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Article

The Prognostic Value of Tumor Fibrosis in Patients Undergoing Hepatic Metastasectomy for Colorectal Cancer: A Retrospective Pooled Analysis

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Abstract: BACKGROUND: Colorectal cancer (CRC) is a significant global health burden, with liver metastases representing a key prognostic factor. Neoadjuvant chemotherapy (NAC) has improved outcomes in metastatic CRC (mCRC), and tumor regression is commonly assessed using the Rubbia-Brandt classification. The Poultsides classification defines $\geq 40\%$ fibrosis as an independent prognostic factor, particularly in patients treated with cetuximab (45.71%). However, the predictive value of this threshold remains under debate, warranting further investigation. METHODS: This study evaluates the extent of fibrosis ($\geq 40\%$) induced by NAC plus anti-epidermal growth factor receptor (anti-EGFR) therapy versus NAC plus anti-vascular endothelial growth factor (anti-VEGF) therapy in mCRC patients. It also examines the prognostic relevance of the Poultsides and Rubbia-Brandt classifications. A total of 108 patients undergoing liver resection for CRC metastases were included. Statistical analyses were performed using SPSS and R software to compare fibrosis rates and survival outcomes. RESULTS: From September 2005 to January 2023, 108 patients were analyzed: 54 received chemotherapy plus anti-EGFR (Cohort 1) and 54 received chemotherapy plus anti-VEGF (Cohort 2). Fibrosis was significantly higher in Cohort 1 (median 40.0%, IQR: 25.4–53.2) than in Cohort 2 (median 20.6%, IQR: 8.07–36.9), $p < 0.001$. Overall survival was better in Cohort 2 ($p = 0.003$), with a median follow-up of 41.6 months. CONCLUSIONS: Anti-EGFR therapy is associated with greater fibrosis than anti-VEGF, despite similar survival outcomes. The Poultsides classification may be a useful prognostic tool for resected liver metastases in mCRC.

Keywords: liver metastases; colorectal cancer; pathological response; fibrosis

Introduction

Colorectal cancer (CRC), including anal cancer, represents a significant global health concern. In 2020, an estimated 1.9 million new cases and 935,000 deaths were reported, accounting for approximately 10% of all cancer cases and fatalities worldwide. In Spain, the observed survival rate between 2008 and 2013 was approximately 52.1% [1]. The prognosis of CRC is strongly influenced by the presence of metastases (M1), most commonly in the liver, which plays a critical role in determining patient outcomes following surgical treatment for metastatic disease.

The surgical resection of hepatic metastases from metastatic colorectal cancer (mCRC), whether open or laparoscopic, has been consistently associated with an overall survival benefit over time [2–6].

Neoadjuvant chemotherapy (NAC) has demonstrated beneficial effects in patients with unresectable or borderline resectable mCRC [7–9]. Studies have highlighted the prognostic significance of pathological responses to NAC [10–13], with the Rubbia-Brandt classification [10] being the most widely used system for categorizing these responses into five Tumor Regression Grades (TRG). Notably, multivariate analyses suggest that TRG is an independent prognostic factor

for both Disease-Free Survival (DFS) and Overall Survival (OS). This finding underscores the significance of TRG in predicting patient outcomes.

Although no universally accepted threshold for fibrosis or residual tumor exists to differentiate tumor regression grade (TRG) categories, a study conducted by Poultides *et al.* has identified a fibrosis level of $\geq 40\%$ as an independent prognostic marker in the context of NAC for liver metastases from mCRC [14]. However, the reliability of this threshold remains contentious, as it was established from analyses employing various fibrosis cut-offs. This raises concerns regarding potential statistical bias in designating 40% as a significant predictor. This classification has come to be known as the Poultides classification.

This study included only patients who underwent NAC—either chemotherapy alone or in combination with bevacizumab—prior to liver metastasis surgery. Interestingly, only 18.8% of patients treated with bevacizumab exhibited fibrosis $\geq 40\%$. In contrast, a subsequent study comparing fibrosis levels across different treatment groups—chemotherapy alone, chemotherapy with bevacizumab, and chemotherapy with cetuximab—revealed a significantly higher incidence of fibrosis $\geq 40\%$ in the cetuximab-treated group (45.71%) compared to the bevacizumab group (24.4%) [15].

Given the potential prognostic value of increased fibrosis and its higher prevalence in cetuximab-treated patients, further evaluation of cetuximab in combination with chemotherapy as a neoadjuvant treatment strategy is warranted. The simplicity of fibrosis assessment methods—such as reticulin staining, trichrome staining, and hematoxylin-eosin staining—could facilitate its integration as a readily available prognostic marker in clinical practice. Further studies are necessary to validate these findings and optimize treatment strategies to improve survival and disease progression outcomes in mCRC patients.

Materials and Methods

Hypothesis

The primary objective of this study is to demonstrate that NAC combined with cetuximab or panitumumab (anti-Epidermal Growth Factor Receptor, or anti-EGFR)) induces a higher degree of fibrosis ($\geq 40\%$) compared to NAC with bevacizumab (anti-Vascular Endothelial Growth Factor, or anti-VEGF)). Moreover, the primary objective of this study is to investigate differences in overall survival based on the presence or absence of $\geq 40\%$ fibrosis in liver metastases resected after NAC, as proposed by de Poultides classification [14]. The secondary objective is to compare the prognostic significance of the Poultides classification, which considers fibrosis $\geq 40\%$ as a favorable prognostic indicator, with that of the Rubbia-Brandt classification, which has been reclassified into two categories based on TRG.

Study Population

The current study retrospectively included mCRC patients who underwent liver resection between September 2005 and January 2023 at the Hepatopancreatobiliary Surgery Department of University Hospital of Girona, Dr. Josep Trueta. All data were collected and analyzed retrospectively. The study was approved by the Ethics Committee of Hospital Dr. Josep Trueta and performed according to the Declaration of Helsinki. The inclusion criteria were as follows: 1) pathologically confirmed CRC; 2) considered a resectable or potentially resectable liver metastases by a multidisciplinary team (MDT) at diagnosis; 3) Having received at least 4 cycles of neoadjuvant chemotherapy and anti-EGFR or anti-VEGF targeted therapy. 4) Having undergone radical surgery for hepatic metastases (cR0), regardless of whether surgery for extrahepatic metastases was performed. According to the criteria, this retrospective observational study included 108 patients (Figure 1).

Hepatopathy prior to hepatic surgery was classified according to the following criteria: 0 (none) = no history of liver disease; 1 (mild) = history of abnormal liver enzyme levels with negative hepatitis

virus serology and no signs of chronic liver disease; 2 (moderate) = history of abnormal liver enzyme levels with positive or negative hepatitis virus serology, with clinical or imaging signs of chronic liver disease but without cirrhosis; 3 (severe) = liver cirrhosis of any etiology, classified as Child-Pugh A.

The Clinical Risk Score (CRS) is based on the classification by Fong *et al* [16], which includes five categories. For the purposes of this study, these were reclassified into two groups: CRS 0–2 (low risk) and CRS 3–5 (intermediate or high risk).

The Lymph Node Ratio (LNR) is defined as the ratio of positive locoregional lymph nodes to the total number of lymph nodes retrieved in the colectomy or rectal resection specimen.

Statistics

Sample Size Calculation: Based on the data from Stremitzer's study (15), in which mCRC patients treated with cetuximab achieved fibrosis $\geq 40\%$ in 45.7% of cases (16/35), while those treated with bevacizumab achieved fibrosis $\geq 40\%$ in 24.2% of cases (24/99), a sample size calculation was performed. Assuming an alpha error of 0.05 and a statistical power of 0.80, a total of 52 patients in the anti-EGFR group (Cohort 1 or EGFR inhibitor) and 52 in the anti-VEGF (Cohort 2 or VEGF inhibitor) group is required to detect a significant difference in the fibrosis rate (45% vs. 25%) [17–21].

Statistical Analysis: Categorical variables will be described using absolute and relative frequencies (%) and analyzed using the Chi-square test or Fisher's exact test, as appropriate. Quantitative variables will be summarized using the mean and standard deviation (SD) or the median and interquartile range [IQR], depending on data distribution. Normality will be assessed using the Shapiro-Wilk test. Quantitative variables with a normal distribution will be analyzed using the Student's t-test for independent samples and one-way analysis of variance (ANOVA), while non-normally distributed variables will be analyzed using the Mann-Whitney U test or the Kruskal-Wallis test. Logistic regression modeling will be used to assess the factors associated with fibrosis $\geq 40\%$. First, a univariate analysis will be performed, and significant and clinically relevant factors will be selected for inclusion in the multivariate regression model. For survival analysis, Kaplan-Meier curves will be generated and compared using the log-rank test. If necessary, Cox regression analysis will be performed to evaluate the effect of independent and/or confounding variables. Hazard ratios (HR) and their corresponding 95% confidence intervals (CI) will be reported. For the comparative analysis of the prognostic informative value of survival models, the Akaike Information Criterion (AIC) has been employed.

All analyses will be conducted using SPSS and R statistical software, with a significance level set at 5%.

Pathological Specimen Analysis

The pathological examination of liver resection specimens was performed in accordance with guidelines established by the Spanish group [22]. All nodules ≤ 2 cm in diameter were fully embedded. For nodules measuring between 2 and 5 cm, at least one complete panoramic section was required, accompanied by an inclusion diagram. For nodules larger than 5 cm, an additional tissue block was included for each additional centimeter beyond this threshold. To adequately capture tumor heterogeneity, both central and peripheral regions of large nodules were sampled. Additionally, non-tumor liver tissue was obtained at least 2 cm away from the metastasis whenever possible. If this distance was not feasible, the most distant available sample was collected.

Histological staining protocols included standard hematoxylin-eosin staining. Non-tumor liver tissue was stained with at least Masson's trichrome and reticulin stains, and additional staining techniques such as Periodic Acid-Schiff (PAS) and Perl's iron stain were utilized when appropriate.

The macroscopic evaluation of resected specimens involved quantifying the number of metastases, measuring their maximum diameters, and assessing resection margins. Pathological reports were generated by three expert pathologists (M.R. Ortiz, C. Meléndez, and A. Aula) without a secondary blind review.

The percentage of tumor, fibrosis, and residual necrosis within each metastasis was documented. Based on these parameters, two Tumor Regression Grade (TRG) classifications were applied:

The Rubbia-Brandt classification (recoded into two categories): good tumor response (TRG1, TRG2, and TRG3) versus poor tumor response (TRG4 and TRG5) and the Poultsides classification, categorized according to fibrosis level: fibrosis \geq 40% versus fibrosis<40%.

For patients who underwent resection of multiple metastases, the median values of residual tumor, fibrosis, and necrosis across all metastases were used to determine TRG classification.

Results

1. Descriptives

Between September 2005 and January 2023, 437 hepatic surgeries were performed, with 378 patients undergoing liver resection for colorectal cancer metastases. Of these, 108 met the study criteria and were evenly divided in two cohorts: 54 patients (50%) received chemotherapy combined with anti-EGFR therapy (Cohort 1 or EGFR inhibitor), and 54 patients (50%) received chemotherapy combined with anti-VEGF therapy (Cohort 2 or VEGF inhibitor) (Figure 1).

1.1 Baseline Clinical and Tumor Characteristics

Baseline clinical and tumor-related preoperative characteristics for both cohorts are summarized in Table 1. The groups were generally well balanced, except for a significantly higher frequency of RAS or BRAF mutations in Cohort 2 (74%) compared to Cohort 1 (7.41%) ($p<0.001$) and a higher Ca 19.9 level at metastasis diagnosis in Cohort 2 (median: 115 U/mL [IQR: 22.4–650] versus (vs) 34.4 U/mL [IQR: 9.6–149]; $p=0.032$).

The median age at liver surgery was 63 years [IQR: 56.0–70.2]. Most patients had left-sided primary tumors (84.3% vs. 15.7%) and had undergone primary tumor resection (94.4%). The majority of resected primary tumors were locally advanced (pT3–pT4: N=53 (93%) or ypT3–ypT4: N=39 (90.7%)) and frequently had nodal involvement (pN1–pN2: N=45 (79%) or ypN1–ypN2: N=27 (62.8%)).

Of the 66 patients analyzed for BRAF mutations, only one patient (1.52%) harbored a mutation (data not shown in tables). Among 31 patients analyzed for mismatch repair (MMR) protein expression, three (9.68%) exhibited deficiency in at least one MMR protein (*MLH1*, *MSH2*, *MSH6*, or *PMS2*) (data not shown in tables).

Synchronous metastases were present in 84 patients (77.8%), while 28 patients (25.9%) had extrahepatic metastases, most commonly in the lungs (N=13, 46.4%) (data not shown in tables).

At diagnosis, the median number of liver metastases was 4 [IQR: 2–7], and the median number of affected hepatic segments was 4 [IQR: 2–5]. Large liver metastases (\geq 5 cm) were observed in 47 patients (46.1%). Initially, 70.4% (N=76) of patients were deemed technically unresectable by a multidisciplinary team, and 61.7% (N=58) had an intermediate or high Clinical Risk Score (CRS 3–5).

1.2 Preoperative Treatment Characteristics and Radiologic Tumor Response

Preoperative treatment characteristics and radiologic tumor response are summarized in Table 2.

Most patients (N=69, 63.9%) received oxaliplatin-based chemotherapy, with a median of 7 neoadjuvant chemotherapy cycles [IQR: 5–11] and 6 monoclonal antibody cycles [IQR: 4–9]. The median preoperative chemotherapy dose intensity was 95% [IQR: 90–100].

In terms of radiologic response (RECIST response criteria), the majority of patients (N=74, 69.8%) achieved either a partial or complete tumor response.

Significant differences were observed in monoclonal antibody dose intensity, which was slightly lower in Cohort 1 (97.13%) compared to Cohort 2 (99.91%); $p=0.013$, and in preoperative Ca 19.9 levels, which were significantly lower in Cohort 1 (16.5 U/mL) than in Cohort 2 (32.2 U/mL); $p=0.003$.

1.3 Hepatic Surgery Characteristics and Complications

Table 1 supplementary summarizes the characteristics of hepatic surgery and postoperative complications. Ninety-day postoperative mortality was significantly lower in the VEGF inhibitor group (N=0, 0%) compared to the EGFR inhibitor group (N=9, 16.1%); $p=0.003$.

No significant differences were observed in surgical approach (types of liver surgery), operative time (liver surgery duration), preoperative portal embolization or ligation, or vascular clamping duration. However, the interval between the last monoclonal antibody dose and surgery was longer in Cohort 2 (6 weeks [IQR: 4–9.75]) than in Cohort 1 (4 weeks [IQR: 3–7]), likely reflecting the well-documented challenges of VEGF inhibitor-associated wound healing and bleeding complications [23].

A significant difference was observed in the incidence of postoperative hemoperitoneum, which was more frequent in Cohort 1 (N=10, 18.5%) than in Cohort 2 (N=2, 3.7%); $p=0.032$. However, no significant differences were found in other postoperative complications, including biliary fistula, intra-abdominal abscess, wound infection, pneumonia, or liver failure.

1.4 Postoperative Treatment and Recurrence Characteristics

Regarding postoperative treatment and recurrence (Table 2 supplementary), significant differences were observed in the administration of adjuvant therapy following hepatic surgery or first-line therapy for patients with persistent, unresected metastases. A greater proportion of patients in Cohort 1 (EGFR inhibitor) did not receive postoperative treatment (N=11, 23.9%) compared to Cohort 2 (VEGF inhibitor, N=1, 2.13%); $p<0.001$, possibly due to the higher postoperative mortality in Cohort 1. However, no significant differences were observed in post-treatment progression-free survival (PFS) or in the incidence of hepatic or pulmonary recurrences.

1.5 Pathologic Tumor Response

The median residual fibrosis in resected metastases was significantly higher in the EGFR inhibitor-Cohort 1 (40.0% [IQR: 25.4–53.2]) compared to the VEGF inhibitor-Cohort 2 (20.6% [IQR: 8.07–36.9]); $p<0.001$. Conversely, the median residual necrosis was lower in Cohort 1 (16.1% [IQR: 5.00–25.0]) than in Cohort 2 (33.0% [IQR: 15.0–50.0]); $p=0.001$ (Table 3).

When fibrosis was classified using the Poultsides criteria (2-category response), a significantly higher proportion of patients in Cohort 1 exhibited fibrosis $\geq 40\%$ (N=29, 53.7%) compared to Cohort 2 (N=13, 24.1%); $p=0.003$. Similarly, categorized necrosis $\geq 40\%$ was more frequent in Cohort 2 (N=24, 44.4%) than in Cohort 1 (N=7, 13%); $p=0.001$.

No significant differences were observed between cohorts in pathologic response grading according to the Rubbia-Brandt (2-category) classification ($p=0.438$). Additionally, no differences were found in the weight of resected liver tissue, pathological size of the largest metastasis, or margin clearance distance.

1.6 Non-Tumor Liver Toxicity

In terms of hepatic toxicity associated with preoperative treatment (Table 3 supplementary), sinusoidal injury was less frequent in Cohort 2 (VEGF inhibitor), where the majority of patients exhibited mild or no sinusoidal damage (N=48, 88.9%) compared to Cohort 1 (N=34, 63.0%); $p=0.003$. No significant differences were found between the two cohorts regarding liver steatosis ($p=0.071$), non-alcoholic steatohepatitis ($p=0.330$), or clinical signs of portal hypertension post-hepatic surgery ($p=0.158$).

2. Monoclonal Antibody Use and Fibrosis Degree. Analytics

To further explore factors associated with fibrosis $\geq 40\%$, a logistic regression analysis was performed (Table 4), as univariate analysis does not account for interactions among multiple variables influencing tumor fibrosis.

2.1 Monoclonal Antibody Use and Fibrosis Degree. Univariate Logistic Regression Analysis

In univariate analysis, the following variables were identified as potential predictors of fibrosis $\geq 40\%$: Clinical Risk Score (CRS) ($p=0.025$), Preoperative monoclonal antibody type ($p=0.002$), Primary tumor location ($p=0.062$) and RAS/BRAF mutations ($p=0.006$).

2.2 Monoclonal Antibody Use and Fibrosis Degree. Multivariate Logistic Regression Analysis

Given the strong collinearity between RAS/BRAF mutations and monoclonal antibody type (as nearly all patients in Cohort 1 (92.6%) were RAS/BRAF wild-type), RAS/BRAF mutations were excluded from the final multivariate model. Monoclonal antibody type was the strongest independent predictor of fibrosis $\geq 40\%$ (Odds Ratio (OR)=4.967 for EGFR inhibitors, 95% CI: 1.885–13.087; $p=0.001$). CRS was also a significant predictor, with higher CRS scores (3–5) being associated with a lower likelihood of fibrosis $\geq 40\%$ (OR=0.377, 95% CI: 0.146–0.975; $p=0.044$). Primary tumor location did not reach statistical significance in the multivariate model (OR=4.607 for left-sided tumors, 95% CI: 0.854–24.855; $p=0.076$), but it was retained due to potential clinical relevance, small sample size, and a p-value approaching significance. This multivariate model has a sensitivity of 33.3% and specificity of 89.7%.

2.3 Monoclonal Antibody Use and Fibrosis Degree. Multivariate Model Equation

The probability (P) of fibrosis $\geq 40\%$ can be estimated using the following formula: $\log(P/1-P) = -2.174 + (1.603 \times \text{VEGF inhibitor}=0 \text{ or EGFR inhibitor}=1) + (-0.974 \times \text{CRS } 0-2=0 \text{ or CRS } 3-5=1) + (1.528 \times \text{right colon}=0 \text{ or left colon}=1)$ or equivalently $P = 1 / 1 + e^{-1 \times ((-2.174 + (1.603 \times \text{VEGF inhibitor}=0 \text{ or EGFR inhibitor}=1) + (-0.974 \times \text{CRS } 0-2=0 \text{ or CRS } 3-5=1) + (1.528 \times \text{right colon}=0 \text{ or left colon}=1))}$.

This model defines eight probability groups for fibrosis $\geq 40\%$ based on tumor location, monoclonal antibody type, and CRS (Table 6).

3. Overall Survival Analysis

To assess overall survival (OS), we excluded nine patients who experienced early (90-day) postoperative mortality to minimize confounding effects and better isolate the impact of neoadjuvant treatment, surgical interventions, and pathological outcomes on long-term survival.

The median follow-up period was 41.6 months [IQR 0–215], with 34.9 months [IQR 0–171] in Cohort 1 and 48.6 months [IQR 4–215] in Cohort 2. By the end of the study, 88 patients (81.5%) had died, and 82 patients (75.9%) had experienced disease recurrence or progression. Among the deceased, 24 patients (22.2%) died from non-cancer-related causes, including postoperative mortality.

Two patients were lost to follow-up due to relocation, and their mortality and disease progression status remained unknown. For survival analysis, their OS events were censored at the last available follow-up, meaning they contributed prognostic information only up to that point.

Ultimately, 99 patients were included in the overall survival analysis.

3.1 Univariate OS Cox Regression Analysis

Univariate Cox regression (Table 4-6 supplementary) identified the following variables as potentially associated with improved prognosis: Female sex (Hazard Ratio (HR)=0.60, 95% CI 0.37–0.97, $p=0.033$), Primary tumor resection (HR=0.22, 95% CI 0.09–0.56, $p=0.009$) and Fibrosis $\geq 40\%$ ((Poultides classification) (HR=0.61, 95% CI 0.38–0.97, $p=0.034$)).

Conversely, variables associated with a potentially worse prognosis included: Lymph node ratio ((LNR) (HR=3.99, 95% CI 1.25–12.7, $p=0.032$)), Preoperative CEA level (HR=1.001, 95% CI 1.0004–1.0024, $p=0.006$), Liver metastasis size ≥ 10 cm (M1 ≥ 10 cm) (HR=2.34, 95% CI 1.11–4.92, $p=0.044$), and Degree of hepatopathy at diagnosis (moderate/severe) (HR=2.63, 95% CI 1.63–4.26, $p<0.001$).

Although tumor pathological response based on the Rubbia-Brandt classification (2 categories) was not statistically significant in the univariate model, we developed two multivariate overall survival models, incorporating significant univariate predictors and each pathological response classification separately.

3.2 Multivariate OS Models

Poultides Classification Model (Table 5): In the survival model based on the Poultides classification, the following variables had a positive impact on OS: Fibrosis $\geq 40\%$ (HR=0.488, 95% CI 0.270–0.880, $p=0.014$) and Female sex (HR=0.505, 95% CI 0.281–0.909, $p=0.019$). Conversely, the following variables were associated with worse overall survival: Moderate or severe hepatopathy (HR=2.678, 95% CI 1.535–4.672, $p<0.001$) and Lymph node ratio (LNR) (HR=4.190, 95% CI 1.222–14.36, $p=0.033$).

Rubbia-Brandt Classification Model (Table 5): In the multivariate survival model using the Rubbia-Brandt classification, the following variables were associated with better overall survival: TRG responders (TRG1–3) (HR=0.549, 95% CI 0.321–0.938, $p=0.028$) and Female sex (HR=0.531, 95% CI 0.294–0.960, $p=0.031$). In contrast, the following variables were associated with worse overall survival: Moderate or severe hepatopathy (HR=2.821, 95% CI 1.612–4.936, $p<0.001$) and Preoperative CEA level (HR=1.002, 95% CI 1.000–1.003, $p=0.029$).

Both models were compared using the Akaike Information Criterion (AIC) to assess their relative explanatory power. The AIC for the Poultsides model is 407.284 and the AIC value for the Rubbia-Brandt model is 408.493. The small difference in AIC values suggests that both models provide equivalent predictive value, with no clear preference for one over the other.

A calculator has been developed (in Excel format) to estimate survival probability (%) based on both pathological tumor regression models (Poultsides and Rubbia-Brandt), incorporating the significant variables identified in each model as well as their corresponding hazard ratios for each estimated time point (Figure 1. Supplementary).

Discussion

Several classification systems have been proposed for assessing pathological response in resected hepatic metastases from colorectal cancer (CRC). Many of these classifications are subject to interobserver variability among pathologists or involve overlapping categories that are difficult to distinguish. This study aims to determine whether the pathological response in resected metastases differs between anti-VEGF and anti-EGFR therapies, focusing on fibrosis $\geq 40\%$ (Poultsides classification) and the Rubbia-Brandt criteria (in two categories). Additionally, we assess the prognostic value of each classification within a multivariate overall survival model.

The characteristics of our cohort highlight a population with poor prognosis: 70.4% of patients were initially deemed unresectable by a multidisciplinary team, 61.7% had high or intermediate clinical risk scores (CRS 3–5), and 46.1% had metastases larger than 5 cm. Notably, in the anti-EGFR group (Cohort 1), early postoperative mortality was 16.1%, compared to 0% in the anti-VEGF group (Cohort 2). This was primarily due to hemoperitoneum (44.4% vs. 8.08%) and ascites (66.7% vs. 9.09%), leading to hepatic failure (66.7% vs. 9.09%). This discrepancy may be explained by the protective effects of bevacizumab (anti-VEGF) on hepatic sinusoids, reducing portal vascular resistance in patients treated with oxaliplatin and lowering the risks of intraoperative bleeding and portal hypertension-related ascites, as previously reported [24–27].

Regarding our primary objective, a Chi-square analysis revealed a significantly higher proportion of fibrosis $\geq 40\%$ (Poultsides classification) in patients treated with chemotherapy and anti-EGFR compared to those receiving chemotherapy and anti-VEGF (53.7% vs. 24.1%, $p=0.003$). However, no significant differences were found when pathological response was assessed using the Rubbia-Brandt criteria ($p=0.438$). The results of the logistic regression analysis (both univariate and multivariate, Table 7) revealed several significant associations with the likelihood of having fibrosis $\geq 40\%$. Specifically, anti-EGFR treatment was positively associated with an increased likelihood of fibrosis $\geq 40\%$, (OR=4.967, 95% CI: 1.885–13.087; $p=0.001$). Additionally, the presence of a left-sided primary tumor was also associated with a higher likelihood of fibrosis $\geq 40\%$, though this association was less statistically significant, as evidenced by an OR of 4.607 (95% CI: 0.854–24.855; $p=NS$). On the other hand, a Clinical Risk Score (CRS) of 3–5 was inversely associated with the likelihood of fibrosis $\geq 40\%$, suggesting a resistance effect to the development of fibrosis in high-risk patients (OR=0.377, 95% CI: 0.146–0.975; $p=0.044$).

These findings may be influenced by selection bias, as most patients receiving anti-EGFR therapy were RAS/BRAF wild-type (92.6%), compared to only 26% in the anti-VEGF group (Table 1). Consequently, these results do not support a universal preference for anti-EGFR over anti-VEGF therapy in RAS/BRAF wild-type patients based solely on the probability of fibrosis $\geq 40\%$. Overall, right-sided CRC liver metastases are less likely to exhibit fibrosis $\geq 40\%$ compared to left-sided CRC metastases. However, in resected liver metastases from right-sided CRC, anti-EGFR therapy

increases the likelihood of fibrosis $\geq 40\%$ (17.5%–36.1%) compared to anti-VEGF therapy (4.1%–10.2%) (Table 6), according to a multivariate logistic regression model. This finding aligns with the guidelines from the Pan-Asian and European Society for Medical Oncology (ESMO) [27–29], which recommend considering anti-EGFR therapy for potentially resectable right-sided colorectal cancer metastases in patients with wild-type RAS/BRAF, although our recommendation is based on pathological response.

In our view, strategies aimed at enhancing pathological response, particularly fibrosis, should be explored to improve overall survival in patients undergoing resection of colorectal cancer liver metastases. For this analysis, patients with early postoperative mortality (N=9) were excluded, as their outcomes were more reflective of surgical complications or preoperative clinical conditions rather than the impact of treatment strategy on survival.

Univariate overall survival analysis suggested that fibrosis $\geq 40\%$ (Poultides classification) could be a significant prognostic factor (fibrosis $\geq 40\%$ vs. $<40\%$, HR=0.61, 95% CI: 0.38–0.97; $p=0.034$). However, overall survival was independent of the monoclonal antibody used (anti-VEGF vs. anti-EGFR; HR=0.99, 95% CI: 0.63–1.55; $p=0.96$, Table 8). This underscores the complexity of metastatic CRC, where overall survival depends on multiple factors beyond the type of biological therapy administered.

In our cohort, univariate survival analysis identified female sex as a favorable prognostic factor for overall survival (HR=0.6, 95% CI: 0.37–0.97; $p=0.033$), as well as primary tumor resection (HR=0.22, 95% CI: 0.09–0.56; $p=0.009$). However, the survival benefit associated with tumor resection may reflect selection bias, as patients with better prognoses are more likely to undergo surgery. Adverse prognostic factors included elevated Preoperative carcinoembryonic antigen (CEA) levels (HR=1.001, 95% CI: 1.0004–1.0024; $p=0.006$), higher lymph node ratio (HR=3.99, 95% CI: 1.25–12.7; $p=0.032$), the presence of liver metastases ≥ 10 cm (HR=2.34, 95% CI: 1.11–4.92; $p=0.044$), and moderate-to-severe preoperative hepatopathy (HR=2.63, 95% CI: 1.63–4.26; $p<0.001$) (Table 8).

Regarding the secondary objective—assessing the prognostic value of pathological response using the Poultides classification (fibrosis $\geq 40\%$ vs. $<40\%$) compared to the Rubbia-Brandt classification (categorized into two groups)—we constructed two multivariate overall survival models. Both models incorporated the same prognostic variables that were significant in the univariate analysis, including gender, lymph node ratio, pre-operative CEA levels, and preoperative hepatopathy, while substituting one pathological response classification for the other. The primary tumor resection variable (yes vs. no) was excluded due to its potential confounding effect.

The analysis demonstrated that both models provided equivalent prognostic information, as indicated by nearly identical Akaike Information Criterion (AIC) values. In the absence of direct comparative studies between these two classification systems, our findings suggest that assessing pathological response using the Poultides classification yields prognostic information comparable to that obtained with the Rubbia-Brandt classification in patients undergoing surgical resection of liver metastases (M1) from mCRC.

Given the increasing use of semi-automated histopathological analysis [30], the Poultides classification, with its specific fibrosis cutoff, may offer a more objective and reproducible approach for assessing pathological response. Its standardized threshold could facilitate its adoption in clinical practice and research, particularly in studies evaluating treatment response in hepatic metastases from mCRC.

These findings highlight the need for further validation through prospective studies, particularly to determine whether fibrosis-based classifications can enhance treatment stratification and guide therapeutic decisions in metastatic colorectal cancer.

One of the main limitations of the present study is its retrospective nature. Data has been collected over an extended period during which both surgical and non-surgical management strategies have evolved. These changes may have influenced overall survival outcomes. Additionally, although pathological assessments were conducted by expert digestive pathologists, no blinded review of the specimens was performed, which may limit the external validity of the findings.

Conclusions

While anti-EGFR therapy was associated with a higher likelihood of tumor fibrosis $\geq 40\%$ compared to anti-VEGF therapy, this did not translate into improved overall survival for patients undergoing resection of hepatic M1 metastases from mCRC. Fibrosis $\geq 40\%$ was identified as an independent prognostic factor for survival in patients with resected hepatic metastases, regardless of the type of monoclonal antibody used prior to surgery. Furthermore, fibrosis $\geq 40\%$ provided prognostic information comparable to that of the Rubbia-Brandt classification. The standardized threshold used in the Poultides classification, combined with its potential for easier, more objective, and reproducible assessment, suggests that it may offer a practical and reliable alternative for evaluating pathological response in future studies and clinical practice.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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