Two-stage hepatectomy for colorectal liver metastases: Pathologic response to preoperative chemotherapy is associated with second-stage completion and longer survival

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Running head: Pathologic response and longer survival

ABSTRACT

Background

Two-stage hepatectomy (TSH) of bilobar colorectal liver metastases (CRLM) is widely used and shows encouraging survival results. However, the risk of drop-out after the first-stage remains high and associated with poor survival. The objective of our study was to evaluate the factors associated with long-term survival based on the pathologic response to preoperative systemic chemotherapy in CRLM patients who underwent TSH.

Methods

The pathologic response to preoperative chemotherapy and its effect on second-stage completion and survival were retrospectively evaluated in 67 patients treated between 2003 and 2013.

Results

Fifty-six patients underwent TSH for initially non-resectable CRLM. Chemotherapy was combined with a biotherapy in 32 cases. The Tumor Regression Grade (TRG), modified-TRG and Blazer grade were used to classify patients as responders (TRG and mTRG 1-3, Blazer 0-1) or non-responders (TRG and mTRG 4-5, Blazer 2) after the first stage. Tumor response in the three classifications was associated with second-stage completion (TRG 1-3: OR=4.01 95% CI: 1.12-14.36 p=0.033; mTRG 1-3: OR=3.8 95% CI:1.13-12.6 p=0.03; Blazer 0-1: OR=5.45 95% CI: 1.66-17.85 p=0.005). Triple chemotherapy was also associated with responders. The median overall survival of responders was significantly higher (Blazer 0-1: 42.9 months *versus* Blazer 2: 20.1 months p=0.018; TRG 1-3: 42.9 months *versus* TRG 4-5: 25.1 months, p=0.04).

Conclusion

A pathologic response to chemotherapy is associated with second-stage completion and

longer survival. Furthers studies are needed to try to early identify the patients for whom the benefit of the second surgical stage is less straightforward.

Keywords: colorectal cancer, liver metastases, two-stage resection, pathologic response, survival

INTRODUCTION

Liver metastases occur in approximately 50% of patients affected by colorectal cancer and are the most common cause of death in this population[1,2]. Recent advances in multidisciplinary approaches have changed the prognosis of patients with colorectal liver metastases (CRLM)[3,4] and technical improvements such as the two-stage hepatectomy (TSH) now allow a higher number of patients with CRLM to be eligible for surgery[5,6].

The TSH strategy is widely used since the 2000s[6] for patients with initially unresectable metastases, becoming resectable after neoadjuvant chemotherapy. This potentially-curative strategy is associated with longer overall survival for patients who completed the two surgical stages. However, the risk of drop-out before the second stage is high, mainly due to disease progression[7]. Patients who drop-out before the second surgical stage have poorer survival rates, close to those obtained in unresectable patients, and surgery may thus be inappropriate[7,8]. Studies have suggested that increased exposure to chemotherapy prior to the first hepatectomy may predict failure to complete the second surgical stage[9] and that the pathologic response to intensive preoperative chemotherapy is a favourable prognostic factor[10]. However, the prognostic impact of the pathologic response to systemic chemotherapy is probably still underestimated. We thus conducted a retrospective study to demonstrate a possible correlation between the pathologic response to preoperative chemotherapy and survival in CRLM patients who underwent TSH. The objective of our study was to evaluate the factors associated with the completion of the second surgical stage, and, consequently, of progression-free and overall survival, based on the pathologic response to systemic chemotherapy administered preoperatively.

PATIENTS AND METHODS

Patients

From January 2003 to January 2013, 899 patients were treated for CRLM in our institution. Among these, patients with multiple, bilobar CRLM initially considered unresectable with a one-stage procedure, and who underwent at least the first stage of a planned TSH, were retrospectively selected for analysis on an intent-to-treat basis. Initial unresectability was defined as the inability to resect all metastases with tumor-free margins while saving a sufficient remnant liver volume to prevent postoperative liver failure. A sufficient remnant liver volume was defined as 30% of the residual liver parenchyma on CT-scan[11] or by estimation of the future remnant liver function with ^{99m}Tc-mebrofenin 3D SPECT-CT. Patients with a ^{99m}Tc-mebrofenin clearance rate <2.69%/min/m^2 were considered at high risk of postoperative liver failure[12].

Patients with metachronous metastases diagnosed at least three months after primary tumor diagnosis were also included. Patients with unplanned repeated hepatic resection for recurrence, who underwent a one-stage resection, or who presented with an unresectable extrahepatic abdominal disease were excluded from the study. A limited lung metastatic involvement was not considered a contraindication for hepatic resection. The protocol was approved by the local ethics committee review board. The study was conducted according to the Declaration of Helsinki and European Good Clinical Practice requirements. Patients were informed that their clinical and scientific data could be used for scientific purpose prior to their care in our institution.

Preoperative chemotherapy and therapeutic strategy

All patients were treated with intensive chemotherapy before liver resection, with oxaliplatin, irinotecan, 5-fluorouracil (5-FU), leucovorin and capecitabine (5-FU oral form) as doublet or triplet regimens. From 2008, a biotherapy (bevacizumab, cetuximab or panitumumab)[13–15]

was frequently added, depending on the *RAS* status. The Response Evaluation Criteria In Solid Tumor (RECIST) evaluation was used to select patients responding to preoperative chemotherapy[16].

Surgery

During the first operative stage, the future remnant liver was cleared from tumor tissue using wedge resection and/or anatomical resection and/or radiofrequency ablation. Resection of the primary colorectal tumor was performed concomitantly if the surgical liver procedure was not too important and in case the primary tumor was symptomatic. To prevent tumor progression, interval chemotherapy was for most patients administered between the two surgical stages. A portal vein ligation or embolization was performed to prevent postoperative liver failure according to volumetric CT-scan and mebrofenin hepatoscintigraphy data. Resection was mostly a right hepatectomy to achieve a curative resection. Postoperative morbidity at 30 days was graded according to the Clavien-Dindo classification[17]. Postoperative mortality was defined as death occurring within 90 days following surgery.

Pathologic response

All tumor metastases were sampled for pathology examination. Three-µm thick sections of formalin-fixed paraffin-embedded tissues were mounted on SuperFrost Plus glass slides (Menzel, GmbH, Germany) deparaffinized before Hematein-Eosin-Saffron (HES) staining.

Three pathologists with hepatobiliary expertise and blinded to the clinical data evaluated the response in all resected metastases, using the Tumor Regression Grade (TRG), the modified TRG and the Blazer classification[10,18,19]. Challenging evaluation cases were reviewed jointly for final consensus. Patients were classified as pathologic responders (TRG and mTRG 1-3, Blazer 0-1) or non-responders (TRG and mTRG 4-5, Blazer 2) after the first stage. The mean

percentage of residual tumor cells and regression features in the different metastases were used to define the pathologic response. Differentiation, tumour size, number of metastases and resection margins were also reported for each patient.

Statistical analyses

Patients' characteristics were compared using the $\chi 2$ or the Fisher's exact tests for discrete variables. The Student's t-test and Wilcoxon rank-sum test were used for continuous variables. Factors associated with second-stage completion were analysed using univariate and multivariate logistic regression models. Factors significant at the $p<0.05$ level in the univariate analysis and the appropriate pathologic response variable were included in the classical multivariate model, validated using a stepwise automatic method. Survival rates were estimated from the first surgery until the event of interest, death of any cause for OS and recurrence for RFS, using the Kaplan-Meier method, and compared using a log-rank test. Median survival was presented with its 95% confidence interval (95% CI). Patients lost to follow-up were censored at the last documented visit, and those who died without recurrence at the date of death. All statistical analyses were performed using the STATA 11.0 software (StataCorp, College Station, Texas).

RESULTS

Patients' characteristics

TSH strategy was planned for 67 patients, among whom 11 were excluded because of the lack of pathological sample: 8 because they only had radiofrequency and 3 because the samples were not found (Figure 1 and Table 1). The median age was 59 years (range: 38-77) and there were 25 (44.6%) women. The primary tumor site was colon in 80.4% cases. All CRLM were bilobar at diagnosis, and 90.9% were synchronous. The median time from initial diagnosis

and occurrence of metachronous metastases was 18.3 months (95% CI: 11.8-28.7). *RAS* was mutated in 30.6% patients and 25.5% had a carcinoembryogenic antigen (CEA) level >200ng/mL.

Preoperative chemotherapy

All patients received downstaging chemotherapy. The median number of cycles was 8 (4-25). A doublet regimen, FOLFIRI (5FU+irinotecan) or FOLFOX (5FU+oxaliplatine) was administered to 60.7% patients and FOLFIRINOX (5FU+oxaliplatine+irinotecan) to 33.9% patients. A biotherapy was associated with chemotherapy for 55.3% patients.

First-stage hepatectomy

The median hospital stay was 11 days (6-162) (Table 2). A median of 3 (1-5) radiofrequency ablations and 1 (1-6) non-anatomical resection were performed. The median size of the largest metastasis resected was 18mm (3-60). The primary colorectal tumor was resected during this first surgery in 22 patients (39.3%). Postoperative grade ≥3 morbidity at 30 days was reported in 8 (14.3%) patients (Table 2), mainly hepatic abscesses (n=3) and complications linked to the primary colorectal tumor (n=3). There was no postoperative mortality at 90 days.

Second-stage hepatectomy

The TSH was completed for 35 (62.5%) patients. Twenty-one patients dropped-out after the first surgical stage. The main reason was disease progression (n=16), including hepatic (n=14) or extra-hepatic (n=1) progression, or both (n=13). Two patients experienced severe complications after the first surgery (one rectal fistula and one abdominal wall complication) which prevented undergoing the second stage; one patient refused the second surgery, and 3 were lost to follow-up.

The median time between the two surgical stages was 6 months (2-24), mainly due to our deliberate policy to administer systemic chemotherapy between the two stages. Interval chemotherapy was administered to 50 (89.3%) patients, with a median of 6 cycles (4-20) (Table 1). Six patients (11%) did not receive interval chemotherapy, mostly patients who had undergone radiofrequency ablations with non-anatomic resections. A portal vein embolization was reported in 17.6% patients. The median hospital stay was 11 days (5-49). A standard right hepatectomy was performed in 31.4% patients, and 23.4% underwent a right lobectomy (Table 2). Postoperative grade ≥3 morbidity was reported in 6 (17.1%) patients mainly hepatic abscesses (n=3) and complications linked to the primary colorectal tumor (n=2). Two patients died of disease progression within 90 days following the second stage. There was no postoperative mortality reported.

Pathologic response

A pathologic response (TRG, mTRG 1-3, and Blazer 1-0) was observed in 21 (37.5%), 24 (42.9%), and 30 (53.6%) patients, respectively (Table 3). Patients who completed the two stages were significantly better responders than those who dropped-out, according to the three classifications: 48.6% *vs* 19.0%, 54.3% *vs* 23.8% and 68.6% *vs* 28.6% responders *vs* non-responders for the TRG, mTRG and Blazer, respectively.

The pathologic response was associated with second-stage completion according to the three classifications: TRG 1-3: OR=4.01; 95% CI: 1.12-14.36; p=0.033; mTRG 1-3: OR=3.8; 95% CI: 1.13-12.6; p=0.03; Blazer 0-1: OR=5.45; 95% CI: 1.66-17.85; p=0.005 in univariate analysis. In multivariate analysis, among the three classifications, only the Blazer grade was associated with second stage completion (Blazer 0/1: OR=5.42; 95% CI: 1.53-19.15; p=0.006), together with the initial number of metastases ≤5 (OR=4.06, 95% CI: 1.14-14.47, p=0.009) (Table 4).

The neoadjuvant FOLFIRINOX regimen was associated with a pathologic response according to the three classifications (TRG 1-3:73.7% *vs* TRG 4-5: 26.3%; $p<0.001$; mTRG 1-3: 73.7% *vs* mTRG 4-5: 26.3%; $p<0.0001$; Blazer 0-1: 84.2% *vs* Blazer 2: 15.8%; $p=0.002$) (Table 5).

Survival

After a median follow-up of 66 months, the median overall survival (OS) of the whole population was 39 months (95% CI: 25.1-42.9), with 3-year and 5-year OS rates of 46.7% (95% CI: 32.6-59.6) and 17.1% (95% CI: 7.1-30.8). In patients who completed TSH, the median OS was significantly higher than that of patients who did not: 50 months (95% CI: 36.2-59.3) *versus* 18.4 months (95% CI: 12.0-22.2) respectively, with a 3-year OS rate of 71% (95% CI: 51.4-83.8) *versus* 9.5% (95% CI: 1.6-26.1), hazard ratio (HR)=0.17 (95% CI: 0.08-0.35, $p<0.001$) (Figure 2). The median relapse-free survival (RFS) of patients who completed the two stages (n=43) was 16.9 months (95% CI: 14.5-21) (Figure 2). The median OS was significantly higher in responders according to the TRG and Blazer classifications: Blazer 0-1: 42.9 months *versus* Blazer 2: 20.1 months, HR= 2.14 (95% CI: 1.13-4.04), $p=0.018$, and TRG 1-3: 42.9 months *versus* TRG 4-5: 25.1 months, HR=2.08 (95% CI: 1.03-4.19), $p=0.04$ (Figure 3).

DISCUSSION

The present study is, to our knowledge, the first to demonstrate a direct correlation between a favorable pathologic response to chemotherapy and second-stage completion, and thus longer survival. In unresectable CRLM patients, previous studies have shown that a major pathologic response after preoperative treatment was a favorable prognostic factor[10,18,19]. Yet, its importance is not fully taken into account when taking therapeutic decisions. Among the three classifications, the TRG[18] considers fibrosis as a characteristic feature of cellular response,

whereas the mTRG[19] also takes into account the infarct-like necrosis (ILN); the Blazer score[10] is exclusively based on the percentage of residual tumor cells relative to the total tumor area, whatever the type of regression, and may thus be more efficient to evaluate the impact of intensive induction treatments. Probably because of that reason, the pathologic response was higher assessed with the Blazer score than with both the TRG and mTRG in our series, and it was the only classification associated with second-stage completion in multivariate analysis. Our study also allowed identification of patients treated with FOLFIRINOX as better responders than those treated with the standard regimen. Correlatively, Ychou *et al.* showed that this triplet systemic chemotherapy was associated with a better response, higher resectability and OS rates[20].

Very few series have evaluated the impact of the pathologic response in the setting of TSH strategies. Faitot *et al.* showed that the pathologic response was associated with clinical outcome but not with TSH feasibility, contrary to our study[21]. Another study, Mentha *et al.*, showed in 22 CRLM patients treated with neoadjuvant therapy and TSH that a "dangerous halo" in the pathologic findings was a bad prognosis factor but didn't correlate the intensity of the pathologic response to survival[22]. Recently, Pietrantonio *et al.* showed that bevacizumab induced significant better pathological response rates and complete responses compared with cetuximab even if OS was not significantly different[23]. Moreover, a significant correlation with pathological response was found between the number of resected metastases and bevacizumab allocation.

Numerous studies have suggested that regenerative growth factor levels increased immediately after the first-stage hepatectomy and contributed to tumor recurrence[24–26]. In this context, administering interval chemotherapy allowed a longer period of natural liver regeneration while preventing tumor progression. It thus was our deliberate policy to administer interval systemic chemotherapy, although its benefits are still controversial.

Tanaka *et al.*[27] showed decreased tumour growth and growth factor expression with perioperative chemotherapy, while Muratore *et al.*[28] showed no benefit of interval chemotherapy on disease progression between the two surgical stages.

The main drawback of the TSH strategy is that more than 30% patients drop-out after the first-stage procedure (36% patients in our study)[7,8]. They have a poor prognosis, with survival no longer than that of patients undergoing chemotherapy alone[7]. The median OS of patients who only underwent the first stage was of 18.4 months (95% CI: 12-22.2) compared to almost 50 months for those who completed the second stage; 3-year OS rates were 9.5%, and 71%, respectively. Completion of the whole procedure allows a similar benefit than that of the single stage reported in patients with initially resectable liver metastases[29]. It was shown that the main cause of drop-out is disease progression between the two stages[7]. It is thus essential to identify patients who will not reach the second surgery[30]. Several studies have identified predictive factors of drop-out[8,9,30–32], among which major postoperative complications after the first stage[31], older age, CEA level >200ng/ml before portal vein embolization, 3 or more tumours in the future remnant liver[8], high number of metastases, and increased exposure to chemotherapy prior to the first hepatectomy[8]. Mise *et al.* showed that a *RAS* mutation should be taken into account to predict response to chemotherapy[33]. In this study, a pathologic response was more common in patients with wild-type *RAS* compared with patients with *RAS* mutations. Passot *et al.* showed that the *RAS* mutation independently predicts the oncologic efficacy of TSH[34].

A pathologic response was shown to be a strong prognostic factor after preoperative chemotherapy and surgical resection of CRLM[35,36] and, in another field, of colorectal distant metastasis such as peritoneal carcinomatosis[37]. However, its use for treatment decision remains limited. Our study shows some limitations as it was retrospective and monocentric,

and with a relatively limited number of patients. However, it is the first to show a significant correlation between a pathologic response to chemotherapy and second stage completion and consecutively, a better survival and, considering the design limitations, proofs of these correlations seem quite robust. We hope it to be a first step towards further investigations correlating histologic and radiologic findings. Among these, identifying non-responders patients before the first surgical step, which is unfortunately not yet possible, would allow sparing some patients from a not necessarily-needed surgery.

CONCLUSION

A pathologic response to preoperative chemotherapy after the first-stage hepatectomy is associated with completion of the second surgical stage and of longer survival. The accurate assessment of the pathologic response to induction treatments represents an important contribution from pathologists for treatment decision for these patients, and may allow avoiding resections for patients for whom the TSH strategy benefit seems less straightforward.

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Authors' contributions

Study design: FQ, FB.

Collection of data: FQ, MHP, HG, LK, SC, OS, PR, ED, MY, FB.

Analysis and interpretation of data: LR, FQ, FB, MHP.

Drafting of the manuscript: FQ, MHP, HF, FB

Revising critically the manuscript: FQ, MHP, FB, HF, OS, PR, SC, LR

Final validation of the manuscript: FQ, MHP, HG, LR, SC, OS, PR, HF, LK, ED, MY, FB.

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Figure legends

Figure 1

Flowchart of the study

Figure 2

A: Overall survival in patients who completed or did not complete the two-stage resection; B: Relapse-free survival of patients who completed the second stage.

Figure 3

Overall survival depending on the pathological response, according to the TRG (A), mTRG (B) and Blazer (C) classifications. A: TRG classification: responders (TRG 1,2,3) *versus* non-responders; B : mTRG classification: responders (mTRG 1,2,3) *versus* non-responders (4,5) ; C : Blazer classification: responders (Blazer 0,1) *versus* non-responders (Blazer 2).

Tables

Table 1: Patients' characteristics

Table 2: Surgical characteristics of the first and second stages

Table 3: Patients' and tumor characteristics, and histologic response in the "first-stage only" and the "two-stages completed" groups

Table 4: Factors associated with second-stage completion (uni- and multivariate analyses)

Table 5: Correlation between the neoadjuvant FOLFIRINOX regimen and the pathologic response

Table 1: Patients' characteristics

Patients' characteristics		**n=56**
Age (years)	*med (range)*	59 (38-77)
Sex, n (%)	*n (%)*	
Male		31 (55.4)
Female		25 (44.6)
Site of primary tumor	*n (%)*	
Colon		45 (80.4)
Rectal		11 (19.6)
N+ stage	*n (%)*	42 (75.0)
Metastases,	*n (%)*	
Synchronous		50 (90.9)
Metachronous		5 (9.1)
Missing		*1*
Initial number of metastases	*n (%)*	
0-5		28 (50.9)
5-10		7 (12.7)
>10		20 (36.4)
Missing		*1*
Neoadjuvant chemotherapy regimens	*n (%)*	56 (100)
Doublet therapy (Folfox or Folfiri)		34 (60.7)
Triplet therapy (Folfirinox)		19 (33.9)
Biotherapy		31 (55.3)
Bevacizumab		14 (25)
Cetuximab		16 (28.6)
Panitumumab		1 (1.5)
Missing		*3*
Number of cycles,	*med (range)*	8 (4-25)
Missing		*5*
Interval chemotherapy regimens	*n (%)*	50 (89.3)
Doublet therapy (Folfox or folfiri)		42 (75)
Triplet therapy (Folfirinox)		6 (10.7)
Biotherapy		37 (66.1)
Number of cycles,	*med (range)*	6 (4-20)
Preoperative CEA plasma level (ng/ml)	*n (%)*	
> 200		13 (25.5)
Missing		*5*
RAS status	*n (%)*	
Mutated		15 (30.6)
Missing		*7*

CEA: Carcinoembryonic antigen

Table 2: Surgical characteristics of the first and second stages

First-stage perioperative characteristics		**n=56**
Length of stay (days)	*med (range)*	11 [6-162]
Associated resection of primary tumor	*n (%)*	22 (39.3)
Side of hepatic clearance resection Right Left Right + Left	*n (%)*	 6 (10.7) 40 (71.4) 10 (17.9)
Anatomic minor liver resection (segmentectomy)	*n (%)*	15 (22.3)
Wedge resection *Median number per patient*	*n (%)* *med (range)*	46 (82.1) 1 [1-6]
Radiofrequency, *Median number per patient*	*n (%)* *med (range)*	25 (44.6) 3 [1-5]
Largest diameter of the resected metastases (mm), *Missing*	*med (range)*	18 [3-60] *2*
Resection margins (mm), Contact margins with tumor, *Missing*	*med (range)* *n (%)*	2 [1-25] 10 (17.8) *10*
Morbidity grade ≥3	*n (%)*	8(14.3)
Second-stage perioperative characteristics		**n=35**
Length of stay (days)	*med (range)*	11 [5-49]
Delay between the 2 stages (months)	*med (range)*	6.7 [2.1-24.5]
Portal embolization	*n (%)*	6 (17.6)
Right hepatectomy[1]	*n (%)*	11 (31.4)
Right lobectomy[2]	*n (%)*	8 (22.8)
Left hepatectomy[3]	*n (%)*	2 (5.7)
Segmental resections (≥2)*	*n (%)*	6 (17.1)*
Morbidity grade ≥3	*n (%)*	6 (17.1)

[1] Segments 5 to 8; [2] Segments 4 to 8; [3] Segments 2 to 4

* Two patients had a single segmentectomy, but also had a right hepatectomy extended to segment 4 during the first surgical stage.

Table 3: Patients' and tumor characteristics, and histologic response in the "first-stage only" and the "two-stages completed" groups

n (%)	**First stage only n=21**	**2 stages completed n=35**	**Total n=56**	***p*-value**
Age: < 60	8 (38.1)	21 (60)	29 (51.8)	ns
≥ 60	13 (61.9)	14 (40)	27 (48.2)	
Sex Male	13 (61.9)	18 (51.4)	31 (55.4)	ns
Female	8 (38.1)	17 (48.6)	25 (44.6)	
Primary tumor localization				
Colon	17 (81)	28 (80)	45 (80.4)	ns
Rectum	4 (19)	7 (20)	11 (19.6)	
T stage I/II	1 (4.8)	5 (14.3)	6 (10.7)	ns
III/IV	16 (76.2)	28 (80)	44 (78.6)	
X	4 (19)	2 (5.7)	6 (10.7)	
N stage N0	2 (9.5)	9 (25.7)	11 (19.6)	***p*=0.03**
N+	16 (76.2)	26 (74.3)	42 (75)	
Nx	3 (14.3)	0	3 (5.4)	
Metastases				***p*<0.01**
Number ≤ 5	6 (28.6)	22 (64.7)	28 (50.9)	
Number > 5	15 (71.4)	12 (35.3)	27 (49.1)	
Missing	0	1	1	
Type				ns
Synchronous	19 (90.5)	31 (91.2)	50 (90.9)	
Metachronous	2 (9.5)	3 (8.8)	5 (9.1)	
Missing	*0*	*1*	*1*	
CEA level				
<200 ng/dl	13 (76.5)	25 (73.5)	38 (74.5)	ns
>200 ng/dl	4 (23.5)	9 (26.5)	13 (25.5)	
Missing	*4*	*1*	*5*	
RAS status				
Mutated	5 (29.4)	10 (31.3)	15 (30.6)	ns
Non-mutated	12 (70.6)	22 (68.8)	34 (69.4)	
Missing	*4*	*3*	*7*	
Chemotherapy				
Neoadjuvant	21 (100)	32 (91.4)	53 (94.6)	ns
Interval	19 (90.5)	31 (88.6)	50 (89.3)	ns
All-grade morbidity after the first stage (Clavien-Dindo)	6 (28.6)	9 (25.7)	15(26.8)	ns
Histologic response				
TRG				***p*=0.045**
Responders (1/2/3)	4 (19.0)	17 (48.6)	21 (37.5)	
Non-responders (4/5)	17 (81.0)	18 (51.4)	35 (62.5)	
mTRG				***p*=0.026**
Responders (1/2/3)	5 (23.8)	19 (54.3)	24 (42.9)	
Non-responders (4/5)	16 (76.2)	16 (45.7)	32 (57.1)	
Blazer				***p*= 0.004**
Responders (0/1)	6 (28.6)	24 (68.6)	30 (53.6)	
Non-responders (2)	15 (71.4)	11 (31.4)	26 (46.4)	

CEA: Carcinoembryonicantigen; TRG: Tumor Regression Grade; mTRG: modified TRG

Table 4: Factors associated with second-stage completion (uni- and multivariate analyses)

Univariate analysis	**Odd ratio**	**95% CI**	***p*-value**
TRG 1/2/3	4.01	1.12-14.36	**0.033**
mTRG 1/2/3	3.8	1.13-12.67	**0.030**
Blazer 0/1	5.45	1.66-17.85	**0.005**
Initial number of metastases ≤5	4.6	1.4-14.9	**0.011**
Multivariate analysis			
Blazer 0/1	5.42	1.53-19.15	**0.006**
Initial number of metastases ≤5	4.06	1.14-14.47	**0.009**

TRG: Tumor Regression Grade; mTRG: modified TRG; 95% CI: 95% Confidence interval

Table 5: Correlation between the neoadjuvant FOLFIRINOX regimen and the pathologic response

	No FOLFIRINOX n=43	**FOLFIRINOX n=24**	**Total n=67**	***p*-value**
TRG Responders (1/2/3) Non-responders (4/5) *Missing*	 7 (18.9) 30 (81.1) *6*	 14 (73.7) 5 (26.3) *5*	 21 (37.5) 35 (62.5) *11*	***p*<0.001**
mTRG Responders (1/2/3) Non-responders (4/5) *Missing*	 10 (27.0) 27 (73.0) *6*	 14 (73.7) 5 (26.3) *5*	 24 (42.9) 32 (57.1) *11*	***p*=0.001**
Blazer Responders (0/1) Non–responders (2) *Missing*	 14 (37.8) 23 (62.2) *6*	 16 (84.2) 3 (15.8) *5*	 30 (53.6) 26 (46.4) *11*	***p*=0.001**

TRG: Tumor Regression Grade; mTRG: modified TRG

Figure 1: Flowchart of the study

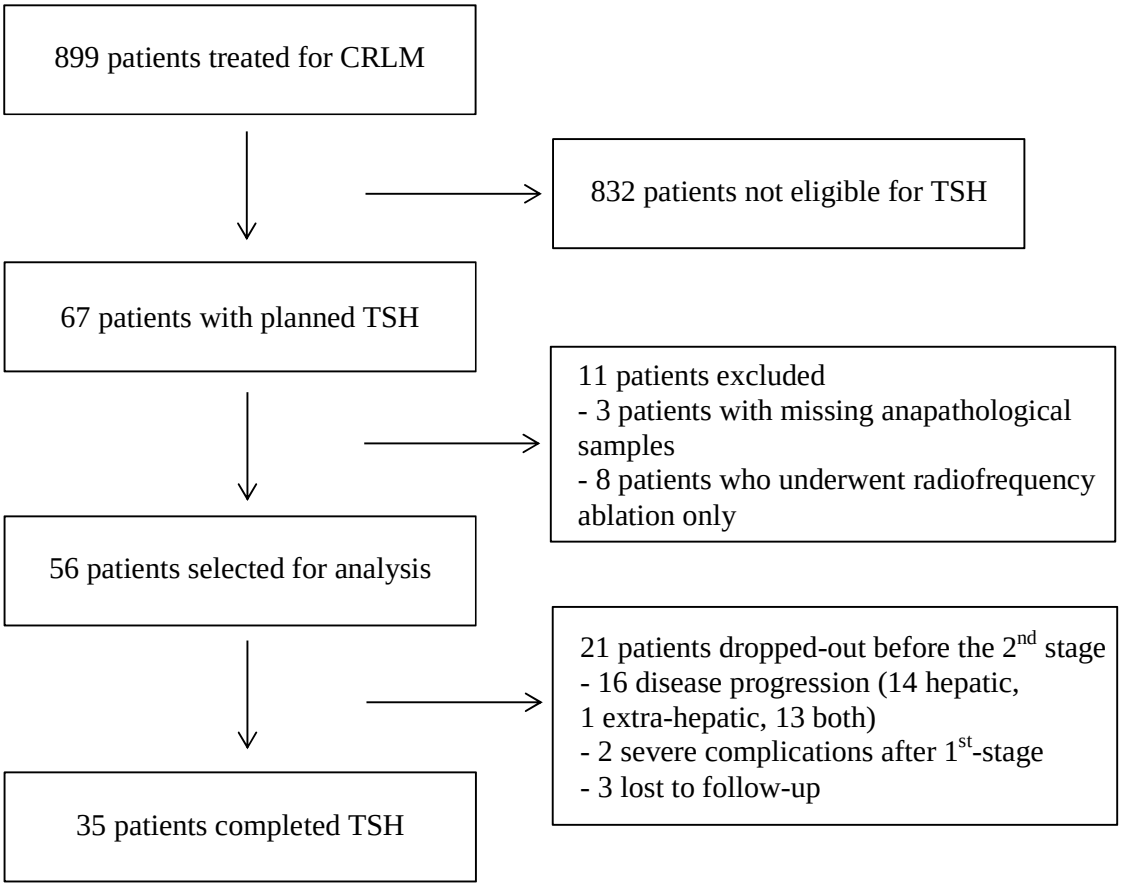
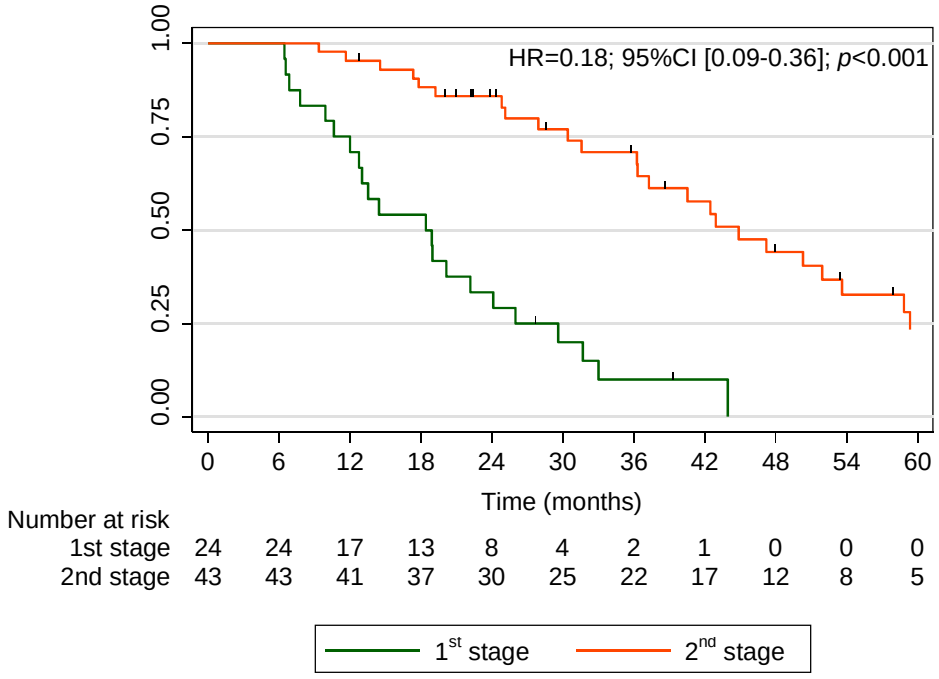


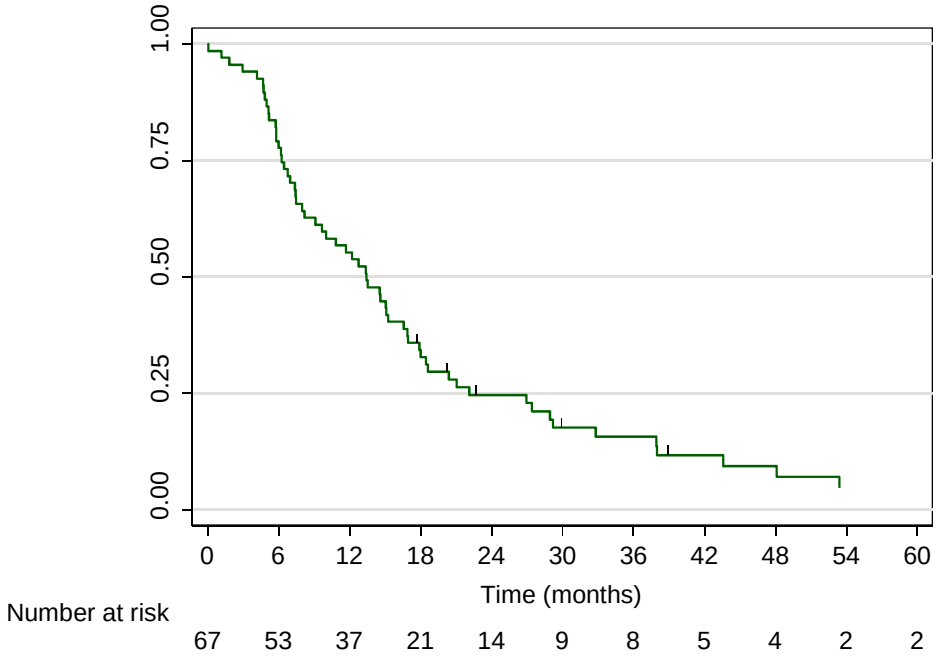
Figure 2

A. Overall survival (OS) in patients who completed or did not complete the two-stage resection



** OS was defined as the time between the date of first surgery to death. The Kaplan-Meier estimates of probabilities of OS were estimated in all patients who completed or not the two-stage resection*

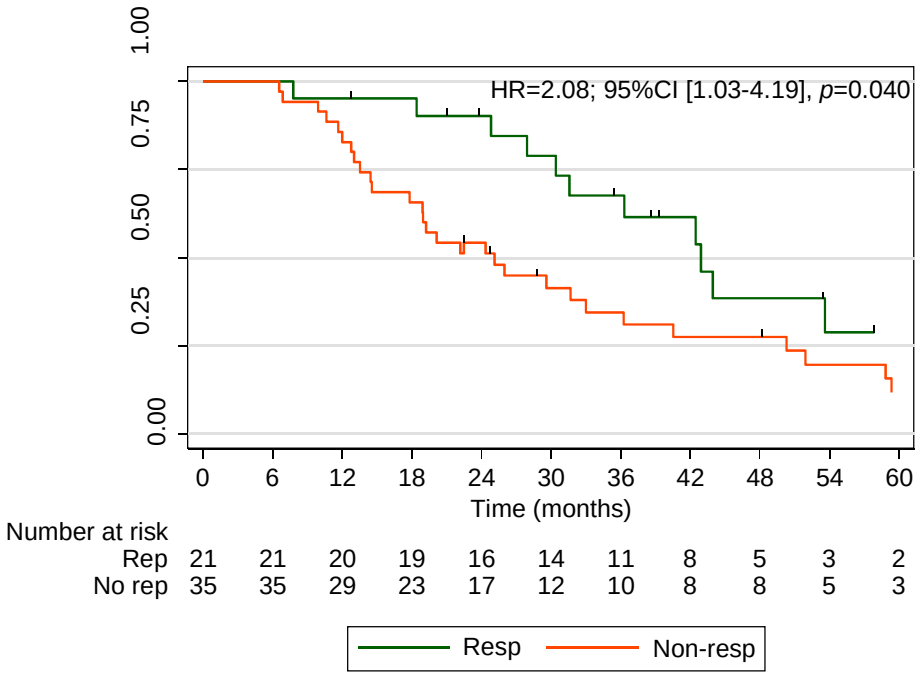
B. Relapse-free survival (RFS) of patients who completed the second stage



** RFS was defined as the time between the date of first surgery to the relapse event. The Kaplan-Meier estimates of probabilities of RFS were estimated in all patients who completed the two-stage resection*

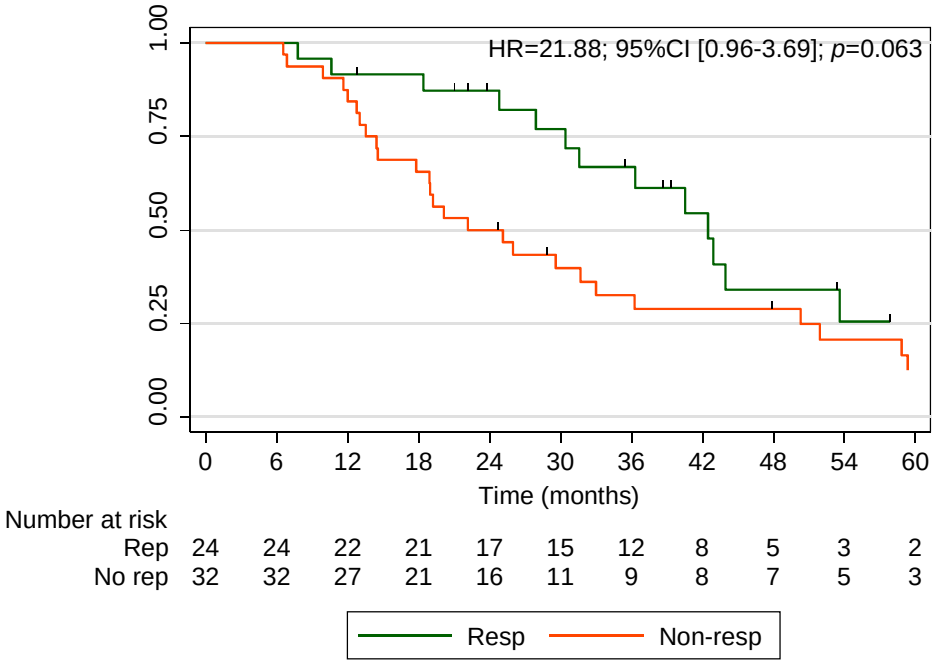
Figure 3: Overall survival depending on the pathological response, according to the TRG (A), mTRG (B) and Blazer (C) classifications.

A. TRG classification: responders (TRG 1,2,3) *versus* non-responders (4,5)



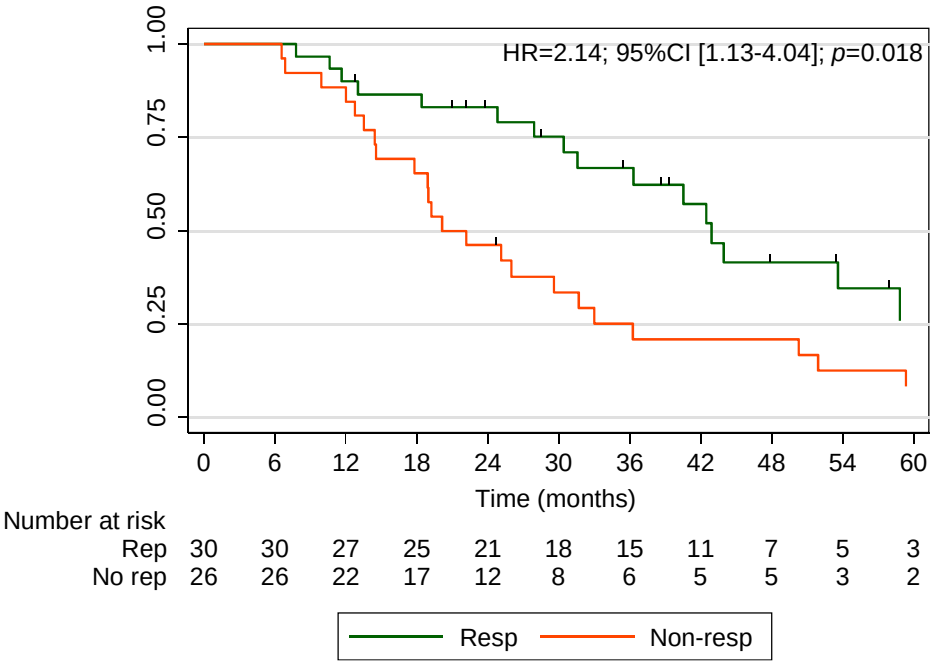
** OS was defined as the time between the date of first surgery to death. The Kaplan-Meier estimates of probabilities of OS depending on the TRG pathological response were estimated for responders (TRG 1,2,3) versus non-responders (TRG 4,5)*

B. mTRG classification: responders (mTRG 1,2,3) *versus* non-responders (4,5)



** OS was defined as the time between the date of first surgery to death. The Kaplan-Meier estimates of probabilities of OS depending on the mTRG pathological response were estimated for responders (TRG 1,2,3) versus non-responders (TRG 4,5)*

C. Blazer classification: responders (Blazer 0,1) *versus* non-responders (Blazer 2)



** OS was defined as the time between the date of first surgery to death. The Kaplan-Meier estimates of probabilities of OS depending on the Blazer pathological response were estimated for responders (Blazer 0,1) versus non-responders (Blazer 2)*