- | 19402 | (14.4) | 9712 | (13.8) | 3993 | (13.6) | 5697 | (16.5) | |
| Total | 134558 | (100.0) | 70511 | (100.0) | 29449 | (100.0) | 34598 | (100.0) | |
| Regions | | | | | | | | | 0.000 |
| HIA | 77272 | (57.4) | 36560 | (51.9) | 10958 | (37.2) | 29754 | (86.0) | |
| LIA | 57286 | (42.6) | 33951 | (48.1) | 18491 | (62.8) | 4844 | (14.0) | |
| Total | 134558 | (100.0) | 70511 | (100.0) | 29449 | (100.0) | 34598 | (100.0) | |
| Urban/Rural Residence | | | | | | | | | 0.000 |
| Urban | 15433 | (11.5) | 7655 | (10.9) | 4445 | (15.1) | 3333 | (9.6) | |
| Rural | 119125 | (88.5) | 62856 | (89.1) | 25004 | (84.9) | 31265 | (90.4) | |
| Total | 134558 | (100.0) | 70511 | (100.0) | 29449 | (100.0) | 34598 | (100.0) | |
| Cigarette Smoking | | | | | | | | | 0.000 |
| Yes | 60740 | (45.1) | 31959 | (45.3) | 13825 | (46.9) | 14956 | (43.2) | |
| No | 73818 | (54.9) | 38552 | (54.7) | 15624 | (53.1) | 19642 | (56.8) | |
| Total | 134558 | (100.0) | 70511 | (100.0) | 29449 | (100.0) | 34598 | (100.0) | |
| Alcohol Consumption | | | | | | | | | 0.000 |
| Yes | 41987 | (31.2) | 21614 | (30.7) | 9561 | (32.5) | 10812 | (31.3) | |
| No | 92571 | (68.8) | 48897 | (69.3) | 19888 | (67.5) | 23786 | (68.7) | |
| Total | 134558 | (100.0) | 70511 | (100.0) | 29449 | (100.0) | 34598 | (100.0) | |
| Cancer Family History | | | | | | | | | 0.000 |
| Positive | 55152 | (41.0) | 22827 | (32.4) | 8649 | (29.4) | 23676 | (68.4) | |
| Negative | 79287 | (59.0) | 47565 | (67.6) | 20800 | (70.6) | 10922 | (31.6) | |
| Total | 134439 | (100.0) | 70392 | (100.0) | 29449 | (100.0) | 34598 | (100.0) | |
| Tumor Location# | | | | | | | | | 0.000 |
| Upper | 21831 | (17.0) | 11370 | (17.2) | 3971 | (14.3) | 6490 | (18.9) | |
| Middle | 82543 | (64.3) | 41492 | (62.7) | 19048 | (68.4) | 22003 | (64.0) | |
| Lower | 24019 | (18.7) | 13286 | (20.1) | 4844 | (17.4) | 5889 | (17.1) | |
| Total | 128393 | (100.0) | 66148 | (100.0) | 27863 | (100.0) | 34382 | (100.0) | |

*(Continued)*

TABLE 2 Continued

| Characteristics | No. of The Patients Examined | | Hospital Volume | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Patients Examined | | Low | | Medium | | High | | P |
| Differentiation | | | | | | | | | 0.000 |
| Well | 19026 | (15.2) | 12712 | (19.5) | 3076 | (11.6) | 3238 | (9.7) | |
| Moderate | 77673 | (62.1) | 40736 | (62.6) | 16410 | (61.7) | 20527 | (61.3) | |
| Poor | 28451 | (22.7) | 11639 | (17.9) | 7105 | (26.7) | 9707 | (29.0) | |
| Total | 125150 | (100.0) | 65087 | (100.0) | 26591 | (100.0) | 33472 | (100.0) | |
| Incisal Edge Residue | | | | | | | | | 0.000 |
| Negative | 105773 | (96.4) | 54813 | (97.3) | 23880 | (97.3) | 27080 | (93.8) | |
| Positive | 3980 | (3.6) | 1524 | (2.7) | 659 | (2.7) | 1797 | (6.2) | |
| Total | 109753 | (100.0) | 56337 | (100.0) | 24539 | (100.0) | 28877 | (100.0) | |
| Lymph Node Metastasis | | | | | | | | | 0.000 |
| Negative | 80312 | (59.7) | 42192 | (59.8) | 17787 | (60.4) | 20333 | (58.8) | |
| Positive | 54245 | (40.3) | 28318 | (40.2) | 11662 | (39.6) | 14265 | (41.2) | |
| Total | 134557 | (100.0) | 70510 | (100.0) | 29449 | (100.0) | 34598 | (100.0) | |
| Pathological Stage | | | | | | | | | 0.000 |
| I | 13111 | (9.7) | 6432 | (9.1) | 3017 | (10.2) | 3662 | (10.6) | |
| II | 24377 | (18.1) | 12830 | (18.2) | 5297 | (18.0) | 6250 | (18.1) | |
| III | 97070 | (72.1) | 51249 | (72.7) | 21135 | (71.8) | 24686 | (71.4) | |
| Total | 134558 | (100.0) | 70511 | (100.0) | 29449 | (100.0) | 34598 | (100.0) | |
| Surgical Approaches$ | | | | | | | | | 0.000 |
| Left | 33157 | (80.1) | 7612 | (63.8) | 8277 | (93.7) | 17268 | (83.6) | |
| Right | 8260 | (19.9) | 4314 | (36.2) | 553 | (6.3) | 3393 | (16.4) | |
| Total | 41417 | (100.0) | 11926 | (100.0) | 8830 | (100.0) | 20661 | (100.0) | |
| Operative Deaths* | | | | | | | | | 0.554 |
| Yes | 286 | (0.2) | 155 | (0.2) | 55 | (0.2) | 76 | (0.2) | |
| No | 134272 | (99.8) | 70356 | (99.8) | 29394 | (99.8) | 34522 | (99.8) | |
| Total | 134558 | (100.0) | 70511 | (100.0) | 29449 | (100.0) | 34598 | (100.0) | |
| Death in Hospital& | | | | | | | | | 0.665 |
| Yes | 734 | (0.5) | 380 | (0.5) | 155 | (0.5) | 199 | (0.6) | |
| No | 133824 | (99.5) | 70131 | (99.5) | 29294 | (99.5) | 34399 | (99.4) | |
| Total | 134558 | (100.0) | 70511 | (100.0) | 29449 | (100.0) | 34598 | (100.0) | |

HIA, high incidence area; LIA, low incidence area.
#:Because of the small number, cervical esophageal cancer was divided into the upper segment.
$:Left: Sweet procedure. Right: Ivor-Lewis procedure+Mckeown procedure.
*: Operative Death: Death within 14 days of esophagectomy or death during the hospitalization in which the primary procedure was performed.
&: Death in Hospital: Death within the same hospital admission or within 30 days.

volume hospitals had a reduced risk of death, compared with those at low volume hospitals. The 3-year survival rates in low, medium and large volume hospitals were 66.0%, 61.6% and 75.6%, respectively. The 5-year survival rates were 55.8%, 52.6% and 64.4%, respectively (Figure 3A). This trend also existed in patients for diagnosed between 1997 and 2020(log-rank $P = 0.000$)(3-year survival rates: 57.8%, 56.8% and 60.2%; 5-year survival rates:46.8%, 46.4% and 48.8%) (Figure 3B).

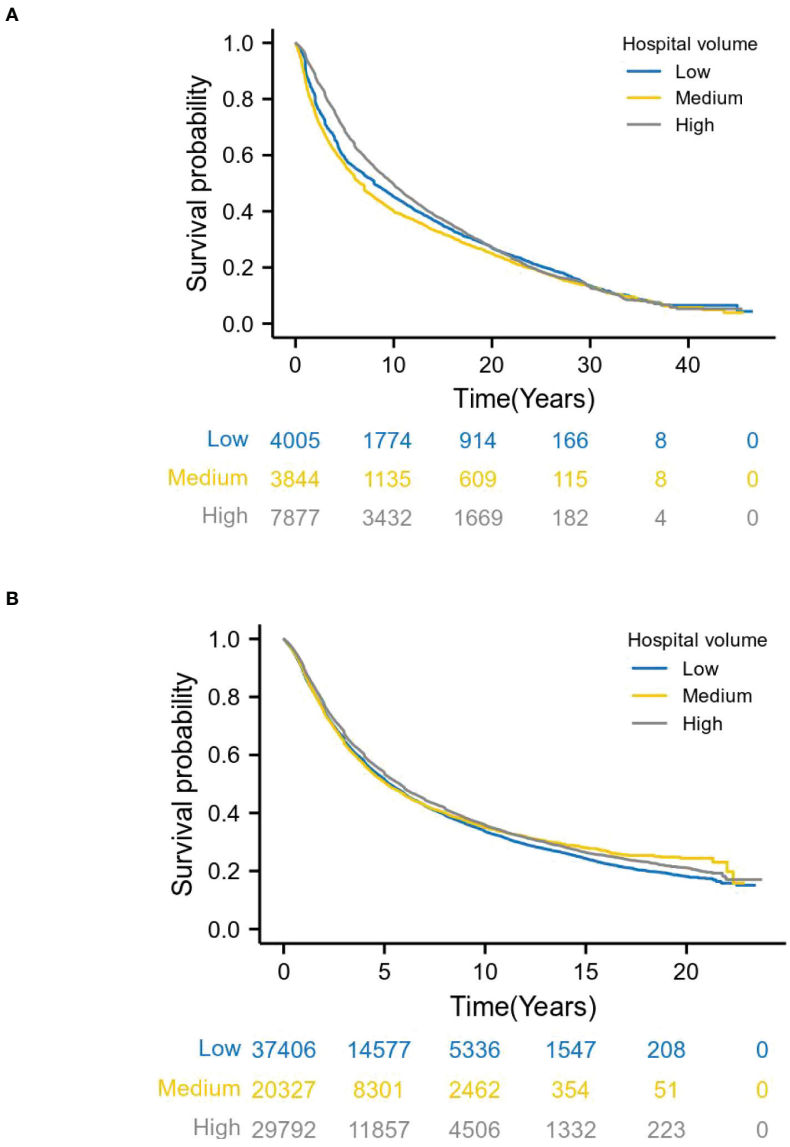


FIGURE 3
Relationship between hospital volume and overall survival by year. **(A)** shows patients with esophageal cancer from 1973 to 1996, and **(B)** from 1997 to 2020. Long-term survival analysis, Kaplan-Meier curve showed that patients with stage I-III esophageal cancer who underwent surgery in high volume hospitals had better survival than patients in low volume hospitals (log-rank P =0.000).

## Multivariable analysis

Patients diagnosed between 1973 and 1996 in this study, multivariate analysis demonstrated that after adjusting for patient/tumor-related mixed factors (age, sex, regions, urban/rural residence, cigarette smoking, alcohol consumption, cancer family history, incisal edge residue, tumor location, differentiation and pathological stages), the overall survival rate of medium and high volume hospitals was better than that of low volume hospitals (HR 0.797, 95% Cl 0.637-0.999; HR 0.518, 95% Cl 0.456-0.589). This confirmed the survival benefit of treatment at a high volume hospital. Older age, later pathological stage, poor differentiation, male(HR 0.883, 95%Cl 0.805-0.968), negative family history of cancer (HR 0.872, 95% Cl 0.787-0.967) were associated with a poorer prognosis (Figure 4A).

For patients diagnosed between 1997 and 2020, the results of our multivariate Cox proportional hazards model also confirmed the survival benefit of treatment in a high volume hospital. Older age, later pathological stage, poor differentiation, male(HR 0.879, 95%Cl 0.853-0.907) and upper+cervical tumor were independent influencing factors for poor prognosis (Figure 4B).

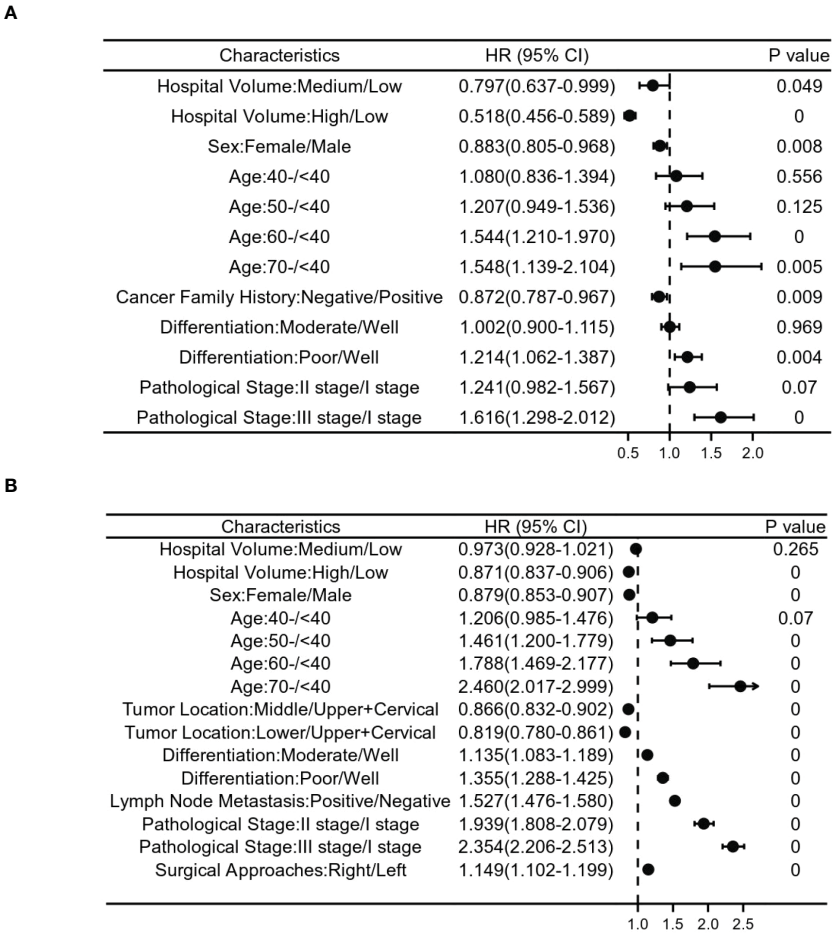


FIGURE 4
Relationship between clinicopathological features and postoperative survival risk in patients with stages I-III esophageal cancer. **(A)** shows patients with esophageal cancer from 1973 to 1996, and **(B)** ITom 1997 to 2020. Risk ratios based on hospital volume, age, sex, cancer family history, differentiation,tumor location, lymph node metastasis, surgical approaches and pathological stage.

## Hospital volume and all-cause mortality

The relationship between hospital volume and the risk of all-cause mortality was half-U-shaped on a continuous scale. However, the overall hospital volume was still a protective factor for postoperative esophageal cancer patients (HR<1). In multivariable adjusted analyses, the hospital volume associated with the lowest risk of all-cause mortality was 1027 cases/year (Figure 5).

# Discussion

Based on a large sample size of 158,618 patients spanning 47 years (1973-2020) in China, this paper systematically summarizes the relationship between hospital volume and the treatment effect of ESCC patients with stage I-III in China in two time periods. The hospital volume with the lowest risk of all-cause mortality was found to be 1027 cases/year. These results may provide an important basis for patients to choose hospitals and may have an impact on the centralized management of hospital surgery. As expected, the unlimited increase in hospital volume does not always benefit patients after surgery.

We found that high volume hospitals were both independent predictors of improved survival for stage I-III patients with esophageal cancer in two time periods. Several studies in the United States have shown that treatment in high volume hospitals is better for the long-term survival of patients with esophageal cancer (4, 8, 17–21). Relevant studies in Korea, Switzerland, Australia, Japan and the Netherlands also suggest that centralized surgery for esophageal cancer can improve the clinical prognosis of patients (6, 7, 22–30). However, four other studies in the United States and Sweden found no effect of hospital volume on the postoperative survival of patients with

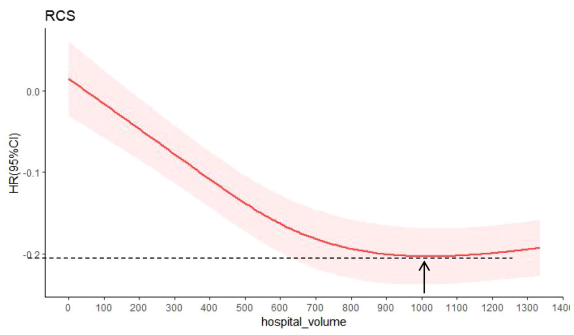


FIGURE 5
Hazard ratios were multivariable adjusted for death from all causes according to hospital volume. The solid red line is the multivariable adjusted hazard ratio, and the shaded red is the 95% confidence interval obtained from the restricted cubic spline regression. Arrows indicate the hospital volume with the lowest risk of death from all causes. Analyses were adjusted for sex, age, region, urban/rural residence, smoking history, drinking history, cancer family history, incisal edge residue, tumor location, differentiation, pathological stage and contirmed time.

esophageal cancer (9, 10, 31, 32). Our results are consistent with those of most studies. The reason for the inconsistency of our results with those of the Swedish and American studies may be the inclusion of different ethnicities and esophageal cancer subtypes (97% of patients have esophageal squamous cell carcinoma in China, compared with Western countries dominated by esophageal adenocarcinoma). In many tumors and complex procedures, we generally agree that a good survival of patients is strongly associated with hospital volume and the number of thoracic surgeons. Studies have shown that by choosing surgeons who often perform surgery and larger hospitals, patients often can significantly improve their chances of survival (18, 31, 33). Large volume hospitals tend to have better facilities, wider departments and better staffed intensive care units, and other resources, which are not available in small volume hospitals. With these resources, large volume hospitals can better reduce perioperative mortality for cancer patients or high-risk surgical patients (34).

Our results also showed that surgery by the left approach was independent factors of good prognosis in 1997-2020 patients, but not in 1973-1996 patients. One possible explanation for the inconsistent results is that our study included only 7,029 patients with a well-defined surgical approach in the first period (much less than the 41,417 patients in the second period), and the results were statistically biased. It is necessary to enroll more patients with a clear surgical approach for validation. In fact, controversy exists between open esophagectomy by the left approach(Sweet procedure) and surgery by the right approach(Ivor-lewis procedure and Mckeown procedure). In China, left-side approach surgery is the main traditional surgical method, and Sweet procedure is widely used because of its simplicity, speed and relatively small trauma (35, 36). Although it has been criticized for failing to clear or completely clear the upper thoracic lymph nodes (37). In contrast, the right-side approach surgery offers better visualization of the thoracic esophagus, and a skilled surgeon can clean the chest from top to bottom of all lymph nodes. However, the operation time was prolonged and related postoperative complications were increased (38). In this study, the left-side approach was the main operation in both time periods, and in the second time period, the left-side approach was an independent factor influencing the prognosis of patients with ESCC. This suggests that a left-side approach with limited lymphadenectomy remains a priority in China for nearly 20 years. However, as it is popular to perform minimum invasive surgery and postoperative adjuvant therapy in recent years. Minimally invasive surgery is promising with less trauma and fewer complications, but its applicability is limited. In order to better understand the influence of different treatment methods on postoperative prognosis of patients with ESCC, we searched the database for all patients underwent minimally invasive surgery, surgery and surgery + adjuvant therapy in 2014-2015 to analyze their 5-year survival rates, and found that patients underwent minimally invasive surgery had the best survival, followed by surgery and surgery + adjuvant therapy (Supplementary Figure S1). This is consistent with the findings of two other studies (39, 40). Therefore, for patients with ESCC, minimally invasive surgery can be preferred if there are indications for minimally invasive surgery. However, no matter it is minimally invasive surgery or open surgery, it is most important to select the treatment approach suitable for the patient based on the patient's own conditions and ensure the complete resection of the tumor and thorough dissection of the lymph nodes, which will affect the prognosis of the patient.

It is well known that medical equipment of the hospital, the quality of resection and perioperative management of esophageal cancer can also affect patient outcomes. In order to better evaluate the prognosis of patients undergoing esophagectomy in different hospitals, we divided hospitals into tertiary hospitals and secondary hospitals for prognostic analysis according to hospital size, hospital technical level, medical equipment, hospital management level and hospital quality (i.e., hospital grade). The survival of patients undergoing surgical treatment in tertiary hospitals was better than that in secondary hospitals during the period 1973-1996, but the results were reversed in the latter period (Supplementary Figure S2). We carefully compared the composition of hospitals with two levels in two periods, and found that although some hospitals were secondary hospitals from 1997 to 2020, their annual operation volume of esophageal cancer had reached the level of high volume hospitals. Because these hospitals are located in the high incidence area of esophageal cancer (Linzhou) and have a large number of patients, the level of thoracic surgery, ICU and anesthesiology departments in the hospitals has been

significantly improved. This suggests that it may be necessary to develop specialized cancer hospitals in China.

Hazard ratios were multivariable adjusted for death from all causes according to hospital volume, we found that hospital volume with the lowest risk of death from all causes was 1,027 cases/year. However, the volume threshold of 1,027 cases/year appears to be higher than the high volume definition in previous studies (4, 6–10). It is important to emphasize that half of the annual new esophageal cancer cases are from China (1, 2), and many of the hospitals included in this study were in the high incidence areas of esophageal cancer in China. Our threshold number of cases was objectively determined based on the adjusted correlation between hospital volume and postoperative outcomes. Despite the intuitive appeal of using surgical volume as a predictor and quality measure of surgical outcome, the methodological rigor of many surgical volumetry-outcome studies has been questioned (41). In this study, the number of surgical procedures was not arbitrarily classified, but was based on the Cox hazards model and RCS, and multiple confounding factors were adjusted for data grouping. Therefore, we believe that the average annual hospital operation volume is a reliable predictor of the prognosis of patients with esophageal cancer.

This study is retrospective and has some limitations. First, the AJCC staging system was updated during the large time span of our study data. However, to overcome this limitation, we used the uniform earlier (2002) clinical staging with fewer errors. Second, as with many large data registries, although we checked every medical record, we are not immune to errors in data entry. Finally, the study did not record the average annual ESCC operation volume of each surgeon in the hospital, so it is uncertain whether the difference in hospital volume is caused by the surgeon volume because surgeon experience is also widely believed to be a key factor affecting the prognosis of complex surgery (42–45). However, the medium and high volume thresholds used in this study (>277 cases/year, >689 cases/year and >596 cases/year, >1005 cases/year) are unlikely to be accurate for surgeons with a low annual ESCC volume.

Our findings suggest that high volume hospitals improve long-term survival for patients with stage I–III ESCC and identify hospital volume thresholds with the lowest risk of death from all causes. Therefore, hospital volume can be used as an indicator of the postoperative prognosis of patients with esophageal cancer. It also suggests the importance for health care providers and policy-makers to advocate regionalization or surgical centralization in areas with high mortality.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

L-DW and L-LL designed and wrote the paper. L-LL, R-HX, M-XW, LS, P-PW, M-MY, J-FH, KZ, W-LH, X-NH, Z-MF, RW, BL, X-ZW, L-GZ, Q-DB, Y-RQ, Z-WC, J-WK, H-JY, LY, J-LR, X-ML performed data collection, interpretation and follow-up. L-LL, X-KZ and XS contributed to data analysis. F-YZ and L-DW revised the manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher’s note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.1056086/full#supplementary-material

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# A potential link between aberrant expression of ECRG4 and atrial fibrillation

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Esophageal cancer-related gene-4 (ECRG4), a 148-amino acid propertied and new tumor suppressor, is initially cloned from the normal esophageal epithelium. ECRG4 was found to be expressed not only in esophageal tissues but also in cardiomyocytes. Previous studies demonstrated that ECRG4 is constitutively expressed in esophageal epithelial cells, and its degree of downregulation is directly proportional to prognosis in patients with esophageal cancer. In the heart, ECRG4 shows greater expression in the atria than in the ventricles, which accounts for its heterogeneity. Downregulation of ECRG4 expression level correlates with esophageal cancer, as well as myocardial injuries and arrhythmias. As a result, this review summarizes the possible susceptibility gene, ECRG4 and its associated molecular mechanisms in cancer patients with atrial fibrillation and myocardial injury. The review begins by describing ECRG4's biological background, discusses its expression in the cardiovascular system, lists the clinical and animal research related to the downregulation of ECRG4 in atrial fibrillation, and focuses on its potential role in atrial fibrillation. Downregulation of ECRG4 may increase the risk of atrial fibrillation by affecting ion channels, MMPs expression and inflammatory response. We will then discuss how ECRG4 can be used in the treatment of tumors and arrhythmias, and provide a novel possible strategy to reduce the occurrence of perioperative cardiovascular adverse events in patients with tumors such as esophageal cancer and gastric cancer.

KEYWORDS

**esophageal cancer-related gene-4, myocardial injury, tumor suppressor gene, atrial fibrillation, radical surgery for esophageal cancer**

## Introduction

Esophageal cancer-related gene-4 (ECRG4) is a newly identified tumor suppressor gene and a sentinel molecule for maintaining tissue homeostasis. Recent research has revealed that ECRG4 expression is quite distinct and is present in esophageal squamous epithelial cells as well as the sinoatrial node, atrioventricular node, atrial and ventricular cells. Additional investigations further concluded that ECRG4 could maintain cardiac

homeostasis and regulate cardiac rhythm while it downregulation may contribute to atrial fibrillation (AF) (1). Moreover, ECRG4 is most likely a hypoxic sensor and may be related to myocardial ischemia (1). Notably, ECRG4 is also a tumor suppressor gene that can prevent esophageal cancer cell proliferation (2). Downregulation of this gene expression increases the risk of esophageal cancer and is strongly linked to a poor patient prognosis (2–4). Epidemiological studies also suggest a strong correlation between AF and various tumors. For instance, the risk of colorectal cancer in AF patients was ten times greater than in those without AF, according to a case-control study (5). Furthermore, reduced ECRG4 expression in esophageal cancer patients is associated with an increased incidence of myocardial injury and atrial fibrillation (2, 6). Collectively, the above studies show that ECRG4 may be implicated in both tumorigenesis and cardiovascular adverse events. Herein, we describe the ECRG4's biological background, discuss its expression in the cardiovascular system, list the clinical and animal research related to the downregulation of ECRG4 in atrial fibrillation, and focus on its potential role in atrial fibrillation. We will then discuss how ECRG4 can be used in the treatment of tumors and arrhythmias, and provide a novel possible strategy to reduce the occurrence of perioperative cardiovascular adverse events in patients with tumors such as esophageal cancer and gastric cancer.

## The biological background of ECRG4

Su et al. first discovered ECRG4 in normal human esophageal epithelial cells (7), and it was eventually localized in the c2orf40 locus of chromosome 2, which consists of four exons spanning approximately 14.9 kilobases (4, 8). The initial bioinformatic analysis and subsequent biochemical characterization indicate that the protein encoded by ECRG4 is a hormone-like secretory protein. The ECRG4 gene also encodes a protein with a molecular weight of 17KDA, and peptides with different molecular weights associated with the ECRG4 protein were also identified. Most of the tumor suppressor genes are usually intracellular or membrane proteins. Unlike most tumor suppressor genes, which are usually membrane or intracellular proteins, ECRG4 is a 148 amino acid propeptide that is covalently linked to the amino end on cell surfaces (9, 10). Since ECRG4 is attached to the cell surface, it acts as a "sentinel" in maintaining tissue homeostasis (Figure 1). The presence of ECRG4 on the cell surface suggests that its homeostasis is maintained. After tissue injury, ECRG4 can quickly detach from the cell surface (within 24 hours), thereby increasing tissue injury responses like vascular leakage, immune cell infiltration, and cell proliferation (in 2-4 days). The injury response gradually disappears during the healing process as ECRG4 returns to the cellular surface (usually in 6-7 days) (11–13).

Previous studies demonstrate that ECRG4 is constitutively expressed in esophageal epithelial cells, and its degree of downregulation is directly proportional to prognosis in esophageal cancer patients (Figure 1) (2). Studies have shown that ECRG4 may induce the downregulation of COX-2 through the NF-KB pathway, thereby inhibiting tumor growth in esophageal squamous cell carcinoma (ESCC) (2). Other Studies have found that ECRG4 can directly interact with ECRG1 to up-regulate the expression of p21, induce G1 phase arrest of cell cycle, and inhibit the proliferation of cancer cells (3). Further research revealed that ECRG4 was down-regulated to varying degrees in gastric cancer (14, 15), breast cancer (16, 17), hepatocellular cancer (18, 19), nasopharyngeal cancer (20–22), laryngeal cancer (23), bladder cancer (24, 25), glioma (26), colorectal cancer and prostate cancer (26–28). These findings suggest that ECRG4 plays a tumor-suppressive role. Various other cells/tissues, such as the adrenal gland, choroid plexus, cardiomyocytes, and conduction system, also express ECRG4. ECRG4 is known to regulate inflammation (11–13), induce neuronal senescence (29), participate in the formation of atrial fibrillation (30), and possibly act as a hypoxia sensor, contributing to myocardial injury.

## ECRG4: Expression in the cardiovascular system

In 2017 Mirabeau et al. uncovered ECRG4 as a new secretory peptide in mouse endocrine tissues as well as in other locations, including the pituitary, adrenal gland, pancreas, choroid plexus, and the atrioventricular node of the heart (8). Notably, ECRG4 mRNAs are expressed in heart (31). It was found that ECRG4 was expressed in the sinoatrial node, atrium and ventricle, and ECRG4 expression was higher in atrium (30, 31). Porzionato et al. used immunohistochemical analysis to show that ECRG4 was uniformly expressed in normal rat atrial myocytes while only expressed in sporadic ventricular myocytes (31). Professor Dang further discovered that down-regulating ECRG4 in atrial myocytes activated pro-inflammatory cascade and genes involved in heart

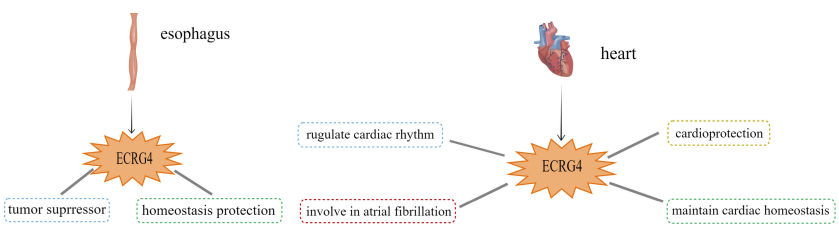


FIGURE 1
Potential functions of ECRG4 in the esophagus and cardiovascular system.

remodeling, which participated in the occurrence of atrial fibrillation, and concluded that the normal expression of ECRG4 could maintain cardiac homeostasis (Figure 1) (30). Other studies have shown that ECRG4 promotes cardiovascular homeostasis and prevents atrial fibrillation by regulating the response to ischemia/hypoxia (32).

## ECRG4 downregulation is associated with atrial fibrillation: Clinical and animal research

Atrial fibrillation is the most common sustained cardiac tachyarrhythmia encountered by physicians, with an ever rising incidence globally. Studies have found that ECRG4 is implicated in the pathogenesis of atrial fibrillation (Figure 2). Moreover, since ECRG4 is a tumor suppressor gene, the downregulation of ECRG4 may also be a risk factor for atrial fibrillation in cancer patients. Extensive epidemiological data show a strong correlation between AF incidence and cancer, which has garnered widespread attention. Erichsen found a tenfold increase in the incidence of colorectal cancer in patients with atrial fibrillation (5). A cohort study found that the cancer diagnosis rate in newly diagnosed AF patients increased 5-fold compared to the expected cancer incidence in the general population after a 3-month follow-up (33). Consistently, Conen found that cancer patients experienced twice the incidence of AF during surgery as non-tumor patients (34). Another clinical control study showed that in esophageal cancer patients with intraoperative hypothermia, the incidence of postoperative myocardial injury could rise to 31.4%, while the incidence of atrial fibrillation (AF) was 14.3% (6), significantly higher than the myocardial injury incidence of 8% in other non-cardiac surgeries (35) and 2.9% incidence of atrial fibrillation in thoracoscopic lung cancer surgery (36). Previous studies reported that the incidence of new-onset atrial fibrillation after radical esophagectomy was 12-37% (37–47). Atrial fibrillation may also be linked to lower ECRG4 expression in other patients besides tumor patients Five suitable atrial appendage specimens from patients with rheumatic heart disease with or without atrial fibrillation were further collected clinically to investigate the expression of ECRG4 in the heart, and immunohistochemistry confirmed the downregulation of ECRG4 in the atrial appendage of patients with atrial fibrillation (30).

Additionally, certain drugs may affect ECRG4 expression and cause atrial fibrillation. Doxorubicin(DOX) is well-known for cardiotoxic effects, including atrial fibrillation. According to a prospective study by Kilickap and colleagues, DOX-containing regimens caused arrhythmia in 19 patients (65.5%) of 29 patients with various cancers, of whom 3 patients (10.3%) had paroxysmal AF (48). In a previous study, Long et al. investigated the role of ECRG4 in AF and myocardial injury induced by DOX (49). DOX decreased endogenous ECRG4 gene expression in the heart and cultured neonatal rat cardiomyocytes. Further, cardiomyocyte-specific conditional ECRG4 knockout mice showed increased sensitivity to DOX-induced cardiotoxicity due to abnormal signaling pathways, including Oxidative phosphorylation, Thermogenesis, Diabetic cardiomyopathy, Cardiac muscle contraction (Figure 3). This study suggests that ECRG4, which is constitutively expressed in the heart, can maintain cardiac homeostasis and protect cardiomyocytes from the cardiac toxicity caused by DOX (49). Taken together, the high incidence of AF in tumor patients may be related to ECRG4 expression. Therefore, it is crucial to investigate how ECRG4 maintains cardiac homeostasis and regulates cardiac rhythm.

Studies on ECRG4 in atrial fibrillation have also been conducted in animal models. Huang et al. previously found that the expression of ECRG4 was significantly decreased in a rapid atrial pacing-induced canine AF model, suggesting that ECRG4 participates in the pathogenesis of AF (44). In addition, another study found that 24 hours after rapid electrical stimulation, ECRG4 was significantly downregulated in mouse atrial myocytes HL1 (50). Furthermore, The International Mouse Phenotyping Consortium revealed that ECRG4 knockout mice have a shorter QRS complex duration, highlighting the role of ECRG4 in heart rate/rhythm regulation. Collectively, the above findings indicate that both tachyarrhythmias and rapid pacing can induce a significant decrease in the expression of ECRG4, and the decrease in ECRG4 further leads to atrial remodeling, which is essential for the generation and maintenance of atrial fibrillation.

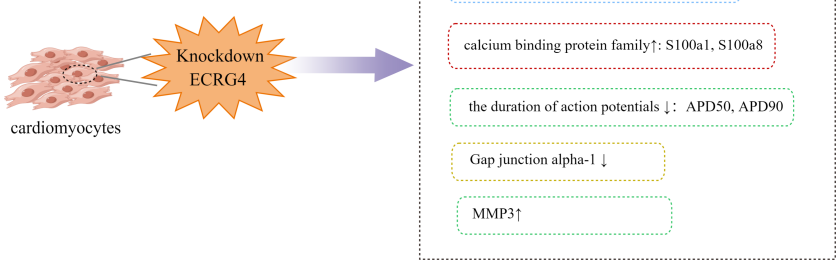


FIGURE 2
Mechanism of atrial fibrillation induced by down-regulation ECRG4.

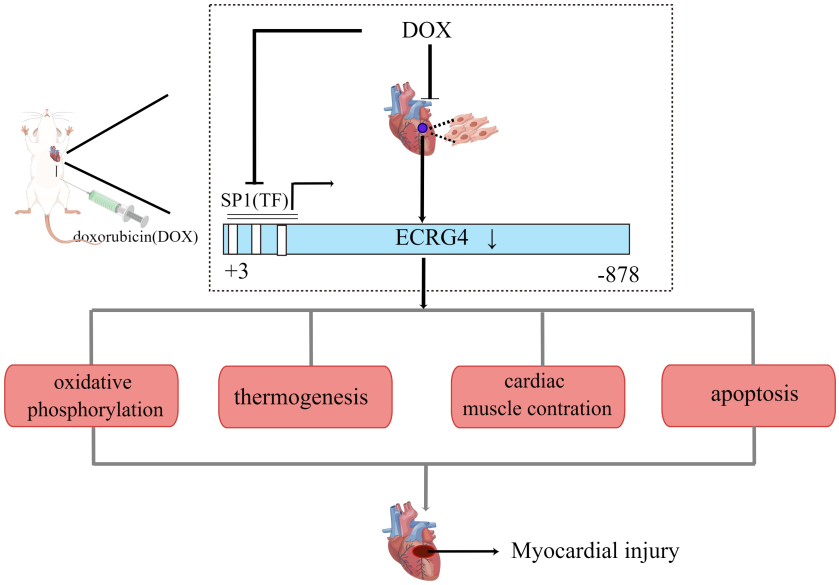


FIGURE 3
ECRG4 is involved in the mechanism of doxorubicin induced myocardial injury. Figure shows the 5'UTR gene sequence of rat ECRG4 -878/+3, which contains 3 potential SP1 transcription factors. Doxorubicin inhibited the expression of SP1 and reduced the binding of SP1 to the rat ECRG4 promoter, thereby decreasing the expression of ECRG4. Cardiomyocyte-specific loss of Ecrg4 significantly enriched the DEPs in the signaling pathways (oxidative phosphorylation, apoptosis, thermogenesis, and cardiac muscle contraction, among others) commonly involved in DOX-induced cardiotoxicity.

## Atrial fibrillation and rhythm control regulated by ECRG4: Possible mechanisms

### ECRG4 affects multiple cardiac ion channels expression

ECRG4 knockdown in neonatal atrial myocytes significantly upregulated the expression of calcium-binding protein family gene (s100a1, s100a8), while downregulating gap channel protein-1(Gjal) expression, leading to a significant shortening of action potential duration (APD50 and APD90) (Figure 2) (30). Calcium homeostasis plays an important regulatory role in myocardial remodeling; s100a1 and sl00a8, members of the S100 calcium-binding protein family and expressed by cardiomyocytes, are key to regulating the $Ca^{2+}$ concentration in cardiomyocytes (51). and in patients with atrial fibrillation, the shortened duration of the action potential can trigger and result in a continuous reentry loop. The shortening of action potential duration is mainly caused by an increase in $K^{+}$ outward current and/or a decrease in inward current induced by $Ca^{2+}$ during repolarization, and in patients with atrial fibrillation, the shortened duration of the action potential can trigger and result in a continuous reentry loop (52). In human-induced pluripotent stem cell-derived cardiac cells (hiPSC-CMs), ECRG4 knockdown using siRNA also altered the expression of multiple ion channels: SCN5A (sodium channel), KCNH2 (HERG channel) and KCND3 (transient outward K channel) were reduced, while KCNN4 (SK4 channel) and HCN2 (funny channel) were increased (53). Taken together, the above studies suggest that ECRG4 may be involved in atrial fibrillation *via* regulating these channels.

### ECRG4 affects the expression of matrix metalloproteinases

Studies have shown that Matrix metalloproteinase play an important role in the development of hypertension and atrial fibrillation by affecting the degradation of the extracellular matrix (54). MMP3 and MMP9 were increased in patients with recurrent AF within one year after electrical cardioversion (55). Studies have shown that MMP-9 is significantly higher in obese patients with paroxysmal atrial fibrillation than in obese patients alone. With the increase of MMP-9 in obese patients exceeding 285ng/ml, the occurrence of AF can be predicted with a sensitivity of 74.5% and specificity of 94% (56). It has been shown that ECRG4 can regulate the expression of MMPs in various organs and tissues. In oral squamous cell carcinoma, ECRG4 down-regulated the expression of matrix metalloproteinases (MMP-9 and MMP-13) (57). In atrial myocytes, ECRG4 knockdown significantly upregulated the expression of matrix metalloproteinase3 (MMP3) (30), which may contribute to atrial fibrillation.

### ECRG4 and immune inflammatory reactions

A substantial amount of evidence suggests that the onset and progression of AF are strongly linked to inflammation (58–61).

Epidemiological studies have shown that compared with subjects with normal sinus rhythm, c-reactive protein and inflammatory cytokines such as TNF-α, IL-1β, IL-8, IL-6 and monocyte chemoattractant protein-1 (MCP-1) were significantly upregulated in patients with atrial fibrillation (59). Inflammatory mediators can disrupt cellular calcium homeostasis, activate and promote fibrosis, inhibit gap junction protein (Gjal)-mediated cell-cell communication (GJIC), and induce cardiomyocyte necrosis and apoptosis (58, 62, 63). ECRG4 knockdown in atrial myocytes significantly increased the production of pro-inflammatory genes. In recent years, a large amount of literature supports the involvement of ECRG4 in inflammation, injury, and infection. ECRG4 is a candidate chemokine that is highly expressed on leukocytes and regulates early neutrophil recruitment and subsequent CD44-mediated inflammatory decline, making ECRG4 a therapeutic target for inflammatory diseases (64). ECRG4 is also expressed on the cell surface of epithelial cells (12, 13, 65, 66). Cell surface ECRG4 is expressed in quiescent tissue and may have a "sentinel" function to monitor homeostasis, measure pro-inflammatory responses to injury and infection, and thus remain quiescent (11–13, 65). When infection or inflammation occurs, the protease activates and initiates the downregulation of ECRG4 gene expression, which is released from the cell surface in a processed form (9, 11, 67). Other studies have shown that ECRG4 has a physiological role in measuring parenchymal and inflammatory responses to traumatic brain injury, with small needle wounds leading to a temporary reduction and full recovery of ECRG4 within 24-48 hours (13, 65). These findings suggest that low expression of ECRG4 may contribute to the development of atrial fibrillation through an inflammatory response.

## ECRG4: Potential clinical target in tumor and arrhythmia

ECRG4 not only has a tumor suppressor effect but also cytokine-like functions. ECGG4 is found in a variety of bodily fluids, including blood, urine, saliva, pleural effusion, and cerebrospinal fluid. Decreased concentrations of ECRG4 in body fluids may indicate cancer development, suggesting that ECRG4 may be a biomarker for predicting cancer occurrence. Previous studies have found that methylated ECRG4 cDNA has promising diagnostic and clinical translational potential (68, 69). Meanwhile, promoter methylation determines ECRG4 expression status, and its aberration could help detect early cancer and predict severity (70). These findings show that ECRG4 can be used as a biomarker for cancer diagnosis as well as predicting staging and invasiveness. The wide distribution and diverse functions of ECRG4 make it an ideal target for drug therapy. ECRG4 has several functions, including tumor suppression, heart rhythm regulation, cardiac homeostasis maintenance, and involvement in the aging process. Overexpression of ECRG4 has been found to increase the sensitivity of gastric cancer cell line, SGC-7901 to 5-FU and NPC cell line, CNE1 to cisplatin, thereby improving the therapeutic effect of chemotherapy drugs (71, 72). Upregulation of ECRG4 expression or activity may also be used to treat diseases characterized by tissue dysfunction caused by attenuated ECRG4 expression. Since down-regulation of ECRG4 is associated with atrial fibrillation, up-regulating ECRG4 expression may aid in treating atrial fibrillation. Moreover, inhibition of ECRG4 can counteract senescence-associated cellular senescence. Interestingly, ECRG4 expression is generally silenced by promoter methylation, which demethylating agents can reactivate. There are currently two types of demethylation drugs, nucleoside DNMT inhibitors and non-nucleoside DNMT inhibitors, the efficacy of which is still being investigated. Furthermore, ECRG4 is a secretory protein that attaches to the cell surface and undergoes proteolysis to achieve biological activation. Therefore, high-affinity receptor agonists or protease inhibitors of ECRG4 are attractive targets for future drug development.

## Perspectives

ECRG4 was initially known for its antitumor function, but as research progressed, its role in various physiology and pathology was gradually revealed, as was its role in the heart. The literature on atrial fibrillation induced by down-regulation of ECRG4 was summarized, and it was found that down-regulation of ECRG4 could induce atrial fibrillation through affecting ion channels, MMPs expression, and activating inflammatory response. ECRG4 is involved in the mechanism of doxorubicin-induced myocardial injury, which suggests that ECRG4 has myocardial protective function. In conclusion, ECRG4 can regulate rhythm, maintain cardiac homeostasis and protect myocardium. This study comprehensively reviewed the biological background of ECRG4 gene and its expression in cardiovascular system, focusing on the possible mechanism of ECRG4's involvement in the formation of atrial fibrillation, which provides a new idea for reducing perioperative atrial fibrillation and myocardial injury in patients with esophageal cancer, gastric cancer and other tumors.

The uniqueness of ECRG4 makes it a potential target for precision medicine. Current research is primarily based on *in vitro* experiments, such as studies in KO or transgenic mouse models, which are extremely useful in determining the role of ECRG4 *in vivo*. The transition from basic research to clinical application necessitates a lengthy process of clarification and validation. Future research will need to decipher the mechanisms of action of ECRG4 and its signaling pathways. Continued development of new targeted drugs is expected to benefit not only the treatment of esophageal cancer, gastric cancer, and other cancers but also the treatment of cardiovascular diseases such as atrial fibrillation and myocardial injury.

## Author contributions

ZZ, WW, and YZ contributed equally to this work. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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