Association of Circulating 25-Hydroxyvitamin D Levels with Colorectal Cancer: An Updated Meta-Analysis

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Summary A higher serum 25-hydroxyvitamin D (25(OH)D) concentration benefits colorectal cancer prevention. However, whether it can improve the prognosis among patients is still under discussion. This study aims to explore the impacts of high level 25(OH)D on the survival of colorectal cancer patients. PubMed, Embase, and Cochrane were searched from January 2000 to August 2017 for relevant articles. Only published studies focusing on the relationship between 25(OH)D levels at or near the time of diagnosis and survival were considered. Two review authors independently assessed the risk of bias for each study, and any disagreement was resolved by discussion or by involving a third assessor. Eleven studies comprising 7,367 patients were included. In these studies, there were considerable differences between the higher 25(OH)D level group and the lower group in terms of overall survival (OS), progression-free survival (PFS) and colorectal cancer-specific survival (CSS) in a random effect model (OS: HR 0.67, 95% CI 0.56–0.80, p<0.00001; CSS: HR 0.73, 95% CI 0.55–0.97, p=0.03; PFS: HR 0.74, 95% CI 0.61–0.90, p=0.003). Moreover, the combined hazard ratios of OS and CSS had considerably significant heterogeneity which may be explained by subgroup analysis. The relationship between 25(OH)D and tumor characteristics/lifestyle factors was also included in the meta-analysis. BMI (p=0.03), smoking (p=0.03) and physical activity (p=0.002) seemed to be associated with circulating 25(OH)D level. Publication bias was undetected. Colorectal cancer patients with higher circulating 25(OH)D level may have a better prognosis.

Key Words 25-hydroxyvitamin D, colorectal cancer, prognosis, meta-analysis

Vitamin D refers to a group of fat-soluble steroids that increase the absorption of calcium, magnesium and phosphate. In humans, the most important compounds in this group are vitamin D3 and vitamin D2 (1). Vitamin D3 is converted into calcifediol in the liver while vitamin D2 is converted into 25-Hydroxyvitamin D2. Metabolites of these two vitamin D in the serum are detected for the state of vitamin D in the body (2). The activated vitamin D metabolites play a role by binding to the vitamin D receptor (VDR), which is mainly in the nucleus of the target cell. Nowadays, more and more scholars recognize the function of vitamin D and its receptor on cell proliferation, survival, and differentiation in cancer growth and progression (3). Vitamin D also affects the immune system, and VDRs are expressed in several white blood cells, including monocytes and activated T/B cells (4).

Colorectal cancer (CRC) is the cancer that develops from the colon or rectum (parts of the large intestine) (5). Most colorectal cancers result from age and lifestyle factors, while only a few cases arise from underlying genetic diseases (6). Diet, obesity, smoking and lack of exercise are the risk factors for this disease (6, 7). CRC is becoming a major cause of cancer mortality and a serious health concern worldwide (8). Although the treatment of colorectal cancer has been improving, no satisfactory therapy exists when surgery is not curative. Thus, prevention and the evaluation of prognosis seem to be extremely important. In 1989, the first report of an inverse association between serum 25-hydroxyvitamin D (25(OH)D) and colorectal cancer was published in the USA (9). Since then, a growing number of observational studies and meta-analyses have confirmed that vitamin D could reduce the risk of colorectal cancer. Colorectal epithelial cells and tumor stromal fibroblasts contain vitamin D receptors. These cells are able to convert the circulating 25(OH)D3 into active 1,25(OH)2D3 metabolites, which in turn bind to the cells’ own vitamin D receptors to produce an autocrine effect by inducing cell differentiation and inhibiting proliferation, invasiveness, angiogenesis, and metastatic potential (10). The stromal cells are also affected by 1,25(OH)2D3. CRC stromal fibroblasts express VDR and the modulation of their gene expression and physiology by 1,25(OH)2D3 contributes to the antitumoural action of 1,25(OH)2D3 on this disease (11). Therefore, low vitamin D levels may increase the risk of colorectal cancer through the potential mechanism above.

At present, most studies have focused on the relationship between vitamin D and colorectal cancer prevention. Only a few studies have investigated the possible

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relationship between serum 25(OH)D status and prognosis of colorectal cancer. Therefore, in this review, we conducted an updated and comprehensive meta-analysis of 12 cohort studies and one RCT on 25(OH)D and colorectal cancer prognosis from January 2000 to August 2017.

METHODS

Search strategy. From January 2000 to August 2017, the relevant literature from the PubMed, Embase and Cochrane databases was systematically screened. The following MeSH terms and textwords were used: “Vitamin D” [Mesh], “Receptors, Calcitriol”, “Vitamin D*”, “Vit D*”, “VD*”, “Colorectal Neoplasms”[Mesh], “Neoplasm*, Colorectal”, “Colorectal Neoplasm*”, “Colorectal Tumor*”, “Tumor*, Colorectal”, “Colorectal Carcinoma*”, “Carcinoma*, Colorectal”, “Colorectal Cancer*”, “Cancer*, Colorectal”, “OS”, “overall survival”, “PFS”, “progression-free survival”, “DFS”, “disease-free survival”. The “AND” or “OR” operator was used to combine these terms in varying combinations. At the same time, references in the articles were also included in the screening. We did not set a language limit during the process. Two authors (Jian-hao Xu and Xuya Yuan) independently reviewed the titles and abstracts identified in the search. In this process, we discussed on the articles to incorporate the differences. If problems still could not be resolved, a third assessor (Yusong Zhang) was invited to make a decision.

Study selection. The inclusion criteria: (1) Participants: patients with colorectal cancer; (2) Research variable: circulating 25(OH)D levels measured at or around the time of diagnosis; (3) Outcomes: estimated hazard ratio (HR) and its 95% confidence interval (CI) for overall survival (OS), colorectal cancer-specific survival (CSS) and progression-free survival (PFS) under the Cox proportional hazard model; (4) Study designs: case-control studies, cohort studies and RCTs. The exclusion criteria: (a) literature published as letters, editorials, abstracts, reviews, case reports or expert opinions; (b) studies not based on patients; (c) articles without the OS/CSS/PFS information; (d) similar and repeated studies; (e) outdated articles with little significance or credibility. Cohen’s kappa statistic was used to assess chance-corrected agreement between reviewers (SPSS version 18.0, SPSS Inc., Chicago, IL).

Data extraction. A detailed form was designed for data extraction. We resolved discrepancies through discussion or, if required, we consulted the third review author (Yusong Zhang). Furthermore, the following clinical data was extracted for each trial: the first author’s last name, publication year, country, study design, number of cases, follow up years, stage, measure of exposure, adjusted HR (95% CI), and the adjustments. OS/CSS/PFS were primary outcomes for this meta-analysis. Tumor characteristics and lifestyle factors were the secondary outcomes.

Assessment of quality. Two review authors (Jian-hao Xu and Xuya Yuan) independently assessed the risk of bias for each study. The Newcastle-Ottawa Quality Assessment Scale (NOS) was applied to assess qualities of cohort studies. A study with NOS ≥ 5 was regarded as a high-quality study (12, 13). The criteria standards for RCT research were according to the criteria outlined in the Cochrane handbook for systematic reviews, including blinding implementation, proportion of withdrawal, and selective reporting.

Data synthesis and statistical analysis. We carried out
Table 1. Characteristics of prospective studies on 25(OH)D and CRC.

<table>
<thead>
<tr>
<th>Author, year, study, region</th>
<th>Age (y)</th>
<th>Sex</th>
<th>No. of subjects</th>
<th>Location</th>
<th>Adjustment</th>
<th>Follow-up (y)</th>
<th>25(OH)D categories (ng/mL)</th>
<th>Adjusted HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ng, 2009, Cohort study, USA</td>
<td>Mean, 66</td>
<td>M/F</td>
<td>1,017</td>
<td>Proximal colon: 19% (n=395) Distal colon: 34% (n=543) Rectum: 22% (n=224)</td>
<td>Age at diagnosis, gender, cancer stage, grade of tumor differentiation, location of primary tumor, and year of diagnosis</td>
<td>8</td>
<td>Quartile 1: 21.6–26.8 Quartile 2: 27.0–28.4 Quartile 3: 28.5–29.9 Quartile 4: 29.9–31.5 Quartile 5: 31.5–36.9</td>
<td>OS: 0.62 (0.42–0.93) CSS: 0.50 (0.26–0.95)</td>
</tr>
<tr>
<td>Mezawa, 2010, Cohort study, Japan</td>
<td>Mean, 65</td>
<td>M/F</td>
<td>257</td>
<td>Proximal colon: 58% (n=149) Distal colon: 36% (n=92) Rectum: 62% (n=159)</td>
<td>Age at diagnosis, gender, calendar month of blood sampling, cancer stage, residual tumor after surgery, time period of surgery, and number of lymph nodes with metastasis</td>
<td>4</td>
<td>Quartile 1: 3.0–7.0 Quartile 2: 8.0–10.0 Quartile 3: 11.0–15.0 Quartile 4: 16.0–36.0</td>
<td>OS: 0.91 (0.84–0.99) CSS: 0.98 (0.89–1.08)</td>
</tr>
<tr>
<td>Ng, 2011, Cohort study, USA</td>
<td>Median, 61</td>
<td>M/F</td>
<td>515</td>
<td>Age: season of blood collection, sex, baseline performance status, treatment arm, body mass index, and metastatic sites</td>
<td>5</td>
<td>Quartile 1: 2.3–13.1 Quartile 2: 13.2–19.9 Quartile 3: 20.0–27.1 Quartile 4: 27.2–75.4</td>
<td>OS: 0.94 (0.72–1.23)</td>
<td></td>
</tr>
<tr>
<td>Tretli, 2012, Cohort study, Norway</td>
<td>Median, 59</td>
<td>M/F</td>
<td>52</td>
<td>Sex, age at diagnosis, season of blood sampling, time between serum sampling and 25(OH)D measurement, and stage of the disease at the time of diagnosis</td>
<td>14</td>
<td>Quartile 1: &lt;18.4 Quartile 2: 18.4–24.3 Quartile 3: 24.4–32.5 Quartile 4: &gt;32.5</td>
<td>OS: 0.40 (0.10–1.60) CSS: 0.37 (0.08–1.71)</td>
<td></td>
</tr>
<tr>
<td>Fedirko, 2012, Cohort study, Europe</td>
<td>Mean, 62</td>
<td>M/F</td>
<td>1,202</td>
<td>Colon: 63% (n=759) Rectum: 37% (n=443)</td>
<td>Age at diagnosis, sex, cancer stage, grade of tumor differentiation, location of primary tumor, smoking status, BMI, physical activity, season of blood collection, and year of diagnosis, stratified by country of residence</td>
<td>6</td>
<td>Quartile 1: &lt;14.5 Quartile 2: 14.5–19.4 Quartile 3: 19.5–24.1 Quartile 4: 24.2–30.7 Quartile 5: &gt;30.7</td>
<td>OS: 0.67 (0.50–0.90) CSS: 0.69 (0.50–0.95)</td>
</tr>
<tr>
<td>Zgaga, 2014, Cohort study, Ireland</td>
<td>Mean, 49</td>
<td>M/F</td>
<td>1,598</td>
<td>Tumor site, surgery, time between definitive treatment and sampling, season of blood collection, body mass index, and level of physical activity</td>
<td>9</td>
<td>Quartile 1: &lt;7.3 Quartile 2: 7.3–13.3 Quartile 3: &gt;13.3</td>
<td>OS: 0.70 (0.55–0.89) CSS: 0.68 (0.50–0.92)</td>
<td></td>
</tr>
<tr>
<td>Obermannova, 2015, Cohort study, Czech Republic</td>
<td>Median, 62</td>
<td>M/F</td>
<td>84</td>
<td>Proximal colon: 17% (n=14) Distal colon: 25% (n=21) Rectum: 58% (n=49)</td>
<td>Season—diagnosis and relapse, age, gender, BMI, stage, KRAS mutation, type of disease, PS, metastasis number, localisation, any surgical procedure, radical metastases resection, chemotherapy type, levels of calcium, haemoglobin, albumin, eGFR, cholesterol total, LDL cholesterol, HDL cholesterol, triacylglycerols</td>
<td>3</td>
<td>Quartile 1: &lt;16.0 Quartile 2: &gt;16.0</td>
<td>OS: 0.45 (0.22–0.93) PFS: 0.59 (0.39–0.98)</td>
</tr>
<tr>
<td>Wesa, 2015, Cohort study, USA</td>
<td>Median, 63</td>
<td>M/F</td>
<td>250</td>
<td>Albumin and ECOG PS</td>
<td>3</td>
<td>Quartile 1: &lt;30.0 Quartile 2: &gt;30.0</td>
<td>OS: 0.61 (0.38–0.98)</td>
<td></td>
</tr>
<tr>
<td>Ng, 2015, Cohort study, USA</td>
<td>NR</td>
<td>M/F</td>
<td>2.043</td>
<td>Sex, race, area, dietary vitamin D intake, ECOG PS, tumoral RAS mutation, body mass index, physical activity, and blood draw season</td>
<td>7</td>
<td>NR</td>
<td>OS: 0.65 (0.51–0.83) PFS: 0.79 (0.63–1.00)</td>
<td></td>
</tr>
<tr>
<td>Facciorusso, 2016, Cohort study, Italy</td>
<td>Median, 68</td>
<td>M/F</td>
<td>143</td>
<td>Colon: 76% (n=110) Rectum: 24% (n=35)</td>
<td>Age, sex, serum albumin, serum bilirubin, INR, CEA, number of nodules, max diameter, primary tumor, timing, ECOG PS</td>
<td>12</td>
<td>Quartile 1: &lt;20.0 Quartile 2: &gt;20.0</td>
<td>OS: 0.35 (0.21–0.59)</td>
</tr>
<tr>
<td>Yang, 2017, Cohort study, China</td>
<td>Median, 63</td>
<td>M/F</td>
<td>206</td>
<td>Colon: 54% (n=112) Rectum: 46% (n=94)</td>
<td>Age, gender, race, and marriage, education, ratio of income to poverty, smoking, alcohol consumption, body mass index, total physical activity, servings of fruit and vegetables, overall fat consumption, percentage of energy from fat, and chemotherapy</td>
<td>5</td>
<td>Quartile 1: &lt;6.2 Quartile 2: 6.2–29.9 Quartile 3: &gt;29.9</td>
<td>OS: 0.56 (0.28–1.11)</td>
</tr>
</tbody>
</table>

1 Vitamin D was analyzed as a continuous variable per ng/mL, the rest were analyzed as a binary variable for the highest serum 25(OH)D concentration level vs the lowest level.
N. of subjects: the number of patients; HR: hazard ratio; M: male; F: female; CEA, carcinoembryonic antigen; ECOG, Eastern Cooperative Oncology Group; PS, performance status; INR, International normalized ratio; OS: overall survival; CSS: cancer-specific survival; PFS: progression-free survival; NR: non reported.
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RESULTS

Search results

Our literature search flow chart is shown in Fig. 1. Three hundred forty articles in total were found in the initial search of the three databases, and 27 duplicate articles were deleted after duplicate checking. Then screening of the titles and summaries was conducted for the remaining 313 articles. After two evaluators’ discussion, 39 articles were regarded related to the topics. After full-text review, 28 of these studies were excluded for the following reasons: serum 25(OH)D concentrations were not measured; several articles were from the same study; articles were not original (review or meta-analysis) or were published as letters, editorials or abstracts; study design was improper. Therefore, this meta-analysis incorporates 11 eligible studies with 7,367 patients in total.

Study characteristics and quality assessment

Eleven cohort studies (15–25) from 2000 to 2017 on the relationship between vitamin D and the prognosis of colorectal cancer were included, with 7,367 patients in total. The main features of the 11 studies included are summarized in Table 1. In short, four (15, 17, 22, 23) studies were conducted in the United States, five (18–21, 24) in Europe and two (16, 25) in Asia. Of the four studies in the United States (15, 17, 22, 23), three (15, 17, 23) were conducted by Ng et al. All the studies were published in the past decade and 5 (20–24) of the 11 studies were the latest findings of the past 3 y. Most patients included were middle-aged and elderly people, with an average age of 49 to 68 y. Most of the studies included no gender restriction. The size of the study sample ranged from 52 to 2,043 while the follow-up years ranged from 3 y to 14 y. Only six studies provided a detailed summary and description of tumor sites (15, 16, 19, 21, 24, 25). Ten articles (15–22, 24, 25) ranked the serum 25(OH)D levels and, although the criteria for division varied, dose-response studies with OS could be conducted on the basis of relevant data. Estimated HR by multivariate analysis along with their 95% CIs for OS, CSS, PFS, and adjustments are also listed in Table 1. The model with most covariates was selected in each study. There were 11 articles (15–25) on OS among the studies: 8 (15, 16, 19–24) of them charged that vitamin D could improve the OS of patients with colorectal cancer, but in contrast another 3 articles (17, 18, 25) showed negative results; 5 articles (15, 16, 18–20) studied CSS:

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>NOS</th>
<th>Selection</th>
<th>Comparability</th>
<th>Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ng (15)</td>
<td>2009</td>
<td>7</td>
<td>★★★★★</td>
<td>★</td>
<td>★</td>
</tr>
<tr>
<td>Mezawa (16)</td>
<td>2010</td>
<td>6</td>
<td>★★★★★</td>
<td>★</td>
<td>★¹</td>
</tr>
<tr>
<td>Ng (17)</td>
<td>2011</td>
<td>7</td>
<td>★★★★★</td>
<td>★</td>
<td>★</td>
</tr>
<tr>
<td>Tretli (18)</td>
<td>2012</td>
<td>7</td>
<td>★★★★★</td>
<td>★</td>
<td>★</td>
</tr>
<tr>
<td>Fedirko (19)</td>
<td>2012</td>
<td>7</td>
<td>★★★★★</td>
<td>★</td>
<td>★</td>
</tr>
<tr>
<td>Zgaga (20)</td>
<td>2014</td>
<td>7</td>
<td>★★★★★¹</td>
<td>★</td>
<td>★</td>
</tr>
<tr>
<td>Obermannova (21)</td>
<td>2015</td>
<td>6</td>
<td>★★★★★</td>
<td>★</td>
<td>★¹</td>
</tr>
<tr>
<td>Wesa (22)</td>
<td>2015</td>
<td>6</td>
<td>★★★★★</td>
<td>★</td>
<td>★</td>
</tr>
<tr>
<td>Ng (23)</td>
<td>2015</td>
<td>7</td>
<td>★★★★★</td>
<td>★</td>
<td>★</td>
</tr>
<tr>
<td>Facciorusso (24)</td>
<td>2016</td>
<td>7</td>
<td>★★★★★</td>
<td>★</td>
<td>★</td>
</tr>
<tr>
<td>Yang (25)</td>
<td>2017</td>
<td>7</td>
<td>★★★★★</td>
<td>★</td>
<td>★</td>
</tr>
</tbody>
</table>

¹The score was produced by discussion.

Table 2. Quality assessment of eligible studies with Newcastle-Ottawa Scale.
3 (15, 19, 20) of them charged that vitamin D could improve the CSS of patients with colorectal cancer, and in contrast the other 2 articles (16, 18) suggested no connection; 2 articles (21, 23) studied PFS and both of them concluded that vitamin D could improve the PFS in patients with colorectal cancer. In addition, most articles explored the risk factors for low 25(OH)D concentration in colorectal cancer patients: age (5 studies (15, 16, 19, 20, 22)), gender (4 studies (16, 19, 20, 25)), stage (5 studies (15, 16, 19, 20, 25)), grade (3 studies (15, 19, 25)), cancer location (4 studies (15, 16, 19, 25)), diagnosis season (3 studies (15, 19, 20)), BMI (4 studies (15, 19, 20, 22)), physical activity (2 studies (15, 19)), smoking (3 studies (19, 20, 25)), and drinking (1 study (25)). None of the 11 cohort studies received a low NOS score (NOS<6), indicating that they had a high level of methodological quality in this meta-analysis (Table 2).

Serum 25(OH)D concentration and overall survival

All of the studies included reported the relationship between serum 25(OH)D levels and overall survival in patients with colorectal cancer. The HR and the 95% CI after multivariate analysis in each study are shown in Fig. 2. Among these studies, vitamin D was analyzed as a continuous variable per ng/mL in Mezawa et al. (16)
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Fig. 4. Forest plot of hazard ratios (HRs) for CSS of high serum 25(OH)D concentration group versus low concentration group in colorectal cancer.

...and Obermannova et al. (21), while in the rest (15, 17–20, 22–25) it was analyzed as a binary variable for the highest serum 25(OH)D concentration level versus the lowest level. The results showed that the pooled HR remained statistically significant (HR 0.67, 95% CI 0.56–0.80, \( p<0.00001 \)) in spite of significant heterogeneity (\( I^2 = 70\% \)), indicating that elevated serum 25(OH)D levels were significantly associated with better OS in colorectal cancer patients.

In view of the obvious heterogeneity, we conducted a subgroup analysis of different characteristics mainly on the following aspects: gender, race, sample size, follow-up time, stage, and lesion location. The included HRs for each study were all multivariate-adjusted estimates. The combined HR and 95% CI for each subgroup are also shown in Fig. 3 to assess whether serum 25(OH)D affected OS under different grouping conditions. When racial factors were considered, the results of the 11 articles (15–25) could be divided into two subgroups: 9 studies (15, 17–24) for Caucasian and 2 (16, 25) for Asian. We found both of the heterogeneities decreased (\( I^2 = 39\% \) and 60\%), which suggested race might be an influence factor. Based on the sample size for each study, three ranks were devised: <250, 250 to 1,000, and >1,000. Each group contained 4 (18, 21, 24, 25), 3 (16, 17, 22) and 4 (15, 19, 20, 23) studies, respectively. \( I^2 \) for the stage III group was 0%, significantly lower than the stage I group (20). \( I^2 \) for the stage III group was 0%, but it increased to 43% in the stage IV group (17, 22, 24). Therefore, it was suggested that the results might not be stable due to the limited inclusion of studies. In this situation, the four phases were merged into two groups, stage I/II and stage III/IV. There were 2 (15, 20) and 7 (15, 17, 20–24) studies involved in each group, with \( I^2 \) decreasing to 0% and 32% respectively. The results showed that tumor stage affected the relationship between serum 25(OH)D and OS, as a confounding factor for heterogeneity. Based on the proportion of colon and rectal cancers reported in each study, we categorized studies into two ranks based on colon cancer/rectal cancer ratio with a cutoff of 1.5. The ratio was less than 1.5 in two studies (21, 25), while three studies (15, 16, 19) showed a greater ratio. \( I^2 \) for these two groups were 0% and 49%, significantly less than 69%. However, two studies (15, 19) reported OS on colon cancer and rectal cancer separately, with significant \( I^2 \) being 69% and 55% respectively, indicating that tumor site contributes little to the heterogeneity.

Serum 25(OH)D concentration and cancer-specific survival

Of the 11 articles screened (15–25), 5 (15, 16, 18–20) reported the relationship between serum 25(OH)D levels and cancer-specific survival in colorectal cancer patients. Figure 4 shows the HR and the 95% CI after multivariate analysis for each study. Among these studies, vitamin D was analyzed as a continuous variable per ng/mL in Mezaw et al. (16), while in the rest (15, 18–20) it was analyzed as a binary variable for the highest serum 25(OH)D concentration group versus the lowest group. The results showed that the pooled HR remained statistically significant (HR 0.73, 95% CI 0.55–0.97, \( p<0.03 \)) in spite of significant heterogeneity (\( I^2 = 69\% \)), indicating that elevated serum 25(OH)D levels were significantly associated with better CSS in colorectal cancer patients.

In view of the obvious heterogeneity, we conducted a subgroup analysis of different characteristics mainly on the following aspects (Fig. 5): gender, race, sample size, follow-up time, stage, and lesion location. Due to the
limited number of articles on the relationship between CSS and age/BMI, the metrics for the CSS subgroup analysis were slightly different from those for the OS. The included HRs for each study were all multivariate-adjusted estimates. The combined HR and 95% CI for each subgroup are also shown in Fig. 5 to assess whether serum 25(OH)D affected CSS under different grouping conditions. In terms of gender, the data for the male and female groups were all from the same studies (15, 19). Although $I^2$ declined to varying degrees ($I^2=0\%$ and $54\%$ respectively), the conclusion was not so credible due to the few studies included and the poor overall representation. When it comes to ethnic factors, only one out of five articles focused on Asian patients (16). So $I^2$ for the Asian group was uncalculated. However, homogeneity was great ($I^2=0\%$) among the remaining four Caucasian-centered studies (15, 18–20), which hinted that the race factor could impact heterogeneity. Taking sample size into account, we classified samples into two groups based on the size of each study: 250 to 1,000 and $>1,000$, containing 2 (16, 18) and 3 studies (15, 19, 20) respectively. $I^2$ for each group fell significantly ($I^2=35\%$ and $0\%$). Relatively speaking, studies with small sample size have poor stability with large heterogeneity. Conversely, studies with large sample size have great stability with little heterogeneity. However, $I^2$ in the two subgroups based on the sample size were both reduced significantly, which was hard to explain logically. Therefore, the sample size was considered to have little effect on heterogeneity. There were two ranks based on the follow-up time: $<9$ y and $\geq 9$ y. $I^2$ for follow-up $\geq 9$ y was 0 (18, 20), and it increased to 74% in follow-up $<9$ y group (15, 16, 19). Heterogeneity among studies (18, 20) with long follow-up time was smaller than among those (15, 16, 19) with short follow-up time. Therefore, follow-up time was seen to impact the heterogeneity in CSS analysis. As for the staging, due to the number of studies included in each of the 4 separate stage subgroups being very limited (one study or none), the four phases were merged into two groups, stage I/II and stage III/IV, with 3 studies (15, 19, 20) involved. Both of the $I^2$ declined to $23\%$, which suggested that tumor stage might contribute to the heterogeneity among studies. In addition, two studies reported the relationship between serum 25(OH)D and CSS in colon (15, 19) and rectal cancer (16, 19) ($I^2=0\%$ and 74% respectively). On the one hand, the number of included studies was limited, which indicated a less representative result. On the other hand, although $I^2$ in the colon cancer group fell to 0%, it increased to 74% in the rectal cancer group. Thus, it was unreasonable to assign the heterogeneity to different tumor sites.

**Serum 25(OH)D concentration and progression-free survival**

Of the 11 articles screened (15–25), 2 (21, 23) reported the relationship between serum 25(OH)D levels and progression-free survival in colorectal cancer.

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**Fig. 5.** Subgroup analyses of multivariate analysis and HR (95% CI) for cancer-specific survival.

**Fig. 6.** Forest plot of hazard ratios (HRs) for PFS of high serum 25(OH)D concentration group versus low concentration group in colorectal cancer.
patients. Figure 6 shows the HR and the 95% CI after multivariate analysis for each study. Among these studies, vitamin D was analyzed as a continuous variable per ng/mL in Obermannova et al. (21), and in the others, it was analyzed as a binary variable for the highest serum 25(OH)D concentration group versus the lowest group. The results showed pooled HR remained statistically significant (HR 0.74, 95% CI 0.61–0.90, p=0.003) with little heterogeneity ($I^2=32\%$), indicating that elevated serum 25(OH)D levels were significantly associated with better PFS in colorectal cancer patients.

### Dose–response meta-analysis

Dose–response curves of seven individual studies (15–20, 25) on the relationship between 25(OH)D concentration near diagnosis and OS of patients with colorectal cancer are shown in Fig. 7. The HR values of all studies showed a decreasing trend with increasing concentration of serum 25(OH)D.

Next, we used STATA to assess the dose–response relationship between serum 25(OH)D levels and OS (Fig. 8). Since the information provided by Mezawa et al. (16) and Ng et al. (17) among seven articles (15–20, 25)
was not complete, these two studies were not included in the statistical analysis. A total of five prospective cohort studies \((15, 18–20, 25)\) with 4,075 colorectal cancer patients were included and results showed a negative correlation between estimated HR and serum 25(OH)D levels after linear and non-linear fitting. The linear regression equation suggests that a 10 ng/mL increment in serum 25(OH)D level conferred an HR of 0.20 (95% CI 0.0662), so the linear dose–response relationship could only provide a referential value.

**Risk factor screening**

For the 11 screened studies \((15–25)\), we divided the patients in each study into a high 25(OH)D concentration group and a low concentration group, then compared the differences according to various characteristics assessed at or around the time of diagnosis with reference to case-control study. The included indicators are listed in Table 3. There were three continuous variable indicators: age, BMI and physical activity, with 5 articles \((15, 16, 19, 20, 22)\), 4 articles \((15, 19, 20, 22)\) and 2 articles \((15, 19)\) included, respectively. A total of 2,746 patients were included in the age groups, and the pooled results showed no significant difference in the age of patients between the two groups \((MD 0.80, 95\% CI −0.88 to 2.48, p=0.35)\). For BMI and physical activity, 2,069 and 889 patients, respectively, were included in the study, and considerably significant differences were found \((MD 2.05, 95\% CI 0.25 to 3.86, p=0.03, Fig. S1; MD −14.64, 95\% CI −23.77 to −5.52, p=0.002, Fig. S2)\) \((Figs. S1 and S2: Supplemental Online Material)\). The heterogeneity of the BMI group was 94%, so we combined the estimates in the random effect model. At the same time, the heterogeneity of the physical activity group was zero and the fixed effect model was applied. A lower BMI and more exercise might lead to a higher blood 25(OH)D level in colorectal cancer patients. A total of six dichotomous variable indicators were included as follows: male \((16, 19, 20, 25)\), smoker \((19, 20, 25)\), diagnosis in summer/fall \((15, 19, 20)\), lesions in the colon \((15, 16, 19, 25)\), stage III/IV \((15, 16, 19, 20, 25)\), and poor differentiation \((15, 19, 25)\). Sample size was adequate among these groups \((972 to 2,276, 1,704 on average)\). The combined results of OR showed no significant difference except for smoker between the high-level and low-level groups \((OR 1.80, 95\% CI 1.06 to 3.08, p=0.03, Fig. S3; Supplemental Online Material)\). Smoking might be associated with low serum levels of 25(OH)D in colorectal cancer patients.

### Sensitivity analysis and publication bias

In order to assess the stability of our results, a sensitivity analysis was performed. In multivariate analysis for OS, the Trim method was used and the results showed no considerable changes between the previous and new HRs \((Fig. S4; Supplemental Online Material)\). Next, we deleted one individual study at a time, and the results of the rest of the studies were checked for any reversal. The statistical outcomes showed that the pooled HRs were all still significant even when one study was excluded, and a more significant new summary HR \((−0.40, 95\% CI −0.51 to −0.29)\) was found when deleting the study Mezawa et al. \((16)\), which supported the positive prognostic value of serum 25(OH)D for OS \((Fig. S5; Supplemental Online Material)\). In the multivariate analysis of CSS, the results of the Trim method showed that the new HR statistics did not flip and remained statistically significant \((p<0.0001 in the random effect model, Fig. S4)\). The statistical outcomes showed that the pooled HRs were all still significant even when one study was excluded, and we found a more significant new summary HR \((−0.42, 95\% CI −0.63 to −0.21)\) when the study Mezawa et al. \((16)\) was removed \((Fig. S5)\). This indicated that serum 25(OH)D was positively correlated with CSS in patients with colorectal cancer. In the multivariate analysis of PFS, the results of the Trim method showed significant differences for the new HR \((p=0.016 in the random effect model, Fig. S4)\). Upon examination of the impact of individual studies on the influence of

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of studies</th>
<th>Data type</th>
<th>Effect measure</th>
<th>No. of cases</th>
<th>Effective index (95% CI)</th>
<th>Heterogeneity, I² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>6</td>
<td>Continuous</td>
<td>MD</td>
<td>1,113</td>
<td>0.80 (−0.88, 2.48)</td>
<td>70</td>
</tr>
<tr>
<td>BMI</td>
<td>4</td>
<td>Continuous</td>
<td>MD</td>
<td>1,042</td>
<td>2.05 (0.25, 3.86)</td>
<td>94</td>
</tr>
<tr>
<td>Physical activity</td>
<td>2</td>
<td>Continuous</td>
<td>MD</td>
<td>446</td>
<td>−14.64 (−23.77, −5.52)</td>
<td>0</td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>Dichotomous</td>
<td>OR</td>
<td>918</td>
<td>0.85 (0.46, 1.57)</td>
<td>85</td>
</tr>
<tr>
<td>Smoker</td>
<td>3</td>
<td>Dichotomous</td>
<td>OR</td>
<td>847</td>
<td>1.62 (1.27, 2.07)</td>
<td>66</td>
</tr>
<tr>
<td>Dx in summer/fall</td>
<td>3</td>
<td>Dichotomous</td>
<td>OR</td>
<td>996</td>
<td>0.62 (0.21, 1.81)</td>
<td>96</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>4</td>
<td>Dichotomous</td>
<td>OR</td>
<td>576</td>
<td>1.14 (0.89, 1.46)</td>
<td>0</td>
</tr>
<tr>
<td>Stage III/IV</td>
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<td>Dichotomous</td>
<td>OR</td>
<td>1,122</td>
<td>1.09 (0.91, 1.31)</td>
<td>0</td>
</tr>
<tr>
<td>Poorly differentiated</td>
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<td>Dichotomous</td>
<td>OR</td>
<td>505</td>
<td>0.93 (0.56, 1.55)</td>
<td>0</td>
</tr>
</tbody>
</table>

No.: the number; Dx: diagnosis.
the combined effects, we found that the remaining combined results after deleting one study were in line with those of the overall three articles (Fig. S5).

In addition, we conducted a publication bias evaluation for the meta-analysis of OS, CSS and PFS (Fig. 9). These funnel plots showed that most of the studies are in the upper part of the inverted funnel and approximately symmetrical, suggesting that no publication bias was obvious. In order to accurately test the symmetry of the graph, we conducted Begg’s test, and found no indication of publication bias (p=0.64, p=1.00 and p=1.00, respectively).

**DISCUSSION**

This study focused on the impacts of high level 25(OH)D on the survival of colorectal cancer patients. Our findings suggest that colorectal cancer patients with higher circulating 25(OH)D level may have a better prognosis for OS, CSS, and PFS. In addition, risk factors for vitamin D deficiency were explored. BMI, smoking, and physical activity seemed to be associated with circulating 25(OH)D level.

In the past 10 y, multiple systematic reviews or meta-analyses demonstrated the inverse association between vitamin D and the risk of colorectal cancer (26–28). Many systematic reviews also showed the impact of vitamin D on tumor prognosis (29–31). These reviews summarized prospective studies of links between vitamin D levels and various types of cancer over the years, including studies on colorectal cancer which were discussed in this article (29–31). However, no quantitative analysis result was given in the above systematic evaluation. In addition, some meta-analyses (32–36) focused on the impact of vitamin D on the prognosis of colorectal cancer. Some of the limitations among these studies are as follows: the articles included are not comprehensive, with only three to five relative articles included (32–36) and the articles included were repeated. For example, two related papers published in 2008 (37) and 2009 (15) respectively were based on the same population. However, reviews by Mohra et al. (33) and Toriola et al. (31) contained both of them. Some articles assessed the prognostic impact of vitamin D on multiple tumors, which meant that there was no detailed discussion on the pooled HR and obvious heterogeneity or publication bias (31, 35); some articles were published in the form of editorials and the specific extraction of data was unknown (33, 34); some articles only emphasized fitting the dose–response curve without providing a detailed analysis of the pooled HR, heterogeneity, sensitivity or publication bias (33, 34); and some articles did not show a fitting result for the dose–response relationship (31, 32, 35). Therefore, we pooled comprehensive and high-quality prospective studies conducted over the past decade to perform a detailed systematic and comprehensive quantitative analysis to fill the gap in this field.

Of the total prospective cohort studies included in this article (15–25), four (17, 18, 22, 25) indicated no relationship between vitamin D and prognosis of colorectal cancer, whereas eight (15, 16, 19–24) suggested the connection. The pooled results proved statistical significance (p<0.00001) on vitamin D and the OS of colorectal cancer patients. For the analysis of heterogeneity (I² = 70%), the decrease after grouping by staging and gender was obvious (Fig. 3). When gender was used as the grouping criterion, only three studies (15, 17, 19) were included. More than one article reported the impact of gender on the prognosis of colorectal cancer patients. For example, in Yang’s meta-analysis (38), the combined results showed that women had significantly better OS (HR 0.87, 95% CI 0.85–0.89) than men, signifying sex as an important factor that affects the outcome of colorectal cancer patients. One study (39) found that female patients had higher expression of some microRNAs regulating the expression of clock genes, which contributed to the differences in OS for female and male patients. Race was also an influencing factor on the prognosis of colorectal cancer. One investigation (40) indicated that Asians and Caucasians had differences in the distributions of individuals with mutation, as well as mutation types in MutL homolog 1 (MLH1) and MutS protein homolog 2 (MSH2), which influenced the prognosis of colorectal cancer. In addition, sulfidogenic bacteria were considered as an environmental risk factor contributing to colorectal cancer (41). Differences in sulfidogenic bacteria between races could be another potential mechanism (41). When it comes to staging, TNM staging remained a strong tool for establishing prognosis and directing therapy (42). Therefore, it was
logical and uncontroversial that staging influenced the prognosis of colorectal cancer.

In five prospective cohort studies on CSS (15, 16, 18–20), three (15, 19, 20) concluded that vitamin D reduced the risk of tumor-specific death in colorectal cancer patients, while two articles (16, 18) indicated no correlation. After a combination of estimates, it was seen that patients with high serum vitamin D concentration suffered lower tumor-specific mortality (p = 0.03). I² analysis showed a significant decrease (I² = 0%/54% in gender subgroups; I² = 74%/0% in follow-up time subgroups; I² = 23%/23% in staging subgroups) when the sex, follow-up time, and stage were used as the grouping criteria.

The pooled results of three prospective studies on PFS showed statistically significance (p = 0.003) and little heterogeneity between studies (I² = 32%). Few relative meta-analysis on vitamin D and colorectal cancer mentioned PFS in the past.

To further demonstrate the correlation between vitamin D and the prognosis of colorectal cancer, we conducted a dose-response study. The fitted curve included data from five studies (15, 18–20, 25). The results of the fit suggested a negative correlation between serum vitamin D levels and overall mortality. However, it is worth mentioning that the linear regression model of the overall test was at a statistically ambiguous level (p = 0.0662), so the linear dose-response relationship could only provide a limited reference value. Thus, we referred to other articles for linear regression models of dose-response study on serum vitamin D and overall survival in colorectal cancer patients. The first study showed that the HR was 0.93 (95% CI 0.88–0.99) per 20 nmol/L increment in blood 25(OH)D levels (34). The second study showed that the HR was 0.84 (95% CI 0.76–0.92) at 41 nmol/L and 0.80 (95% CI 0.67–0.95) at 92 nmol/L (33). The third study revealed that a 20 nmol/L increase in 25(OH)D concentration was associated with a reduction in overall survival (HR 0.91, 95% CI 0.81–1.01) (31). The combination of the above results could be helpful for an objective and precise judgment.

One study (43) pointed out that the majority of CRC patients in Germany (59%) suffered from vitamin D deficiency (serum 25 (OH)D₁ level < 30 nmol/L). Therefore, we further explored the reasons for the differences in vitamin D levels of colorectal cancer patients. According to Table 3, differences in BMI, physical activity, and smoking were found between two groups after meta-analysis of different characteristics of patients. Among these, difference in physical activity was the most obvious (p = 0.002) and showed the least heterogeneity (I² = 0%). After selection of supporting evidence, we found that aerobic exercise could successfully induce a variety of metabolic activities, which adjusted serum vitamin D into an optimal level (44). At the same time, studies in Japan found that endurance training suppressed a seasonal decrease in serum 25(OH)D concentrations (45). Studies have reported that smoking has a strong association with vitamin D deficiency in non-small cell lung cancer (46). In patients with chronic sinusitis, cigarette smoke exposure is also associated with vitamin D3 deficiency (47). Among BMI and vitamin D studies, an Iranian scholar has included 34 articles and a significant negative correlation was found between serum 25(OH)D levels and BMI in adults from developing countries (48). Meanwhile, vitamin D may be an effective drug to reduce the risk of cancer associated with obesity in women; the vitamin inhibits tumor growth by inhibiting the expression of telomerase reverse transcriptase through the leptin pathway (49). In addition, a cross-sectional study by Skaaby et al. (50) showed that a weak increase in alcohol consumption increased the level of vitamin D in the body, whereas another prospective study suggested that alcohol consumption is not associated with serum vitamin D levels (51). Back to this analysis, the only included article relevant to alcohol intake (25) found no difference between the higher and lower vitamin D group (OR 0.94, 95% CI 0.28–3.09).

There were still some limitations to this meta-analysis. First of all, it was impossible to obtain all the original data for each article, such as the death toll per vitamin D dose group in Mezawa et al. (16). We also failed to fit the dose–response relationship curve comprehensively and precisely, which might also be the reason for the ambiguous p value (p = 0.0662). Secondly, “high” and “low” categories were analyzed, and for the RCT, previously untreated metastatic CRC patients were randomized to high-dose vitamin D group and control group. In this way, we acknowledged the considerable variability in definitions across studies as a limitation. Thirdly, although vitamin D could improve the prognosis of patients with colorectal cancer, few articles focused on its relationship with other indicators in the treatment of colorectal cancer. Vitamin D was found correlated with vomiting during oncotherapy (52); whether it had side effects on oncotherapy was still a blank area.

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Conceived and designed the research: Jianhao Xu and Yusong Zhang. Conducted the research: Jianhao Xu and Xuya Yuan. Provided essential reagents: Jianhao Xu, Xuya Yuan, and Runhong Wu. Analyzed data or performed statistical analysis: Jianhao Xu, Xuya Yuan, Jialong Tao, and Na Yu. Wrote the paper: Jianhao Xu, Xuya Yuan, and Yusong Zhang. Yusong Zhang had primary responsibility for final content.

Author contributions
Jianhao Xu and Xuya Yuan contributed equally to this work.

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Supporting Information
Supplemental Online Material is available on J-STAGE.

REFERENCES


