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HISTOPATHOLOGIC PATTERNS AS MARKERS OF PROGNOSIS IN PATIENTS UNDERGOING HEPATECTOMY FOR COLORECTAL CANCER LIVER METASTASES

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Abstract

Introduction: Liver resection combined with neoadjuvant chemotherapy (NAC) has reported notable results in patients with colorectal liver metastases (CRLM). Tumoral response to NAC is associated with specific histopathologic patterns with prognostic implications. The primary objective of this study was to evaluate the influence of pathological findings on overall survival (OS), disease-free survival (DFS) and liver recurrence-free survival (LRFS) and secondarily to analyze the role of NAC and clinical features on patients' outcome.

Patients and Methods: Analysis of clinical and outcome data from 110 patients who underwent first CRLM resection between January 2010 and July 2013 was carried out. Blinded pathological review of histological material of several parameters: resection margin, tumor regression grade (TRG), tumor thickness at the tumor-normal interface (TTNI) and the growth pattern (GP).

Results: The median survival following hepatic resection was 52 months and 3- and 5- year Kaplan-Meier estimates were 69 and 48%, respectively. Seventy-four patients developed recurrent disease. Oxaliplatin-based chemotherapy was significantly associated with a pushing GP. A positive resection margin was an independent predictor of decreased DFS (p=0.018) and LRFS was strongly reduced by the absence of histologic tumor response (p=0.018). The pushing pattern had an adverse impact on both OS (p=0.007) and DFS (p=0.004) on multivariate analysis.

Conclusion: The prognostic value of histopathological features in patients who underwent CRLM's resection is undeniable. The pushing GP was related with worse prognosis. Further studies are required to clarify the biological mechanisms underlying these findings in order to enhance a more personalized and efficient treatment for these patients.

Keywords: liver, metastases, colorectal cancer, hepatectomy, chemotherapy, histopathology, prognosis.

I. Introduction

Yearly, 1.2 million new cases of colorectal cancer (CRC) are diagnosed worldwide and around 50% of them will develop liver metastases.(1) Hepatic resection remains the most efficient treatment for these patients, however, a strategy combining surgery with neoadjuvant chemotherapy (NAC) has gain wide acceptance. Furthermore, most patients are initially unresectable but can be resected after down-staging by conversion chemotherapy.(2-4)

Several clinical variables have been identified as valuable predictors of recurrence and survival in order to provide an enhanced neoadjuvant therapy.(5, 6)

Current research is also focused on finding pathological indicators which may influence treatment response. The role of surgical margin on patients' outcome after colorectal liver metastases (CRLM) resection has been thoroughly investigated (7) and is still subject of debate.(8-10) Recently, new pathologic markers of prognosis have been described. Dipen Maru et al (11) established a pathologic predictor of survival: the tumor thickness at the tumor-normal interface (TTNI). Rubbia-Brant et al (12) documented a pathological tumor regression grade (TRG) system for CRLMs with five histological categories according to the extent of fibrosis and the amount of residual tumor cells.

In 2001, Vermeulen et al (13) carried out one of the most interesting studies in this area and described three histological CRLM growth patterns (GP) with different angiogenesis and invasive potential, labeled as "desmoplastic"; "replacement" and "pushing".

Apart from the prediction of response to chemotherapy, these pathologic patterns can also reflect important tumor-host interactions, as spontaneous necrosis of tumor metastases has been described.

Therefore, this study has two main purposes. The first one was to estimate the value of pathological findings as prognostic factors, analyzing their impact on overall survival (OS), disease-free survival (DFS) and liver recurrence-free survival (LRFS). The second was to evaluate the influence of NAC and clinical parameters on patient's outcome.

II. Patients and methods

1. Study design

The present study reviews clinical and pathological data from a total of 142 patients who underwent hepatic surgery for CRLM from January 2010 to July 2013 at Serviço de Cirurgia A from Centro Hospitalar e Universitário de Coimbra (Head of Department: Prof. Doutor Francisco Castro e Sousa, Coimbra, Portugal).

Demographic and clinical information were collected from patients' medical records. Six of these patients had insufficient clinical data and were excluded. Another six patients were not included due to inadequate histological material for evaluation. Patients undergoing rehepatectomies (20 patients) were also excluded (Figure 1). A formal ethics committee approval was not required considering the retrospective nature of this study.

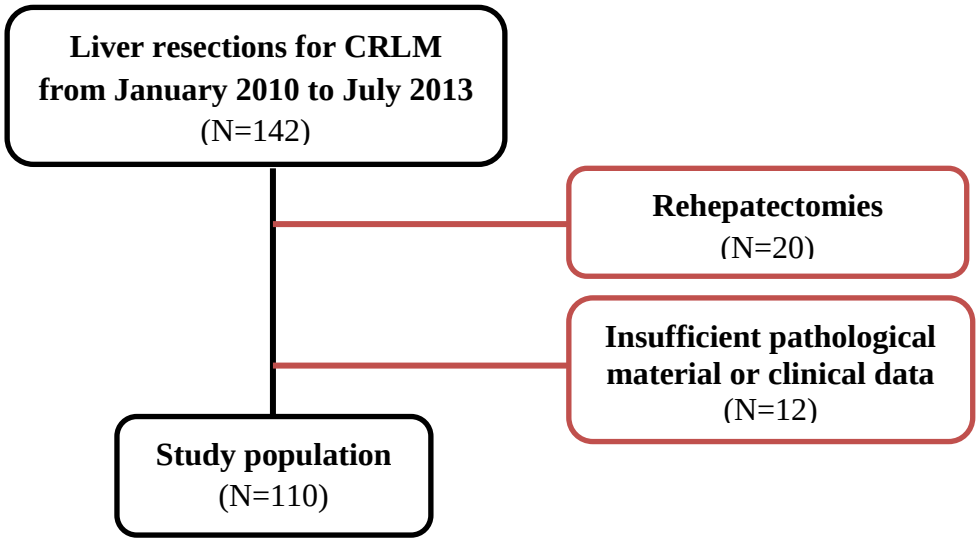

Figure 1. Exclusion criteria and study population.

2. Patients

A total of 110 patients were included, 81 male and 29 female, with a mean age of 63 years ± 10 (range 33-82). Thirty-three patients (30%) were aged 70 years or older.

The primary site of the tumor was the colon in 73 patients (66.4%), the rectum in 32 patients (29.1%) and both locations in five patients (4.5%). Positive lymph nodes were found in more than half of the patients (66.4%).

A single liver metastasis was present in 47 patients (42.7%) versus multiples metastases in 63 patients (57.3%), with a mean of 2.62 metastases per patient (range 1–13).
The maximum diameter of the largest lesion was greater than or equal to three centimeters in 62 patients (56.4%), with a mean size of 4.26 centimeters (range 0.7 to 17). These lesions were located as follows: unilobar in 69 patients (62.7%) (38 in the right and 31 in the left hemi-liver) and bilobar in 41 patients (37.3%).

Fifty-seven patients (51.8%) presented with synchronous liver metastases. In 12 patients a liver resection was simultaneously performed with colorectal surgery, while all others (45 patients) received a metachronous resection. A “Liver First” approach (initial liver resection followed by a colorectal resection) was performed in eight patients (7.3%).
The remaining 53 patients (48.2%) presented with metachronous disease (Table 1).

3. Neoadjuvant Chemotherapy (NAC)

In our series, 52 patients (47.3%) were treated with preoperative systemic chemotherapy. Among them, 44 received one NAC line and seven received two NAC lines. The mean number of cycles administered was 11.2±5.5 (range 4-26 cycles).

A FOLFIRI-based chemotherapy was the option in 37 patients (33.6%), 15 patients (13.6%) received the FOLFOX regimen and 5-fluorouracil (5-FU) was used as monotherapy in one patient (0.9%). Bevacizumab was added in 22 cases (20%) and Cetuximab in 16 (14.5%).

Fifty-eight patients (52.7%) underwent resection without NAC (Table 1).

Table 1. Clinical and pathological characteristics of study population.

Parameters	**No. of patients (%) N = 110**
Patients	
Female	29 (26.4)
Male	81 (73.6)
< 70 (years)	77 (70)
≥ 70 (years)	33 (30)
Primary tumor	
Location	
Colon	73 (66.4)
Rectum	32 (29.1)
Colon + Rectum	5 (4.5)
Node status	
Positive lymph node	73 (66.4)
Negative lymph node	34 (30.9)
Colorectal Liver metastases	
Number of nodules	
Single	47 (42.7)
Multiple	63 (57.3)
Largest diameter	
< 3 cm	45 (40.9)
≥ 3 cm	62 (56.4)
Location	
Unilobar	69 (62.7)
Bilobar	41 (37.3)

Presentation	
Synchronous	57 (51.8)
Metachronous	53 (48.2)
Neoadjuvant Chemotherapy	
No	58 (52.7)
Yes	52 (47.3)
1 Line	44 (40)
2 Lines	7 (6.4)
FOLFIRI	37 (33.6)
FOLFOX	15 (13.6)
5-FU	1 (0.9)
Bevacizumab	22 (20)
Cetuximab	16 (14.5)

4. Operative details

After bilateral subcostal incision (or laparoscopy in selected cases), exploration of the abdominal cavity and intraoperative ultrasound were carried out to thoroughly exclude extra-hepatic disease and to confirm preoperative data. Liver parenchymal transection was performed using the Cavitron Ultrasonic Surgical Aspirator (CUSA®) or Kelly's technique (the clamp-crush technique). The Pringle manoeuvre, in an intermittent clamping strategy as previously described (14) was employed in 74 patients (67.3%) with a mean duration of 27.0±24.2 minutes (range 4-104).

Major hepatectomy, defined as resection of more than two liver segments, was performed in 40 patients (36.4%) while a minor liver resection was accomplished in 70 cases (63.6%), three of them using a laparoscopic approach (2.7%). Surgical procedures are summarized in Table 2.

Twenty-five patients (22.7%) required red blood cells transfusion.

Portal vein ligation or embolization was performed to induce hypertrophy of the future liver remnant in 15 patients (13.6%).

Table 2. List of hepatectomies according to the surgical procedures performed.

Type of hepatectomy	**N (%)**
Right Hepatectomy	
Classic	15 (13.6)
Extended	6 (5.5)
Classic + Left Atypical Resections	2 (1.8)
Extended + Left Atypical Resections	1 (0.9)
Left Hepatectomy	
Classic	4 (3.6)
Extended	3 (2.7)
Classic + Right Atypical Resections	1 (0.9)
Extended + Right Atypical Resections	2 (1.8)
Other anatomical resections	
Segmentectomy	14 (12.7)
Bisegmentectomy	12 (10.9)
Trisegmentectomy	2 (1.8)
Atypical resections	
<4	21 (19.1)
≥4	6 (5.5)
Other anatomical resections + Atypical resections	21 (19.1)

5. Morbidity and Mortality

Surgical complications were graded according to the Dindo-Clavien classification up to the 90th postoperative day (15) into four groups: no morbidity; minor morbidity (grade I and II); major morbidity (grade IIIa-IVb) and mortality (grade V). Postoperative mortality was observed in four patients (3.6%), three of these deaths due to posthepatectomy liver failure (as defined by Balzan et al (16)) and one due to portal vein thrombosis. Nine patients (8.2%) suffered major morbidity. The median length of hospital stay was ten days (range 3-55).

6. Neoadjuvant chemotherapy group vs. Surgery-only group

A comparison between patients treated with NAC vs. patients who underwent surgery alone revealed that synchronous presentation (78.8% vs. 27.6%, p<0.001), multiple nodules (76.9% vs. 39.7%, p<0.001), bilobar distribution (50% vs. 25.9%, p=0.009) and synchronous resection (19.2% vs. 3.4%, p=0.008) of CRLM were more frequent in the NAC group (Table 3).

Table 3. Comparative analysis of clinical characteristics between NAC Group and Surgery-only Group

Parameters	**NAC Group (n =52)**	**Surgery-only Group (n = 58)**	**P**
Patients			
Age (years)	62.13 ± 9.21	64.50 ± 10.94	0.226
Primary tumor			
Colon (%)	32 (61.5)	41 (70.7)	
Rectum (%)	19 (36.5)	13 (22.4)	0.156
Colon+Rectum (%)	1 (1.9)	4 (6.9)	
Node-positive (%)	35 (71.4)	38 (65.5)	0.513
Node-negative (%)	14 (28.6)	20 (34.5)	
Colorectal liver metastases			
Synchronous presentation (%)	**41 (78.8)**	16 (27.6)	**< 0.001**
Metachronous presentation (%)	11 (21.2)	42 (72.4)	
Single nodule (%)	12 (23.1)	35 (60.3)	**<0.001**
Multiple nodules (%)	**40 (76.9)**	23 (39.7)	
Mean largest diameter (range)	47.84 ± 38.99	37.93 ± 22.52	0.118
Unilobar (%)	20 (50.0)	43 (74.1)	**0.009**
Bilobar (%)	**26 (50)**	15 (25.9)	
Synchronous resection (%)	**10 (19.2)**	2 (3.4)	**0.008**
Metachronous resection (%)	42 (80.0)	56 (96.9)	
Minor hepatectomy (%)	29 (55.8)	41 (70.7)	0.104
Major hepatectomy (%)	23 (44.2)	17 (29.3)	

7. Postoperative follow-up

Follow-up schedule in the first postoperative two years included clinical evaluation, abdominal computed tomography (CT) scan and serum carcinoembryonic antigen (CEA) measurement every three months and thoracic CT scan every six months. Between the second and the fifth postoperative year abdominal CT scan and CEA measurements were carried out every six months and once a year following this period.

Overall survival was identified as the time between hepatectomy and the date of tumor-related death or most recent follow-up if the patient was alive.

Recurrence was defined as the period between CRLM resection and the first new lesion detected by imaging studies and supported by biochemical data. Patients with documented liver recurrence or distant recurrence in the first six postoperative months were categorized as having early recurrence.

Accordingly, disease-free survival and liver recurrence-free survival were defined as the time between hepatectomy and the date of any recurrence and liver recurrence, respectively.

Clinical follow-up was obtained from patients' medical records or by telephone interviews.

8. Histopathological evaluation

Archival histologic material from each patient was retrospectively reviewed by two experienced hepatobiliary pathologists. Examination was performed on Haematoxylin and Eosin (H&E), Masson's trichrome and reticulin stained slides observed in light microscope – Nikon Eclipse 50i, and images obtained using a Nikon-Digital Sight DS-Fi1 camera. Tissue samples from each tumor nodule and from the tumor-free liver parenchyma distant from the tumor were evaluated. Pathological analysis was performed without awareness of clinical data, treatment details or patient outcome.

8.1. Non-tumor liver parenchyma

Morphological features assessed in non-tumor tissue were analyzed as described by Martins J. (17) and included sinusoidal dilatation, perisinusoidal hemorrhage, peliosis, nodular regeneration, necrosis, fibrosis and steatosis.

8.2. Tumoral parenchyma

The review of metastatic samples was focused on histological findings as tumor-free resection margin, tumor regression grade, tumor thickness at the tumor-normal interface and the growth pattern of CRLM.

Tumor-free resection margins were measured for all the metastases. The smaller resection margin in each patient was labeled as R0 (margin ≥1 millimeter) and No-R0 (R1: margin <1 millimeter or R2: macroscopic tumor in the surgical resection margin).

Tumor pathologic response of tumor nodules was characterized according to Rubbia-Brandt et al (12) as follows: TRG1 (fibrous tissue with no tumor cells); TRG2 (predominant fibrous tissue with occasional residual tumor cells); TRG3 (predominant fibrous tissue with more residual tumor cells); TRG4 (tumor cells superseding the fibrous tissue); TRG5 (full spread of tumor cells with no evidence of fibrous tissue). The worst tumor response in each patient was categorized as absent (TRG5) or present (TRG1-4) (Figure 2).

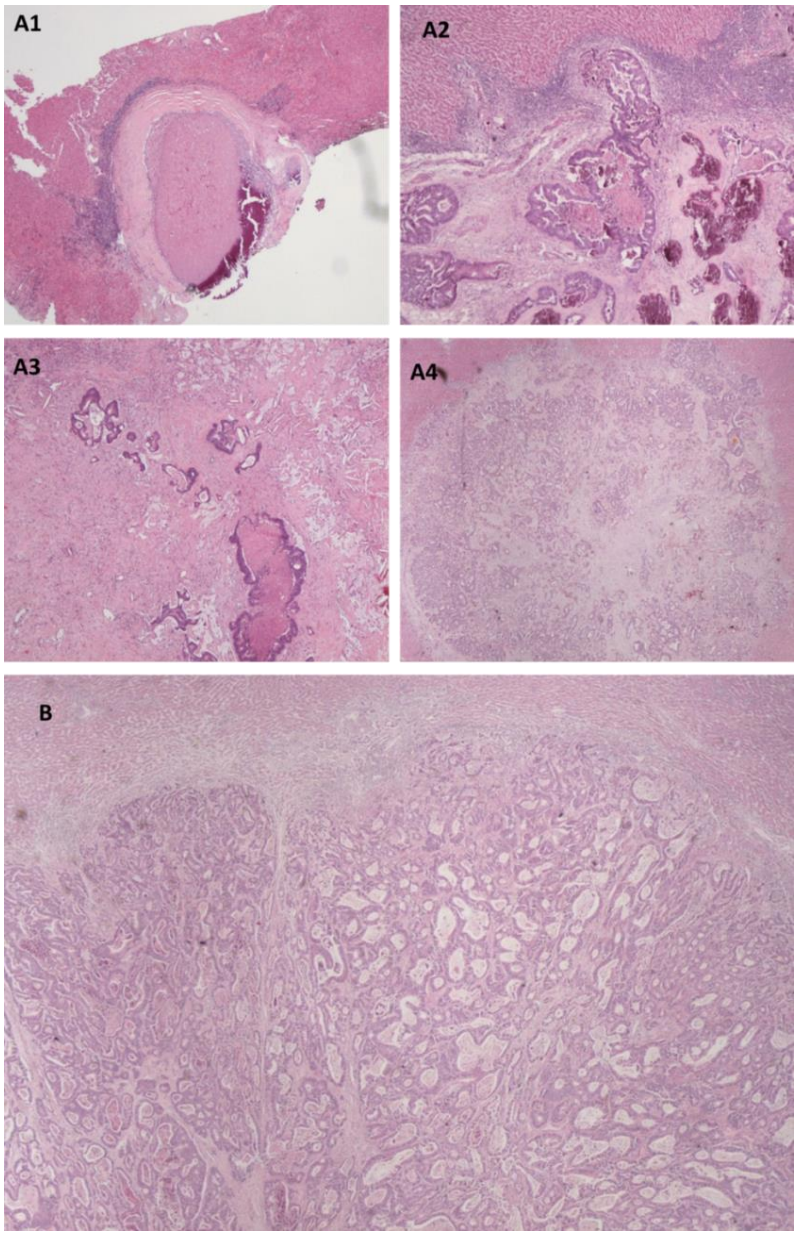

Figure 2. Tumor Regression Grade (TRG) in colorectal liver metastases. (A) CRLM showing pathologic response. (A1) TRG1, H&E 40x; (A2) TRG2, H&E 40x; (A3) TRG3, H&E 40x; (A4) TRG4, H&E 20x; (B) CRLM with no pathologic response, TRG5, H&E 40x.

Tumor thickness at the tumor-normal interface was achieved as described by Dipen Maru et al.(11) In brief, we measured the greatest uninterrupted tumor cell thickness with a ruler (Figure 3) and this measurement in the largest metastasis was stratified into two groups: TTNI <0.5 millimeters and TTNI ≥0.5 millimeters.

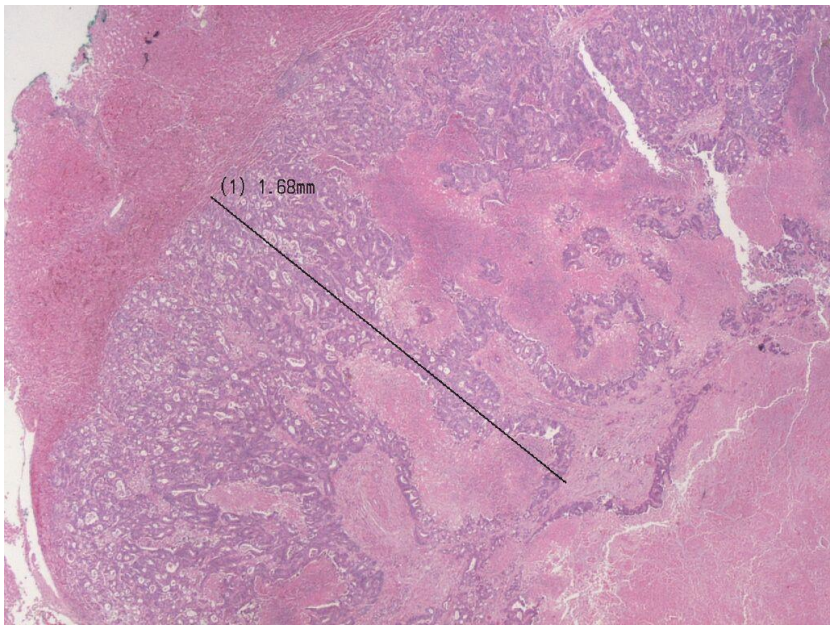

Figure 3. Tumor thickness at the tumor-normal interface (TTNI) measurement, H&E, 20x.

Histological growth pattern of CRLM was described according to Vermeulen et al (13, 18) into four groups. Liver metastases with desmoplastic growth pattern have a connective tissue barrier between the liver parenchyma and the tumor; the pushing pattern is characterized by the compression of the hepatocytes at the tumor-interface; in the replacement growth pattern the liver parenchyma is invaded by tumor cells (Figure 4). If more than one group was observed and each group was present in up to 25% of tumor liver parenchyma interface, the growth pattern was described as mixed.

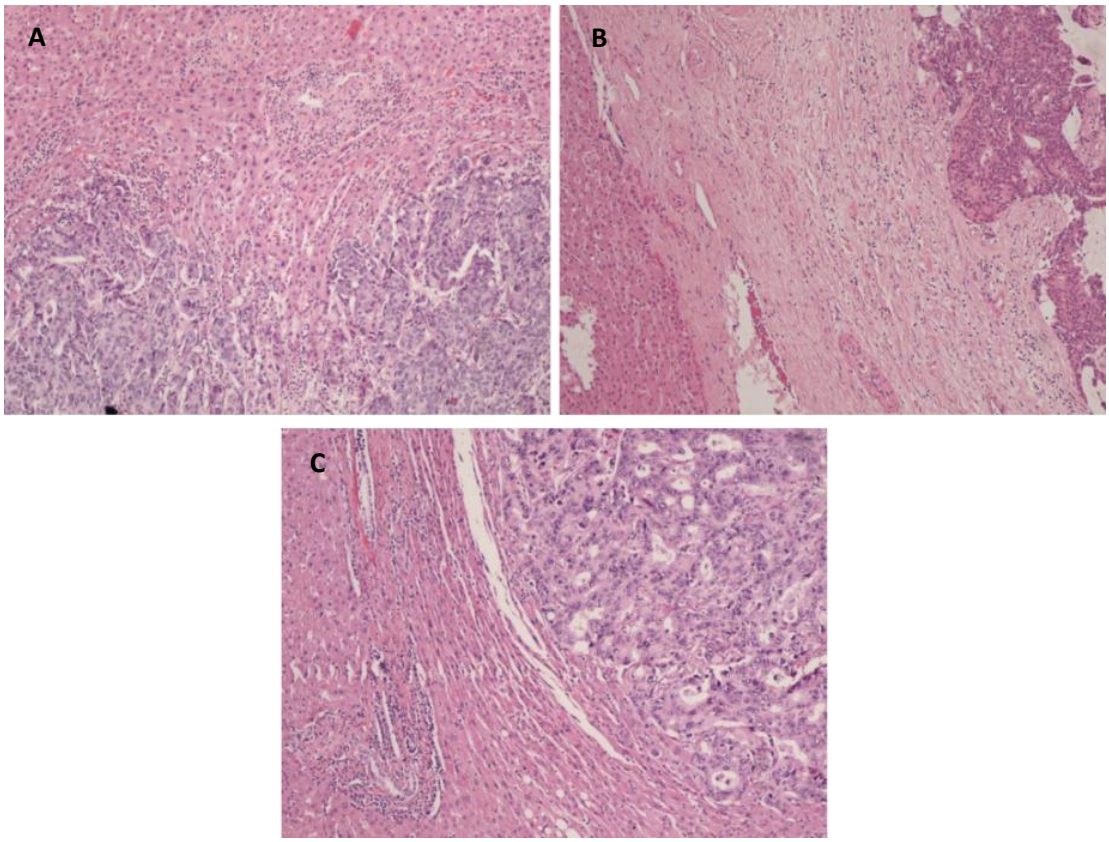

Figure 4. Three different growth patterns in colorectal liver metastases. (A) Replacement pattern, H&E 100x; (B) Desmoplastic pattern, H&E 100x; (C) Pushing pattern, H&E 100x.

9. Statistical analysis

Statistical analyses with SPSS™ software version 21.0 for Windows. Quantitative data were expressed as mean ± standard deviation (SD) and range.

Continuous variables were evaluated using Student's t-test, whereas categorical variables were analyzed performing Chi-square test.

Survival probabilities were evaluated with Kaplan-Meyer method and compared with the log-rank test. Multivariate analysis of factors associated with survival with Cox regression. Statistical significance was defined as $p<0.05$.

III. Results

1. Histopathological findings

Sixty-five patients (59%) underwent R0 resection and a positive surgical margin was achieved in 42 patients (38%).

The majority of the patients (57%) had pathologic response (TRG1-4) but only four patients (4%) had complete replacement with fibrosis (TRG1).

The tumor thickness at the tumor-normal interface was greater than or equal to 0.5 millimeters in 68 patients (62%).

Regarding the growth pattern, the most frequent was the pushing pattern (30%), followed by desmoplastic pattern (21%) with the replacement pattern as the least prevalent (18%) (Figure 5).

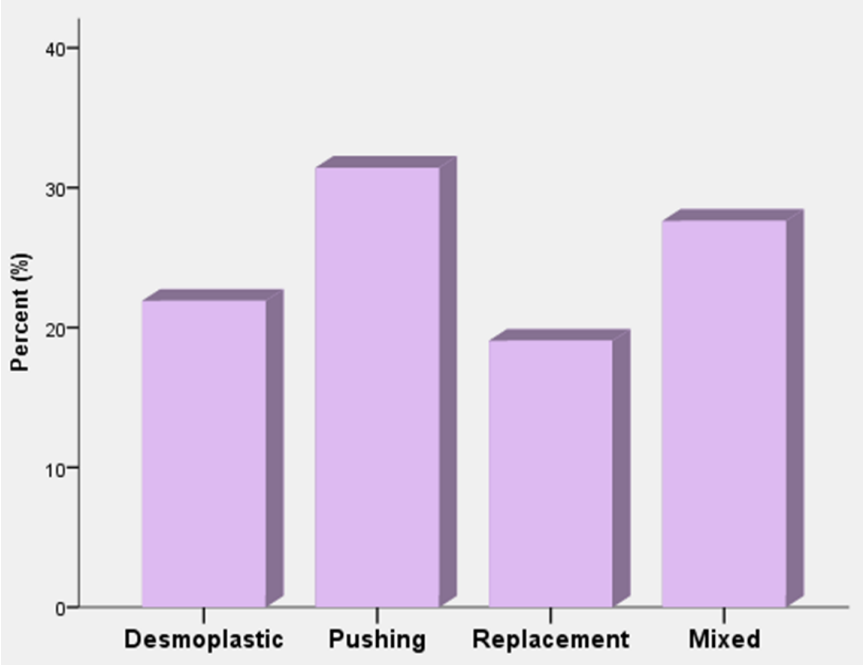

Figure 5. Colorectal liver metastases growth pattern in study population.

3. Impact of neoadjuvant chemotherapy on histopathological findings

The percentage of liver metastases showing pathologic response was significantly higher in patients who received preoperative chemotherapy versus patients undergoing upfront resection (HR 1.667, 95% CI 1.043-2.663, p=0.022). No other differences on histological assessment were found between these two groups.

Pushing growth pattern was predominant in patients treated with FOLFOX-based therapy (HR 2.438, 95% CI 1.087-5.470, p=0.031).

4. Overall, disease-free and liver recurrence-free survival

After a median follow-up period of 31.7 months (range 0-72 months), 74 patients (67%) developed recurrent disease while the remaining 36 patients (33%) were recurrence-free.

The median survival following CRLM resection was 52 months, the 3 and 5-year overall survival was 68.5% and 47.5%, respectively, while 3 and 5-year disease-free survival was 29.9 and 21.7, respectively (Figure 6). Forty patients (36%) died of disease, 29 of them (73%) due to progression of liver metastases and 9 patients (23%) due to distant recurrence. Of the 48 patients (44%) who were alive at the time of the last follow-up, 27 (56%) developed recurrence and 21 (44%) had no evidence of disease.

Liver recurrence was reported in 48 patients (44%) versus other locations in 24 patients (22%). Among these patients, 23 (32%) had an early diagnosis whereas the majority (68%) was diagnosed six months after the liver resection.

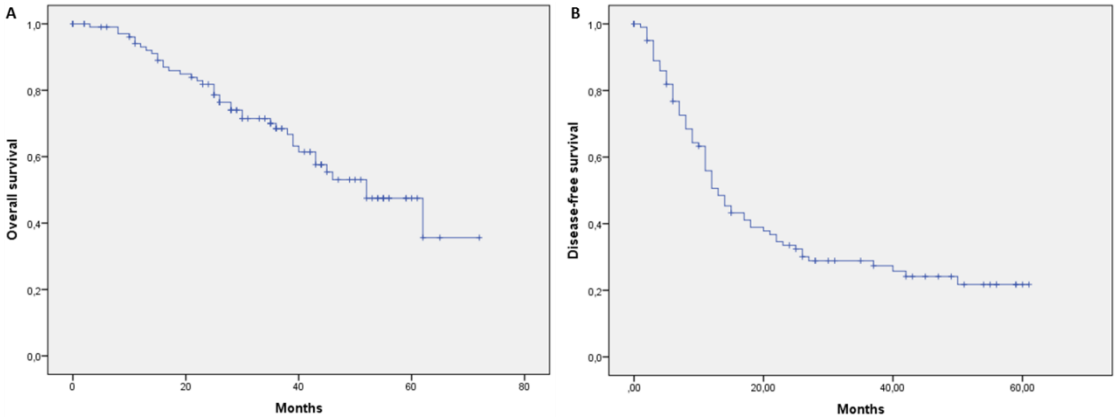

Figure 6. Kaplan-Meier curves of overall survival (A) and disease-free survival (B) of study population.

5. <u>Neoadjuvant chemotherapy as predictor of overall and disease-free survival</u>

Univariate analysis did not identify preoperative chemotherapy treatment as predictor of worse OS (p=0.395), but disease-free survival was significantly lower in the NAC group (9 vs. 23, p<0.001) (Figure 7). This difference between the groups was not significant for LRFS (p=0.179).

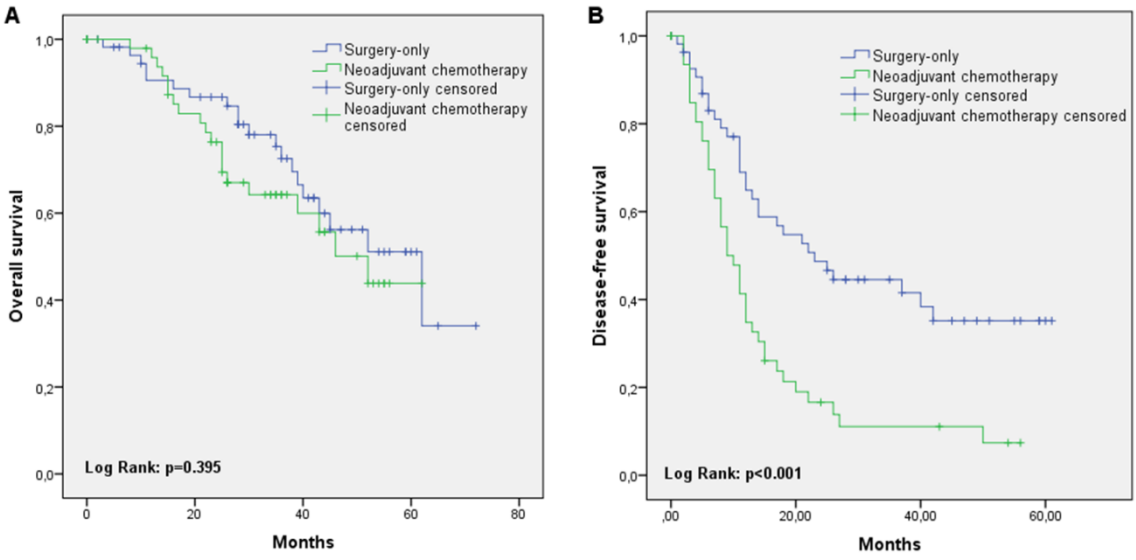

Figure 7. Kaplan-Meier curves of overall survival (A) and disease-free survival (B) in patients treated with neoadjuvant chemotherapy vs. patients underwent surgery-only.

6. Clinical predictors of overall, disease-free and liver recurrence-free survival

On univariate analysis, five parameters were significantly correlated with worse OS: patients aged 70 years and older (HR 5.520, p=0.019), lymph node positivity of the primary tumor (HR 5.746, p=0.017), multiple liver metastases (HR 7.365, p=0.007), largest metastasis greater than or equal to three centimeters (HR 4.578, p=0.032) and major morbidity (HR 4.516, p=0.034).

Disease-free survival was negatively affected by synchronous presentation of CRLM (HR 9.982, p=0.002), multiple liver metastases (HR 17.049, p<0.001), bilobar lesions (HR 8.082, p=0.004), major liver resection (HR 4.294, p=0.038) and major morbidity (HR 4.170, p=0.041).

Liver recurrence was significantly higher in patients with synchronous diagnosis (HR 5.850, p=0.016,) and with bilobar distribution (HR 6.041, p=0.014).

These results are summarized in Tables 4 and 5.

7. Histopathological findings as predictors of overall, disease-free and liver recurrence-free survival

7.1. Non-tumoral liver parenchyma findings

Steatosis was present in 38 patients (35%), 13 of them (34%) with moderate or severe grade. Fifty-five patients presented SOS with moderate to severe lesions in 11 cases (20%). Neither steatosis nor SOS-related lesions were significant predictors of OS, DFS or LRFS.

7.2. Tumor-free resection margin

Overall survival was not significantly decreased in patients with a positive resection margin (HR 2.093, p=0.148). Non-R0 group had a significant reduction in disease-free survival (HR 4.774, p=0.029). However, there was no statistical difference in terms of liver recurrence vs. distant recurrence between the two groups (p=0.584).

7.3. Pathological tumor regression

There was no statistically significant association of tumor response (TRG1-4) with OS (p=0.144), DFS (p=0.488) or LRFS (p=0.158).

7.4. Tumor thickness at the tumor-normal interface

The present study did not show impact of TTNI ≥0.5 millimeters in OS (p=0.463) or LRFS (p=0.375) rates. Disease-free survival was lower in patients with TTNI ≥0.5 millimeters but this difference did not reach statistical significance (p=0.086) (Figure 8).

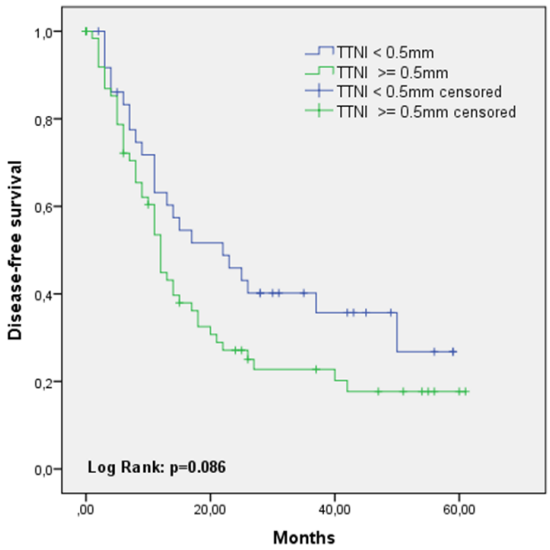

Figure 8. Kaplan-Meier disease-free survival curves between CRLM with TTNI < 0.5 mm vs. TTNI ≥0.5 mm.

7.5. Histologic Growth Pattern

In our study there was a significant impact of GP on overall survival, with pushing GP associated with worse OS when compared to other GP's (HR 6.029, p=0.014) (Figure 9).

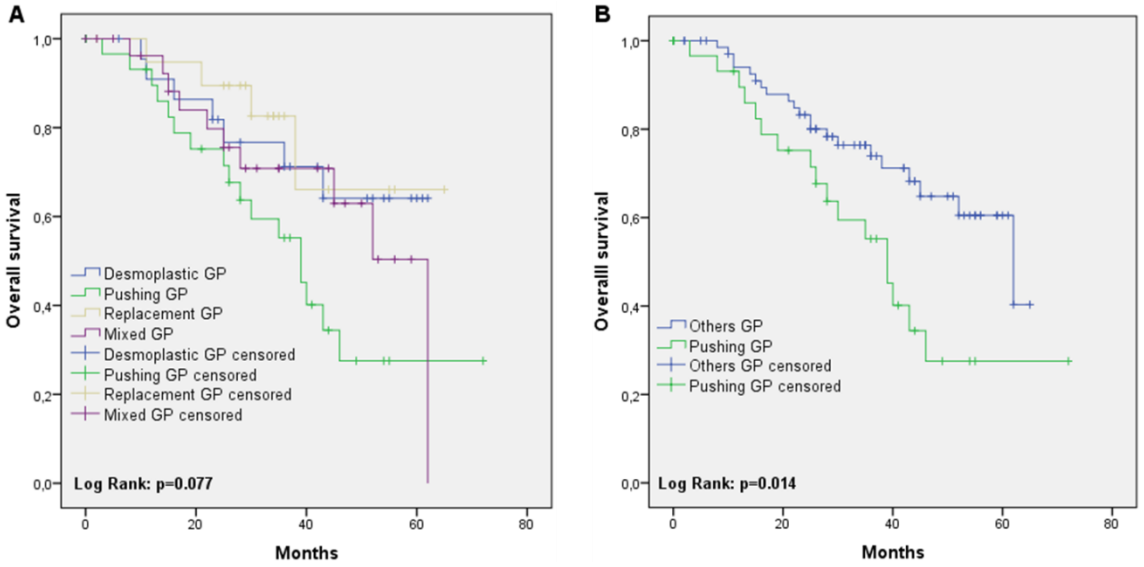

Figure 9. Kaplan-Meier overall survival curves (A) Between the four CRLM growth patterns and (B) Between pushing pattern and the others.

There was also a statistically significant effect of pushing GP on DFS (HR 8.274, p=0.004). Furthermore, desmoplastic GP was associated with a better DFS (HR 0.16, p=0.012) versus other patterns but this difference was not statistically significant for OS (p=0.280). (Figure 10).

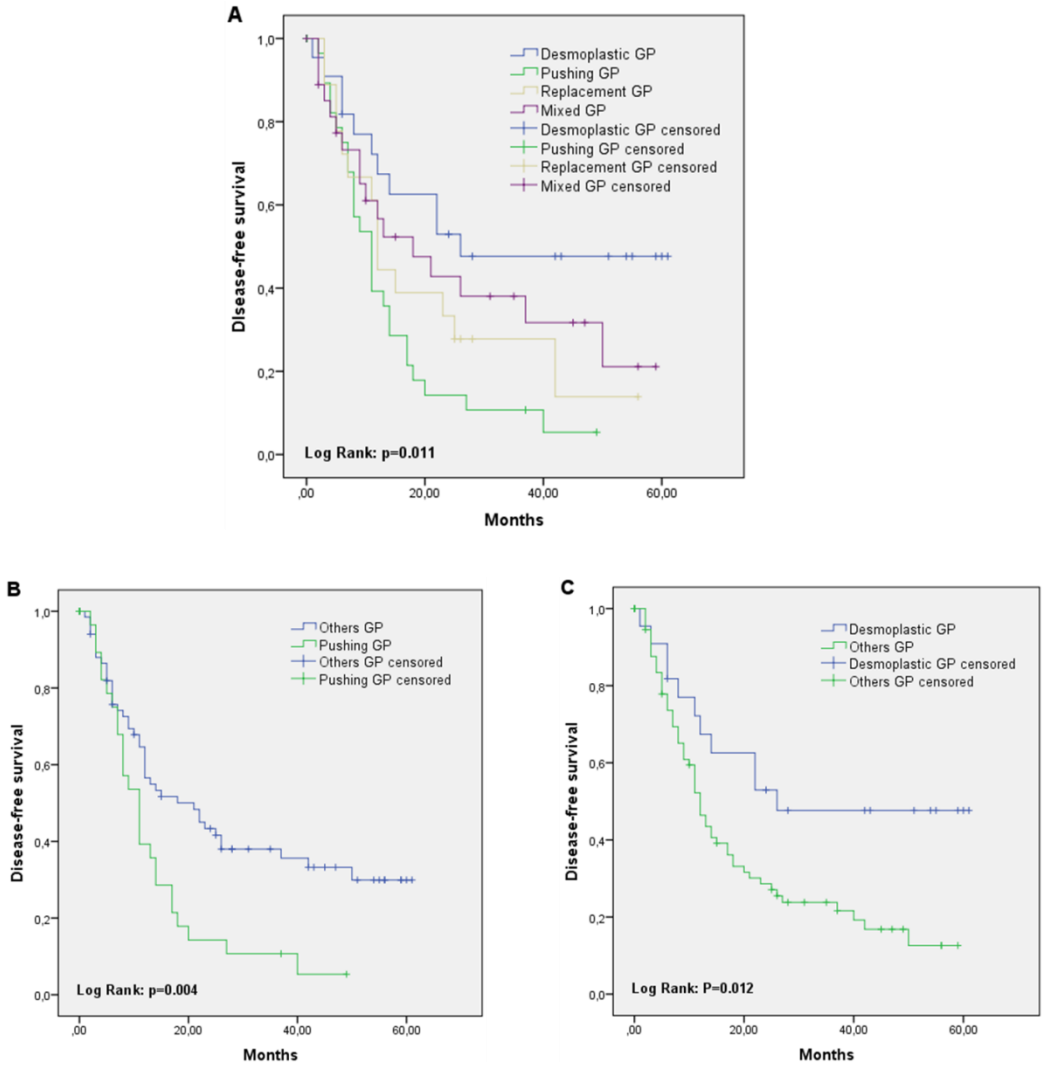

Figure 10. Kaplan-Meier disease-free survival curves (A) Between the four CRLM growth patterns (B) Between pushing patterns and the others (C) Between desmoplastic growth pattern and the others.

Table 4. Clinical and histopathologic parameters as predictors of overall survival (univariate analysis).

Parameters	**No. of patients (%)**	**Overall survival**	
		HR	**P**
Age (years)			
≥ 70	**77 (70)**	**5.520**	**0.019**
< 70	33 (30)		
Lymph node status			
Positive	**73 (66.4)**	**5.746**	**0.017**
Negative	34 (30.9)		
Number of metastases			
Multiple	**63 (57.3)**	**7.365**	**0.007**
Single	47 (42.7)		

Largest diameter (cm)			
≥ 3	**62 (56.4)**	**4.578**	**0.032**
<3	45 (40.9)		
Postoperative course			
Major morbidity	**9 (8.2)**	**4.516**	**0.034**
No major morbidity	97 (88.2)		
Pushing GP			
Yes	**33 (30)**	**6.029**	**0.014**
No	72 (65.5)		

Table 5. Clinical and histopathologic parameters as predictors of disease-free and liver recurrence-free survival (univariate analysis).

Parameters	**No. of patients (%)**	**Disease-free survival**		**Liver recurrence-free survival**	
		HR	**P**	**HR**	**P**
CRLM presentation					
Synchronous	**57 (51.8)**	**9.982**	**0.002**	**5.850**	**0.016**
Metachronous	53 (48.2)				
Number of metastases					
Multiple	**63 (57.3)**	**17.049**	**< 0.001**	-	-
Single	47 (42.7)				
CRLM distribution					
Bilobar	**41 (37.3)**	**8.082**	**0.004**	**6.041**	**0.014**
Unilobar	69 (62.7)				
Neodjuvant chemotherapy					
Yes	**52 (47.3)**	**13.767**	**< 0.001**	-	-
No	58 (52.7)				
Hepatectomy					
Major	**40 (36.4)**	**4.294**	**0.038**	-	-
Minor	70 (63.6)				
Postoperative course					
Major morbidity	**9 (8.2)**	**4.170**	**0.041**	-	-
No major morbidity	97 (88.2)				
Resection margin					
Positive	**65 (59.1)**	**4.774**	**0.029**	-	-
Negative	42 (38.2)				
Pushing GP					
Yes	**33 (30)**	**8.274**	**0.004**	-	-
No	72 (65.5)				
Desmoplastic GP					
No	**23 (20.9)**	**0.16**	**0.012**	-	-
Yes	82 (74.5)				

8. Independent predictors of overall, disease-free and liver recurrence-free survival

On multivariate analysis lymph node positivity of the primary tumor had significant influence on OS (p=0.037).

No-R0 resection margin were independently associated with decreased DFS (p=0.018).

Pushing pattern had significant impact on both OS and DFS (p=0.007 and p=0.004, respectively).

Liver recurrence-free survival was strongly decreased by synchronous presentation of CRLM (p=0.044) and by the absence of histologic tumor response (TRG5) (p=0.018) (Tables 6 and 7).

Table 6. Independent predictors of overall survival (multivariate analysis).

	Overall survival		
	HR	**95% CI**	**P**
Positive lymph node status	**2.590**	**1.061-6.324**	**0.037**
Pushing growth pattern	**2.850**	**1.328-6.117**	**0.007**

Table 7. Independent predictors of disease-free and liver recurrence-free survival (multivariate analysis).

	Disease-free survival			**Liver recurrence-free survival**		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**
Synchronous presentation	-	-	-	**2.119**	**1.021-4.399**	**0.044**
Positive resection margin	**1.908**	**1.119-3.254**	**0.018**	-	-	-
Pushing growth pattern	**2.344**	**1.323-4.155**	**0.004**	-	-	-
Absence of tumor response (TRG5)	-	-	-	**2.546**	**1.174-5.520**	**0.018**

IV. Discussion

Metastatic spread to the liver of colorectal cancer is still a challenging disease. Significant host and tumor factors are at play, and need to be taken into account for individualized multidisciplinary management. The design of this study aimed to identify pathological prognostic factors for these patients and to discuss its value on treatment decisions.

Recent studies proved the importance of chemotherapy as neoadjuvant approach.(19, 20) In our study NAC was provided to patients with more advanced disease compared to the group who received an up-front surgery strategy. Despite these unquestionable differences between the two groups, the administration of NAC was not related with a significant decreased overall survival and the adverse impact on recurrence-free survival was not consistent after a multivariate analysis. We question whether NAC should have been used more liberally in our population, in particular in patients with metachronous disease. These results validate the benefits of NAC therapy and suggest the importance of expanded eligibility criteria for its use in patients with hepatic disease from colorectal cancer.

At the same time, the role of patient characteristics, primary tumor features, operative data and pathologic findings on patients' survival have been brought into focus by several studies. (6, 21, 22)

Based on our analysis, patients aged 70 years or older, positive lymph-node status, multiple metastases and metastatic nodules larger than or equal to three centimeters led to a poorer OS. In addition, synchronous presentation, multiple metastases and bilobar distribution of CRLMs were identified as predictors of recurrence, as previous studies demonstrated. (21, 23)

These results may provide powerful prognostic tools in order to support a multidisciplinary and more personalized approach to these patients. However, these clinical variable-based risk

scores are flawed, and might contribute to denial of resection to patients with many risk factors but favorable tumor biology.(24, 25)

There was a strong correlation between major morbidity and decreased survival outcomes. This finding was previously validated (5) and the impact of immunosuppressive environment caused by inflammatory processes, as responsible for micrometastases progression, as well as tissue hypoxia enhancing tumor angiogenesis have been put forward to explain these facts.(26, 27)

The negative influence of grossly positive surgical margins after CRLM resection is widely accepted, however, the minimal width margin recommended is still under debate.(8-10, 28, 29) Our analysis showed that a positive margin, defined as less than one millimeter from the tumor cells, increases the risk of recurrence, however, without a negative effect on overall survival.

Patients who received preoperative chemotherapy had a significant improvement on histologic tumor response in accordance with Rubbia-Brandt et al.(12) The importance of TRG as a prognostic factor of patient outcome has been recognized in numerous studies but with inconstant categorization criteria.(12, 30, 31) The present study stratified the tumor response as present or absent. On multivariate analysis our results clearly demonstrated that patients without any pathologic tumor response (TRG5) have a decreased liver recurrence-free survival.

Dipen Maru et al (11) recognized in 2010 a new pathologic predictor of disease-free survival: the tumor cells thickness at the tumor-normal interface. In our study the statistical power was

not enough to validate these facts (p=0.086), although we cannot ignore the TTNI as a promising pathologic predictor.

Previous studies have been successful to demonstrate the prognostic value of histological growth patterns of CRLM.(18, 32-34)

Interestingly, our study not only clearly identified the growth pattern with the worst prognosis (the pushing pattern) but it also highlighted the pattern with the best survival results (the desmoplastic pattern). The negative effect of the pushing type of growth was confirmed by multivariate analysis.

Patients with desmoplastic pattern in the largest metastasis had a significant reduced risk of recurrence in comparison to other GPs (p=0.012). This prognostic value was previously reported (32) and is compatible with the thick band of stroma rich in collagen present in the desmoplastic growth pattern which may represent a barrier to tumoral expansion and thus a better prognosis.(13)

This analysis also established the pushing growth pattern on the tumor-liver parenchyma interface as an independent predictor of OS and DFS (p=0.007 and p=0.004, respectively). The pushing pattern has special biologic properties concerning vascularization namely higher levels of endothelial cell proliferation fraction (ECP) (34, 35), evidence of capillarization, upregulation of basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) (36), comprising an angiogenic profile that resembles primary CRC.(37) Also the hypoxic environment of CRC metastasis, detected by higher levels of hypoxia-inducible factor (HIF) (37), will increase VEGF in a synergic manner.(38)

The growth pattern of liver metastases can potentially reflect these complex biologic phenomenona taking place in the tumor microenvironment. As investigation proceeds into these processes, new therapies can translate into the clinical arena. In the meanwhile, the

clinical utility of these findings could be further enhanced if the different GP's could be correlated with specific radiologic patterns on preoperative imaging.(39) Further investigation into this field is mandatory.

V. Conclusion

Multimodal therapy consisting of liver resection and chemotherapy is the cornerstone of colorectal cancer liver metastases management.

Nonetheless, there are biological factors associated with the tumor and the tumor-host interactions, as well as with the tumor response to chemotherapy, which are expressed by different histopathological findings with a promising prognostic value. In this study the pushing growth pattern was an independent predictor of worse overall and disease-free survival.

Further investigation is required to achieve a reliable understanding of these complex biologic mechanisms and to realize how this knowledge can lead to the development of new therapeutic strategies.

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