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RESEARCH

Pathologic Assessment of Response to Chemotherapy in Colorectal Cancer Liver Metastases after Hepatic Resection: Which Method to Use?

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Abstract
Background Patients with metastatic colorectal cancer receive chemotherapy prior liver resection more and more frequently. Histopathologic assessment methods of the resected specimen could evaluate the response to chemotherapy. In this study it is analyzed if these histopathologic changes are specific to preoperative chemotherapy and if these methods have correlation with survival.
Methods Sixty three patients with available pathology slides, resected for colorectal cancer liver metastases were enrolled in this study. 46 patients (73 %) received neoadjuvant chemotherapy. Five pathological evaluation methods were compared according to the literature: [1] residual tumor cell ratio, [2] tumor regression grade (TRG) scoring system, [3] modified tumor regression grade (mTRG) scoring system with the type of necrosis, [4] pattern of tumor regression and [5] the tumor thickness at the tumor-normal interface (TNI).
Results Analyzing the pathological methods between the chemotherapy (CTX) and the non-chemotherapy group (NC), we found that that four evaluation methods showed significant and one showed strong correlation with the use of chemotherapy. (Residual tumor cell ratio: $p=0.08$; TRG: $p<0.01$; mTRG: $p=0.03$; pattern of tumor regression: $p<0.01$; TNI: $p=0.02$). In the chemotherapy group none of the analyzed pathological methods showed significant correlation with progression free survival (PFS) or with overall survival (OS). Residual tumor cell ratio, TRG and the pattern of tumor cells showed positive but not significant correlation with OS and PFS and a slight difference in the group of patients with TNI <2 mm could be documented.
Conclusions Tumor regression grade (TRG) and tumor thickness at the tumor-normal interface (TNI) were the most useful methods for pathological response evaluation and these methods had some correlation with survival. According to these data, authors concluded, that a reproducible and well defined scoring system, based on different histopathological evaluation methods should be developed to predict more accurately the effect of neoadjuvant chemotherapy in CRCLM patients.

Keywords colorectal cancer liver metastases · Preoperative chemotherapy · Pathologic response

Abbreviations

CRCLM	Colorectal Cancer Liver Metastases
TRG	Tumor Regression Grade
TNI	Tumor Thickness at the Tumor-Normal Interface
RECIST	The Response Evaluation Criteria in Solid Tumors
UN	Usual Necrosis
ILN	Infarct-Like Necrosis

Introduction

The first choice of treatment for patients with colorectal cancer liver metastases (CRCLM) is resection. Chemotherapy is not only an option as adjuvant treatment after resection but more frequently used as neoadjuvant treatment before hepatic resection. There are patents receiving preoperative chemotherapy to became resectable (conversion therapy) but even more

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patients with resectable CRCLM receiving a short neoadjuvant chemotherapy. [1–4] Hepatic resection after neoadjuvant chemotherapy is a safe procedure. The new surgical (e.g.: staged hepatectomy) and interventional techniques along with neoadjuvant chemotherapy eventuate that more and more patients become potentially resectable. [5–7] The clue for hepatic resection is based not on technical aspects but the selection of the patients who will benefit from the resection.

The effect of chemotherapy is usually evaluated on radiographic scans according to the Response Evaluation Criteria in Solid Tumors (RECIST) scoring system. Some specialized centrums use the modified RECIST more frequently. [8,9] In patients after hepatic surgery, histopathologic evaluation of the resected specimen could evaluate the response to chemotherapy more accurately and this pathologic response seems to correlate with survival. More histopathological evaluation methods are known in the literature, which were already used to assess pathological response. [10–16]

To our knowledge, only one study by Rubbia-Brandt et al. incorporated control group assessing the histopathological changes in CRCLM patients and this concerned only the TRG system. The aim of this study was to analyze the specificity of these histopathological changes assessing the effect of the preoperative chemotherapy, and to evaluate their correlation with survival in a single institution.

Patients and Methods

Patients and Treatment

Patients undergoing liver resection with curative intent for colorectal cancer liver metastases at the Uzsoki Teaching Hospital in Budapest were identified retrospectively from a prospectively collected database. Patients who underwent resection were divided into two groups. [1] patients who received preoperative chemotherapy (CTX group) and [2] patients without neoadjuvant chemotherapy (Control group).

Preoperative chemotherapy was defined as chemotherapy within 6 months before the hepatic resection independently if this was an adjuvant treatment for the primary tumor or a neoadjuvant chemotherapy for the CRCLM.

Histopathological examination was performed by a gastrointestinal pathologist blinded for clinical data and outcome.

Pathological Evaluation Methods

These methods were listed and compared according to the literature (Fig. 1.)

[1] residual tumor cell ratio according to Blazer et al. where complete-, major-, and minor response were recorded (complete response: no residual tumor cell, major response: <50 % residual tumor cell and minor response: >50 % residual tumor cell) [10].

[2] tumor regression grade (TRG) scoring system according to Rubbia-Brandt et al., where TRG1 corresponded to absence of tumour cells replaced by fibrosis; TRG2 to rare scattered residual tumour cells and abundant fibrosis; TRG3 to a large amount of residual tumour cells with predominant fibrosis; TRG4 to tumour cells predominating over fibrosis; and TRG5 to almost exclusively tumour cells without fibrosis. [11].

[3] modified tumor regression grade (mTRG) scoring system with the type of necrosis according to Chang et al., where usual necrosis (UN) was defined as containing nuclear debris in a patchy distribution, with the necrosis admixed and bordered by viable cells and infarct-like necrosis (ILN) was defined as being composed of large confluent areas of eosinophilic cytoplasmic remnants located centrally within a lesion with absent or minimal admixed nuclear debris. ILN was considered equivalent to fibrosis and so ILN was considered a form of therapeutic treatment effect [12].

4) pattern of tumor regression according to NG JKS et al. where two models for the pattern of tumor regression was defined: in the first model viable tumor cells were more frequent in the periphery of metastases, regardless of chemotherapy exposure. In the second model, residual disease is randomly distributed throughout the original tumor volume [17].

[5] the tumor thickness at the tumor-normal interface according to Maru et al. (TNI). The focus in which the maximum contiguous tumor cell thickness was observed at the TNI was measured by a ruler. This focus was composed of uninterrupted layers of tumor cells without admixed fibrotic stroma, acellular mucin, or nonneoplastic liver parenchyma [14].

Statistical Analysis

The *t*-test was performed to assess differences between continuous variables and the Chi-square- and the Spearman-test was applied to assess the association between categorical variables. Survival probabilities were calculated by the Kaplan-Meier method and compared by the log rank test. A p value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 17 software (SPSS, Chicago, IL, USA).

Results

A total of 128 curative liver resections were performed for CRCLM from December 2006 to December 2010 at the Uzsoki Hospital in Budapest, Hungary. 63 patients were enrolled in this study. From this 63 patients, 46 (73 %) received

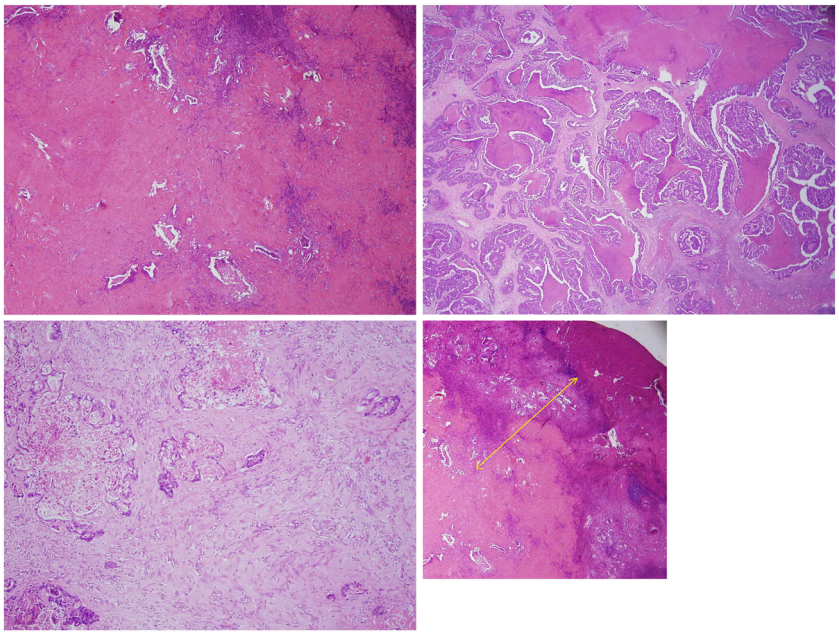

Fig. 1 Examples of histopathological photomicrographs of CRCLM slides; **a** major response (<50 % viable tumor cells), **b** minor response (>50 % residual tumor cells) – TRG4 **c**" tumor regression grade " 3-TRG3, **d** "tumor thickness at the tumor normal interface"-TNI **e** "dirty necrosis"-UN, **f** "infarct-like necrosis"- ILN

neoadjuvant chemotherapy. Patient's characteristics are shown in Table 1. In the preoperative chemotherapy group 6 patients received chemotherapy without bevacizumab and 40 patients received chemotherapy with bevacizumab. Cytotoxic chemotherapy was FOLFIRI in 40 patients FOLFOX in 4 patients and 2 patients received FOLFOXIRI. The median duration of chemotherapy was 9 [3–12] cycles in the chemotherapy group. (Table 1.) In a previous study we already compared the survival data between the chemotherapy and the up-front resected non-chemotherapy control group, and both PFS and OS were significantly worse in the chemotherapy group (data not shown). [18] In this study survival data were analyzed only in the chemotherapy group.

First, we analyzed the pathological methods between the chemotherapy (CTX) and the non-chemotherapy group (Control). (Table 2.) None of the histopathological changes, used to assess the effect of the preoperative chemotherapy were exclusively seen only in the chemotherapy group. Comparing the residual tumor cell ratio between the two groups, there were more patients with <50 % residual tumor cells in the chemotherapy group, but this was not significant (65 vs. 45 %; p=0.08). There was manifest difference in the TRG scores between the two groups, most patients in TRG 1–3 were in the chemotherapy group (48 vs. 6 %; <0.01). In the type of necrosis, we found a significant difference between the groups, "infarct like necrosis" was more common in the

Table 1 Baseline Characteristics

		No preoperative chemotherapy (Control group) N=17	Preoperative chemotherapy (CTX group) N=46	
Age*		61 (48–83)	62 (32–75)	0.08
Type of resection	major minor	5 (29) 12 (71)	22 (48) 24 (52)	0.19
Transfusion		1 (6)	7 (15)	0.32
R-resection	R0 R1	17 (100) -	40 (87) 6 (13)	0.29
Node positive primary		10 (59)	32 (70)	0.42
Synchronous		8 (47)	22 (48)	0.96
Number of metastatic nodules*		1 (1–5)	1 (1–8)	0.38
Size of metastatic nodules (cm) *		3.0 (1.4–7.0)	3.0 (0.9–11.0)	0.49
Chemotherapy	CTX CTX+BV		6 (13) 40 (87)	

Values in parentheses are percentages

*Values are median (range)

CTX+BV preoperative chemotherapy with bevacizumab, CTX, preoperative chemotherapy without bevacizumab

Table 2 Comparison of the different pathological methods (assessing the pathological response in CRCLM patients) between the chemotherapy (CTX) and the non-chemotherapy group (Control)

Methods		No preoperative chemotherapy (Control group) N=17	Preoperative chemotherapy (CTX group) N=46	
Residual viable tumor cells	<50 %	7 (41)	30 (65)	0.08
	>50 %	10 (59)	16 (35)	
TRG	1–3	1 (6)	22 (48)	<0.01
	4–5	16 (94)	24 (52)	
Type of necrosis	UN	14 (82)	25 (54)	0.03
	ILN	3 (18)	21 (46)	
Pattern of viable cells	circle	1 (6)	11 (24)	<0.01
	diffuse	16 (94)	35 (76)	
TNI (mm) *		1.125 (0.75–5.5)	1.00 (0.25–3.13)	0.02

Values in parentheses are percentages

*Values are median (range)

Residual viable tumor cells: major response: <50 % residual viable tumor cell; minor response: >50 % residual viable tumor cell. TRG: tumor regression grade. UN: "usual necrosis" ILN: "infarct-like necrosis". TNI: tumor thickness at the tumor-normal interface

chemotherapy group (46 vs. 18 %; $p=0.03$). The pattern of the tumor cells showed difference between the groups, the circle setting of the remnant viable cells was more common in the chemotherapy group (24 vs. 6 %; $p<0.01$). The tumor thickness at the tumor-normal interface was also narrower in the chemotherapy group (1.00 vs. 1.125 mm; $p=0.02$).

As a second aim of the study, we analyzed the association of these different pathological methods with the survival in the chemotherapy group ($n=46$), (Fig. 2.) The median follow up after liver resection was 30 months. None of the analyzed pathological methods showed significant correlation with progression free survival (PFS) or with overall survival (OS). Residual tumor cell ratio, TRG and the pattern of tumor cells showed positive but not significant correlation with OS and PFS. The PFS and OS curves showed a slight difference in the group of patients with TNI <2 mm ($p=0.17$; $p=0.12$). Analyzing the fibrosis with a cut-off point of 40 % and the residual tumor cell ratio with a cut-off point of 25 %, there was also no significant correlation with survival ($p=0.9$, $p=0.75$).

Discussion

In this study, we analyzed the different evaluation methods of pathologic response in patients who underwent hepatic resection of CRCLM. One would assume that definitive and overall reproducible assessment of the pathological response in CRCLM patients could provide estimate for survival or overall prognosis and perhaps might indicate the necessity for therapy change.

Pathologic response is available only in patients who underwent resection of the metastases. Chun et al. found correlation between pathologic response and the appearance of the lesion on CT scans, and suggested that this morphologic change could be an accurate evaluation of the response to chemotherapy in non-resected CRCLM patients. [9,19] On the other hand, Egger et al. in a recent study found no correlation between RECIST/mRECIST and pathologic response.[20]

The aim of our study was to estimate the clinical value and usefulness of the already known pathological response evaluation methods.

Blazer et al. defined complete, major and minor pathological response according to the residual viable tumor cells. This study showed a significant improvement in survival in complete and major response.[10] In our study in the chemotherapy group there were more patients with major response, but a lot of patients in the control group, who received no chemotherapy prior resection, also showed <50 % residual viable tumor cells during histopathological evaluation. In the chemotherapy group, OS and PFS showed positive correlation with major response, but this was not significant.

Rubbia-Brandt et al. created a tumor regression grade scoring system (TRG) to integrate fibrosis, necrosis and viable tumor cells. This supports the concept, that necrosis is more likely a spontaneous phenomenon and it is not related to chemotherapy. The TRG was equivalent in the different tumor foci in the same patient and in this study histological tumor regression was much more common in patients treated with oxaliplatin based chemotherapy than in patients treated with irinotecan.[11] Histopathological changes, which are used in the TRG system showed a significant difference between the chemotherapy and non-chemotherapy control group in our study (TRG 1–3; 48 vs. 6 %), so this evaluation method was a more accurate and useful method for us, but this was also not 100 % specific to preoperative chemotherapy In the original study by Rubbia-Brandt et al., the histopathological changes combined in the TRG system were only observed in

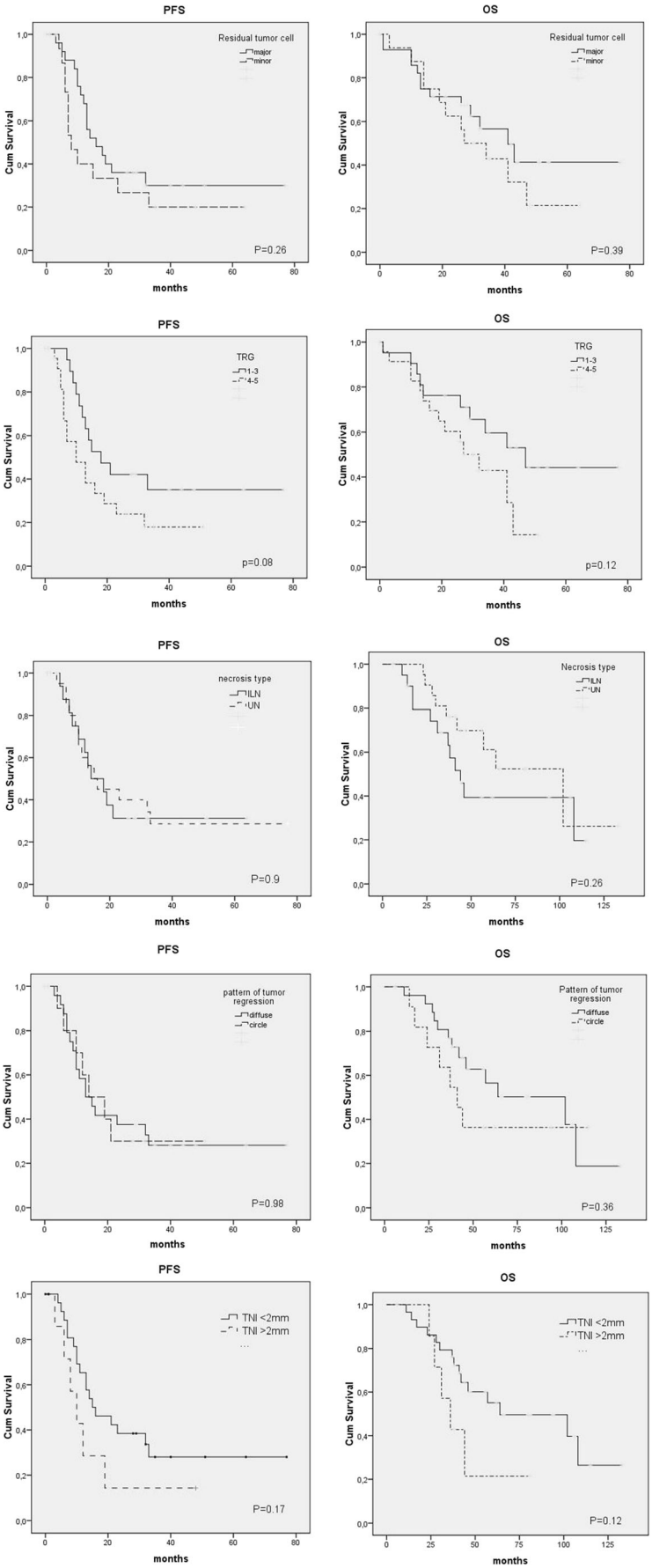

Fig. 2 Association between overall survival (OS) and progression free survival (PFS) and the different pathological assessment methods in patients receiving preoperative chemotherapy (n=46)

the neoadjuvant chemotherapy group, and all the patients in the control group were categorized as TRG4 or TRG5. This was the only study incorporating a control group without receiving preoperative chemotherapy. In the chemotherapy group, the correlation between the TRG and survival was not significant in our study, but showed positive tendency.

Chang et al. tried to modify this TRG system distinguishing two types of necrosis, UN and ILN, where ILN was considered a form of therapeutic treatment effect and additionally bevacizumab may facilitate this. They found that the presence of ILN is positively correlated with survival.[12] In this study ILN was more frequent in the chemotherapy group than in the control group. In the chemotherapy group, the presence of ILN showed no positive correlation with survival.

NG JKS et al. demonstrated, that viable tumor cells were more frequent in the periphery of metastases, regardless of chemotherapy exposure. Central necrosis was prominent in untreated metastases, but disappeared after chemotherapy. They found, that after a response to chemotherapy the distribution of viable cells remained unchanged, suggesting that the probability of cell death is the same in the peripheral and central portions of the tumor, and the replacement of dead tumor cells by fibrosis was the main mechanism responsible for reduction in tumor size.[17] We found similarly to Rubbia-Brandt et al., that in the chemotherapy group, viable cells were more frequently located at the periphery, but this phenomena had no impact on survival.

Maru et al. found that tumor thickness measured at the TNI is a potentially new prognostic factor for therapy response and survival outcome in patients with resected CRCLM. They found that TNI was also correlated with mRECIST and with the response criteria from Blazer. Additional observation of that study was that tumors from patients treated with bevacizumab had smaller tumor thickness at TNI than did tumors from patients who did not receive bevacizumab, and they found significantly thinner median TNI in patients who received oxaliplatin-based therapy than in patients who received irinotecan-based therapy.[14] In our study the median TNI was thinner in the chemotherapy group, and we found positive correlation with survival in the subgroup of patients with TNI <2 mm.

In a recent study, Poultsides et al. concluded, that fibrosis is the predominant chemotherapy related pathologic alteration, and they reported that overall pathologic response ≥75 % and fibrosis ≥40 % were independently correlated with disease-specific survival after hepatectomy.[21] Applying these cut off points there was no significant correlation with survival in the chemotherapy group in our study.

We tried to find out why these response assessment methods showed no significant correlation in our patient population. One reason could be that almost all of the patients received irinotecan based cytotoxic chemotherapy, while most study reported patient population treated with at least 50 % or more with oxaliplatin based chemotherapy. Although there is no clear significant difference in the survival and in the degree of pathologic response for chemotherapy between the irinotecan and oxaliplatin regimens, almost all study showed a slight difference in favor of the oxaliplatin regimen.

There are some evidences that bevacizumab improves pathologic response.[22] Ribero et al. reported that the addition of bevacizumab improved pathologic response rate in tumors ≤4 cm.[13] Chang et al. reported that the use of bevacizumab increased the rate of ILN, which was positively correlated with survival.[12] Dipen et al. also found that TNI was thinner in the bevacizumab group.[14] Loupakis et al. found that the addition of bevacizumab to FOLFOXIRI produces high rates of pathologic responses and necrosis of CRCLM. [23] Klinger et al. reported, that bevacizumab significantly improved tumor regression to chemotherapy and the improved pathologic response significantly correlated with survival. [24] In our study, most patients in the chemotherapy group received bevacizumab, so we could not conclude any additional effect.

There are some limitations of our study: 1. this was a retrospective analysis and 2.the number of patients was relatively small. As a retrospective analysis, the adjuvant chemotherapy protocols and number of cycles after liver resection were not the same in the patients, and this could all affect survival.

In conclusion, comparing the pathologic response evaluation methods used in the literature, in this study tumor regression grade (TRG) and tumor thickness at the tumor-normal interface (TNI) seemed to be the most useful method evaluating pathological response. These two assessment systems showed positive correlation with survival in the group of patients receiving preoperative chemotherapy, although none of them reached statistical significance A reproducible and well defined scoring system based on different histopathological evaluation methods is necessary and should be developed to identify more accurately the effect of neoadjuvant chemotherapy in CRCLM patients.

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