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RESEARCH PAPER

Growth pattern of colorectal liver metastasis as a marker of recurrence risk

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Abstract Despite improved therapy of advanced colorectal cancer, the median overall survival (OS) is still low. A surgical removal has significantly improved survival, if lesions are entirely removed. The purpose of this retrospective explorative study was to evaluate the prognostic value of histological growth patterns (GP) in chemonaive and patients receiving neo-adjuvant therapy. Two-hundred-fifty-four patients who underwent liver resection of colorectal liver metastases between 2007 and 2011 were included in the study. Clinicopathological data and information on neo-adjuvant treatment were retrieved from patient and pathology records. Histological GP were evaluated and related to recurrence free and OS. Kaplan–Meier curves, log-rank test and Cox regression analysis were used. The 5-year OS was 41.8 % (95 % CI 33.8–49.8 %). Growth pattern evaluation of the largest liver metastasis was possible in 224 cases, with the following distribution: desmoplastic 63 patients (28.1 %); pushing 77 patients (34.4 %); replacement 28 patients (12.5 %); mixed 56 patients (25.0 %). The Kaplan–Meier analyses demonstrated that patients resected for liver metastases with desmoplastic growth pattern had a longer recurrence free survival (RFS) than patients resected for non-desmoplastic liver metastases ($p = 0.05$). When patients were stratified according to neo-adjuvant treatment in the multivariate Cox regression model, hazard ratios for RFS compared to desmoplastic were: pushing (HR = 1.37, 95 % CI 0.93–2.02, $p = 0.116$), replacement (HR = 2.16, 95 % CI 1.29–3.62, $p = 0.003$) and mixed (HR = 1.70, 95 % CI 1.12–2.59, $p = 0.013$). This was true for chemonaive patients as well as for patients who received neo-adjuvant treatment.

Keywords Colon cancer · Liver metastasis · Hepatectomy · Histology · Prognosis · Survival · Desmoplasia

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Abbreviations

bev	Bevacizumab
BRAF	v-Raf murine sarcoma viral oncogene homolog B
CEA	Carcinoembryonic antigen
CI	Confidence interval
CLM	Colorectal liver metastasis
CRC	Colorectal cancer
CT	Computed tomography
CTx	Chemotherapy
EGFR	Epidermal growth factor receptor
5FU	5-Fluorouracil
GP	Growth pattern
H&E	Haematoxylin and eosin
HR	Hazard ratio
KRAS	Kirsten rat sarcoma viral oncogene homolog

mCRC	Metastatic colorectal cancer
OS	Overall survival
oxa	Oxaliplatin
pts	Patients
RFS	Recurrence free survival
RTx	Radiotherapy
uPAR	Urokinase-type plasminogen activator receptor
VEGF	Vascular endothelial growth factor

Introduction

Metastatic colorectal cancer (mCRC) is one of the leading causes of cancer-related deaths worldwide. Approximately 15 % of the colorectal cancer (CRC) patients have liver metastases at the time of diagnosis (i.e., synchronous) [1, 2], and another 16–20 % of the patients develop liver metastases within the first 3 years after the diagnosis (i.e., metachronously) [1, 3]. Lung metastases are less frequent and occur predominantly in patients with rectal cancer [4]. Overall metastatic spread is observed in approximately 65 % of the patients during the course of the disease [1]. Recently, it has been described that the majority of metastatic lesions are restricted to one organ, either liver or lung [5]. It is a general point of view that patients with colorectal liver metastases (CLMs) should be evaluated by a liver surgeon at a multi-disciplinary (MDT) conference. At MDT about 20 % of the patients with liver metastases are considered resectable with curative intent [3, 6].

The 5-year survival of patients resected for liver metastasis is about 40 % [7], but up to 58 % in selected groups of patients [8]. Potentially resectable liver metastases may become resectable after neo-adjuvant treatment. The challenge is to find the most beneficial treatment before and after liver surgery with the aim of changing a dismal prognosis into a substantial chance for long term survival [9, 10]. Antineoplastic treatment includes chemotherapy alone or in combination with targeting agents, such as the vascular endothelial growth factor (VEGF) inhibitor, bevacizumab [11, 12] or the epidermal growth factor receptor (EGFR) inhibitors (e.g., cetuximab or panitumumab) [13, 14], tailored to the individual patient. Current focus is on tumour genetics, but histopathological characteristics of the primary colorectal tumour still have pivotal impact on clinical considerations. The identification of specific growth patterns (GP) of liver metastases has nourished the hypothesis that growth pattern and other histological characteristics of liver metastases may carry important biological, predictive and prognostic information. The availability of multiple new treatment options, augments possibilities, but also complicates the choice. To deal with this dilemma, one strategy is to correlate characteristics of liver metastases to the observed prognosis of CRC patients having metastasis surgery [15].

Previously, a number of clinical scoring systems have been developed to evaluate prognosis of liver metastases. The two most frequently applied clinical risk scores are the Fong score and the Nordlinger score [16, 17]. These scores were introduced in the 1990s, when antineoplastic treatment was not as common for mCRC as it is today and the surgical possibilities were limited. Both scores include several prognostic factors such as the number of metastases, the interval between resection of primary tumour and liver metastases, the distribution of metastases and the serum carcinoembryonic antigen level. Based on these prognostic scores, the patients can be categorised into either high or low risk of relapse. Additionally, in the prognostic scoring model described by Köhne et al., including performance status, white blood cell count, alkaline phosphatase and number of metastatic nodules, patients could be divided into three risk groups [18]. The score included patients who received neo-adjuvant chemotherapy. Targeted therapies, such as bevacizumab and cetuximab, were not in use for mCRC when this prognostic scoring model was applied. A prognostic scoring model, considering neo-adjuvant therapies including targeted therapies, would therefore be useful.

Histopathological studies of liver metastases have resulted in the description of three histological GP (Fig. 1a–d). These are: *desmoplastic* GP, where a rim of collagen surrounds the tumour tissue and separates the liver parenchyma from the cancer cells (Fig. 1a, d, g); *pushing* GP, where tumour cells push the liver parenchyma aside, encompassing pressure on the hepatocytes at the tumour margin (Fig. 1b, e, h); and *replacement* GP, where tumour cells replace the hepatocytes hereby maintaining the trabecular architecture of the liver parenchyma (Fig. 1c, f, i) [19–21].

In a number of studies, it has been shown that GP correlates to prognosis [20, 22–28]. Most of these studies included patients before neo-adjuvant treatment was common. In one of the most recent studies that enrolled 205 patients from 1995 to 2005, patients resected for liver metastasis with pushing GP had a significantly poorer two-year survival in comparison to patients with desmoplastic or replacement GP [20]. In a second prognostic study by Nielsen et al. [26], survival was related to GPs, but without considering therapeutic approach. The study found a superior overall survival (OS) for patients resected for liver metastases with desmoplastic GP.

In the present study, we aimed at investigating the impact of GP on the risk of recurrence and survival when concurrently considering treatment before hepatic resection of liver metastases.

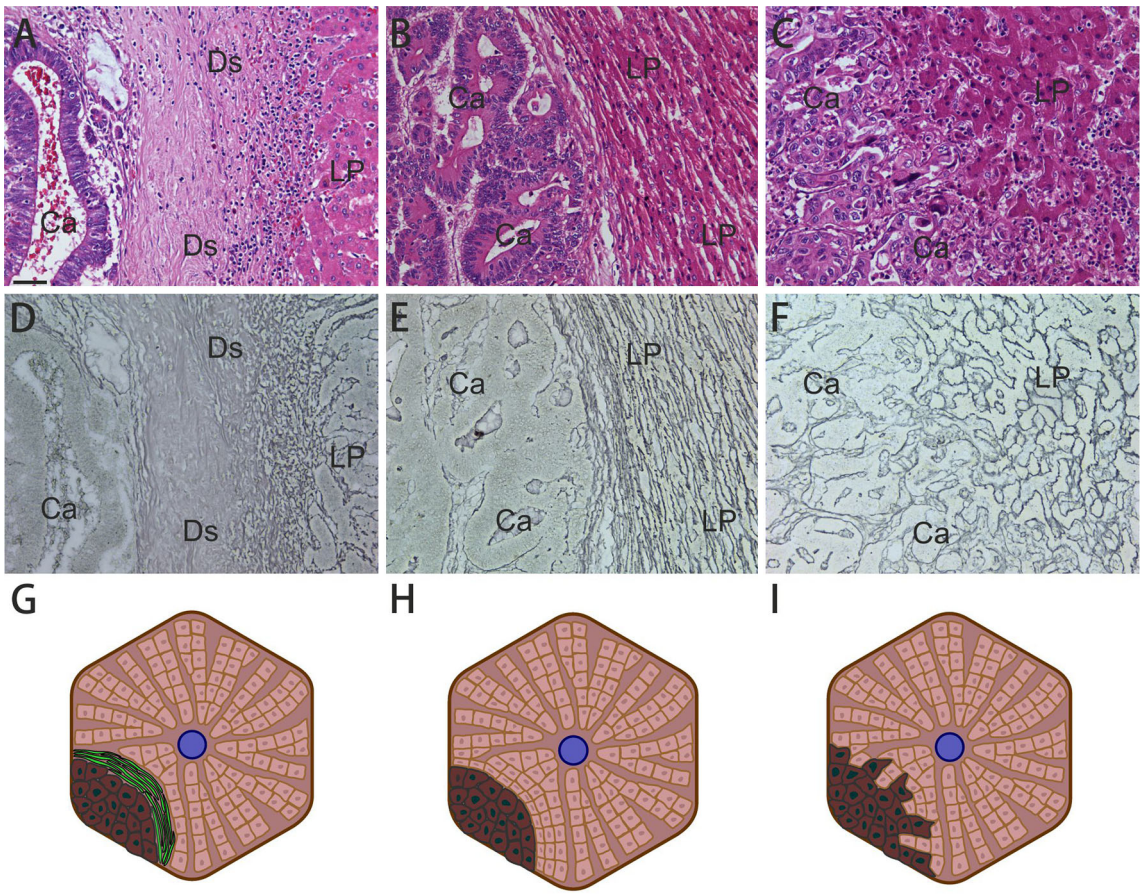

Fig. 1 Illustration of growth patterns in colorectal liver metastases. The different growth patterns are illustrated in **a**, **b**, **g** (desmoplastic growth pattern), **b**, **e**, **h** (pushing growth pattern) and **c**, **f**, **i** (replacement growth pattern). The mixed growth pattern is not shown, but is usually a mixture of two patterns, often including a pushing component

Patients and methods

Patient material

All CRC patients (n = 254) who underwent first hepatic resection of liver metastasis at the Department of Liver Surgery, Rigshospitalet, Copenhagen, Denmark from 2007 to 2011 were included. Eligible for inclusion were individuals with a histopathological diagnosis of CRC with a resected synchronous or metachronous liver metastasis. In patients who underwent more than one liver resection, only tissue specimen from the first resection was included in this study. Patients having a previous percutaneous radiofrequency ablation only were excluded. Patients with other malignancies were excluded unless diagnosed before the liver resection and curatively treated. All patients who fulfilled the inclusion criteria were included, whether they received neo-adjuvant fluoropyrimidine-based cytotoxic chemotherapy with or without targeted drugs such as the VEGF inhibitor, bevacizumab or if no antineoplastic drugs were given before hepatic resection. In the subgroup of untreated patients, twenty-two patients from our previous pilot study were included [21]. The remaining two patients from our pilot cohort did not fulfil the inclusion criteria. Based on pre-operative treatment status, patients were subgrouped as 'untreated' (n = 149), 'neo-adjuvant 5FU ± oxaliplatin' (n = 51) or 'neo-adjuvant 5FU ± oxaliplatin + bevacizumab' (n = 41). A minority of the patients (n = 10) received fluoropyrimidine-based treatment ± irinotecan ± cetuximab (an EGFR inhibitor). The remaining three patients received treatment in an experimental protocol, where chemotherapy was given as a hepatic-artery-infusion. These patients were categorised in the subgroup 'other' (n = 13). The subgroups were used as stratification factors in the survival analyses. An in situ primary CRC was an exclusion criterion, unless the patient had a "liver first approach" (n = 23), i.e., the primary tumour was resected *after* the liver resection.

Patient data were collected based on a retrospective chart review. Clinical variables included were age, gender, relapse, recurrence site and survival. Characteristics of the primary colorectal tumour such as localisation, tumour

(T) stage, microsatellite instability, *KRAS* mutation and tumour differentiation were also retrieved (Table 1). Liver metastasis characteristics such as number and distribution of liver metastases in one or two lobes according to Couinaud [29], surgical margin with R0 as a tumour free margin >1 mm and R1 as a tumour free margin <1 mm [30–32] as described in registry from pathology records, size and timing of liver metastases (synchronous or metachronous metastasis) were registered (Table 2). Synchronous liver metastases were defined as metastatic nodules diagnosed on a computed tomography scan within 6 months from the histologic diagnosis of the primary CRC, in concordance to the definition by Mekenkamp et al. [33]. Additionally, treatment status and recurrence site were retrieved from patient records and imaging descriptions.

The primary endpoint was recurrence free survival (RFS), as described by Punt et al. [34], which was defined as time from liver surgery to date of relapse or date of death of any cause, if no relapse was registered. The secondary endpoint was OS, defined as death of any cause. Four patients died within 30 days after surgery. The causes of death for these patients were septic shock, neutropenia or acute tubular necrosis, respectively. One died of unknown causes.

This retrospective, explorative study was performed in accordance with the REMARK guidelines [35] and in accordance with the Helsinki Declaration of the World Medical Association. It was approved by the Committees of Health Research Ethics in the Capital Region of Denmark (approval no. H-2-2011-045) and the Danish Data Protection Agency (approval no. 2010-41-5623).

Tissue specimens and stainings

A total of 573 liver metastases were identified from first-time hepatic resection of 254 patients. Formalin-fixed and paraffin-embedded liver metastasis tissue blocks (n = 506) and corresponding pathology reports were obtained for 252 patients. The tissue block including the most representative tumour-liver interface was preferred from every metastatic nodule. Moreover, a tissue block from the largest metastatic nodule from the patients, based on measurements described in the pathology records, was available for 250 patients. Slides of 3 μm thickness were cut from each block and stained with haematoxylin and eosin (H&E) and with Gordon–Sweet's staining for reticulin [19, 36, 37].

Growth pattern scoring model

The scoring model for GP has been described previously [19–21]. In concordance with this model, the tumour periphery was assessed for GP in serial sections of H&E and reticulin stains (Fig. 1). A 75 % cut-off value was applied, based on the overall distribution of individual GPs, meaning that if one GP was represented in 75 % or more of the tumour periphery, the liver metastasis was categorised with this particular GP, whereas a mixed GP was registered if more than one GP was present in the liver metastasis and the largest component was represented in less than 75 % of the tumour periphery. In some cases, the liver metastasis consisted only of fibrotic and/or necrotic tissue or lacked the tumour-liver interface. In such cases, the liver metastasis was categorised as non-assessable (NA).

Histological evaluation of GP was performed using a Leica bright field microscope at low magnification (10 × objective). All slides were evaluated blinded with respect to clinical data. The liver metastases from 252 patients (n = 506) were assessed by two observers (RE and MI), and a consensus reading was reached of all lesions. Difficult cases were discussed with two expert pathologists (BV and PBV). The GP from consensus reading was used for survival analysis.

Statistical methods

Kappa statistics were used for inter-observer agreement assessment. The Chi square (χ^2) test was used in contingency table analyses. Uni- and multivariate Cox proportional hazard models were used to investigate associations of patient and tumour characteristics to survival outcomes. The Hazard ratios are present with 95 % confidence intervals (CI) and characterise the relationship between explanatory variables and RFS or OS. A screen of the univariate variables was performed. Variables with $p \leq 0.10$ were selected for the multivariate analysis. Survival probability was estimated using the Kaplan–Meier method and log-rank test was used for comparison between groups. A p value < 0.05 was considered statistically significant. Statistical analysis was performed using the S.A.S. software version 9.3 (SAS Institute, Cary, NC, USA).

Results

Patient characteristics

The median patient age at the time of diagnosis of the liver metastases was 64 years (range 19–92 years). The patient population consisted of 154 (60.6 %) men and 100 (39.4 %) women (Table 1). Of the 254 patients included, 231 had their primary CRC resected before liver resection, whereas the "liver first" approach was applied for the remaining 23 patients. Furthermore, 138 patients (54.3 %)

Table 1 Baseline characteristics

Characteristics	All n = 254	Desmoplastic n = 63 (24.8 %)	Pushing n = 77 (30.3 %)	Replacement n = 28 (11.0 %)	Mixed n = 56 (22.0 %)	NA n = 26 (10.2 %)	Unknown n = 4 (1.6 %)
Age							
<70 year	197 (77.6 %)	50 (79.4 %)	60 (77.9 %)	23 (82.1 %)	43 (76.8 %)	19 (73.1 %)	2 (50.0 %)
>70 year	57 (22.4 %)	13 (20.6 %)	17 (22.1 %)	5 (17.9 %)	13 (23.2 %)	7 (26.9 %)	2 (50.0 %)
Gender							
Women	100 (39.4 %)	21 (33.3 %)	29 (37.7 %)	10 (35.7 %)	27 (48.2 %)	11 (42.3 %)	2 (50 %)
Men	154 (60.6 %)	42 (66.7 %)	48 (62.3 %)	18 (64.3 %)	29 (51.8 %)	15 (57.7 %)	2 (50 %)
Localisation							
Right colon	51 (20.1 %)	13 (20.6 %)	18 (23.3 %)	3 (10.7 %)	11 (19.6 %)	6 (23.1 %)	–
Left colon	125 (49.2 %)	32 (50.8 %)	35 (45.5 %)	15 (53.6 %)	26 (46.4 %)	14 (53.8 %)	3 (75.0 %)
Rectum	78 (30.7 %)	18 (28.6 %)	24 (31.2 %)	10 (35.6 %)	19 (33.9 %)	6 (10.7 %)	1 (25.0 %)
Tumour depth							
T1	5 (2.0 %)	1 (1.6 %)	1 (1.3 %)	1 (3.6 %)	2 (3.6 %)	–	–
T2	17 (6.7 %)	4 (6.3 %)	4 (5.2 %)	3 (10.7 %)	3 (5.4 %)	3 (11.5 %)	–
T3	163 (64.2 %)	40 (63.5 %)	52 (67.5 %)	19 (67.9 %)	35 (62.5 %)	14 (53.8 %)	3 (75.0 %)
T4	65 (25.6 %)	18 (28.6 %)	19 (24.7 %)	5 (17.9 %)	15 (26.8 %)	7 (26.9 %)	1 (25.0 %)
Unknown	4 (1.6 %)	–	1 (1.3 %)	–	1 (1.8 %)	2 (7.7 %)	–
Node status							
Node negative	78 (30.7 %)	27 (42.9 %)	23 (29.9 %)	9 (32.1 %)	12 (21.4 %)	6 (23.1 %)	1 (25.0 %)
Node positive	175 (68.9 %)	63 (57.1 %)	54 (70.1 %)	19 (67.9 %)	44 (78.5 %)	19 (73.1 %)	3 (75.0 %)
Unknown	1 (0.4 %)	–	–	–	–	1 (3.8 %)	–
Vessel status							
Vessel negative	155 (61.0 %)	38 (60.3 %)	44 (57.1 %)	17 (60.7 %)	36 (64.3 %)	18 (69.2 %)	2 (50.0 %)
Vessel positive	92 (36.2 %)	23 (36.5 %)	31 (40.3 %)	11 (39.3 %)	19 (33.9 %)	6 (23.1 %)	2 (50.0 %)
Unknown	7 (2.8 %)	2 (3.2 %)	2 (2.6 %)	–	1 (1.8 %)	2 (3.6 %)	–
Differentiation							
No tumour	1 (0.4 %)	1 (1.6 %)	–	–	–	–	–
Good	39 (15.4 %)	8 (12.7 %)	11 (14.3 %)	8 (28.6 %)	10 (17.9 %)	2 (7.7 %)	–
Moderate	163 (64.2 %)	48 (76.2 %)	45 (58.4 %)	17 (60.7 %)	36 (64.3 %)	15 (57.7 %)	2 (50.0 %)
Poor	19 (7.5 %)	2 (3.2 %)	7 (9.1 %)	2 (7.1 %)	5 (8.9 %)	3 (11.5 %)	–
Unknown	32 (12.5 %)	4 (6.3 %)	14 (18.2 %)	1 (3.6 %)	5 (8.9 %)	6 (23.1 %)	2 (50.0 %)
MSI							
MSS	186 (73.2 %)	41 (65.1 %)	57 (74.0 %)	25 (89.2 %)	42 (75.0 %)	18 (69.2 %)	3 (75.0 %)
MSI	6 (2.4 %)	1 (1.6 %)	4 (5.2 %)	1 (3.6 %)	–	–	–
Unknown	62 (24.4 %)	21 (33.3 %)	16 (20.8 %)	2 (7.1 %)	14 (25.0 %)	8 (30.8 %)	1 (25.0 %)
KRAS status							
KRAS wild-type	71 (28.0 %)	20 (31.7 %)	21 (27.3 %)	9 (32.1 %)	13 (23.2 %)	7 (26.9 %)	1 (25.0 %)
KRAS mutation	45 (17.7 %)	11 (17.5 %)	9 (11.7 %)	6 (21.4 %)	12 (21.4 %)	7 (26.9 %)	–
Unknown	138 (54.3 %)	32 (50.8 %)	47 (61.0 %)	13 (46.4 %)	31 (55.4 %)	12 (46.2 %)	3 (75.0 %)
Tumour budding							
Yes	126 (49.6 %)	38 (60.3 %)	40 (51.9 %)	11 (39.3 %)	28 (50.0 %)	9 (34.6 %)	
No	47 (18.5 %)	12 (19.0 %)	11 (14.3 %)	10 (35.7 %)	8 (14.3 %)	4 (15.4 %)	2 (50.0 %)
Unknown	81 (31.9 %)	13 (20.6 %)	26 (33.8 %)	7 (25.0 %)	20 (35.7 %)	13 (50.0 %)	2 (50.0 %)

The variables included are: age, gender, localisation of primary tumour, tumour depth, node status, vessel invasion status, differentiation, microsatellite instability (MSI), KRAS mutational status and tumour budding

Table 2 Baseline characteristics

Characteristics	All n = 254	Desmoplastic n = 63	Pushing n = 77	Replacement n = 28	Mixed n = 56	NA n = 26	Unknown n = 4
Number							
Single	116 (45.7 %)	27 (42.9 %)	32 (41.6 %)	16 (57.1 %)	27 (48.2 %)	13 (50.0 %)	1 (25.0 %)
Multiple	138 (54.3 %)	36 (57.1 %)	45 (58.4 %)	12 (42.9 %)	29 (51.8 %)	13 (50.0 %)	3 (75.0 %)
Timing							
Synchronous	159 (62.6 %)	41 (65.1 %)	48 (62.3 %)	19 (67.9 %)	44 (78.6 %)	18 (69.2 %)	2 (50.0)
Metachronous	95 (37.4 %)	22 (34.9 %)	29 (36.7 %)	9 (32.1 %)	12 (21.4 %)	8 (28.6 %)	2 (50.0)
Distribution							
Unilobar	181 (71.3 %)	40 (63.5 %)	56 (72.7 %)	19 (67.9 %)	44 (78.6 %)	18 (69.2 %)	4 (100 %)
Bilobar	73 (28.7 %)	23 (36.5 %)	21 (27.3 %)	9 (32.1 %)	12 (21.4 %)	8 (30.8 %)	–
Surgical margin							
R0	144 (56.7 %)	33 (52.4 %)	39 (50.6)	16 (57.1 %)	32 (57.1 %)	21 (80.8 %)	3 (75.0 %)
R1	108 (42.5 %)	30 (47.6 %)	37 (48.1 %)	12 (42.9 %)	23 (41.1 %)	5 (19.2 %)	1 (25.0 %)
Unknown	2 (0.8 %)	–	1 (1.3 %)		1 (1.8 %)	–	–
Size							
<5 cm	197 (77.6 %)	52 (82.5 %)	57 (74.0 %)	23 (82.1 %)	41 (73.2 %)	20 (76.9 %)	4 (100 %)
>5 cm	57 (22.4 %)	11 (17.5 %)	20 (26.0 %)	5 (17.9 %)	15 (26.8 %)	6 (23.1 %)	–
Extrahepatic disease							
Yes	16 (6.3 %)	3 (5.0 %)	4 (5.2 %)	1 (3.6 %)	6 (10.7 %)	1 (3.6 %)	1 (25.0 %)
No	238 (93.7 %)	60 (95.2 %)	73 (94.8 %)	27 (96.4 %)	50 (89.3 %)	25 (89.3 %)	3 (75.0 %)
Treatment before surgery							
No therapy	149 (58.7 %)	34 (54.0 %)	53 (68.8 %)	14 (50.0 %)	38 (67.9 %)	7 (26.9 %)	1 (50.0 %)
5FU ± oxa	51 (20.1 %)	16 (25.4 %)	15 (19.5 %)	7 (25.0 %)	9 (16.1 %)	4 (15.4 %)	–
5FU ± oxa + bev	41 (16.1 %)	12 (19.0 %)	6 (7.8 %)	4 (14.3 %)	7 (12.5 %)	12 (46.2 %)	–
Other	13 (5.1 %)	1 (1.6 %)	3 (3.9 %)	3 (10.7 %)	2 (3.6 %)	3 (11.5 %)	1 (50.0 %)
Recurrence site							
Liver-only	115 (45.3 %)	24 (38.1 %)	35 (45.5 %)	15 (53.6 %)	28 (50.0 %)	11 (42.3 %)	2 (50.0 %)
Lung-only	32 (12.6 %)	5 (7.9 %)	9 (11.7 %)	4 (14.3 %)	12 (21.4 %)	2 (7.7 %)	–
Liver and lung	7 (2.8 %)	2 (3.2 %)	2 (2.6 %)	1 (3.6 %)	2 (3.6 %)	–	–
Other sites	36 (14.2 %)	11 (17.5 %)	11 (14.3 %)	4 (14.3 %)	6 (10.7 %)	4 (15.4 %)	–
No relapse	62 (24.4 %)	21 (33.3 %)	20 (26.0 %)	4 (14.3 %)	8 (14.3 %)	9 (34.6 %)	–
Unknown	2 (0.8 %)	–	–	–	–	–	2 (50.0 %)

The characteristics included are number of liver metastases, timing, distribution, resection margin, siza, extrahepatic disease, neo-adjuvant treatment: 5-fluororuracil (5-FU), oxaliplatin (oxa) and bevacizumab (bev) and site of recurrence

were diagnosed with two or more metastases and one third of these patients presented with metastases in both liver lobes. Baseline characteristics are listed in Table 1. Information based on the primary colorectal tumour, liver metastases and antineoplastic treatment status was registered in the database (Table 1 and 2). A total of 193 patients (76.0 %) experienced relapse during follow-up, whereas 12 patients died before recurrence of the CRC. The median follow-up time of patients censored at the end of the study was 44.6 months (95 % CI 31.9–91.5 months). When OS data were analysed August 26th 2014, 50 % of the patients were still alive.

Primary tumour characteristics

In a univariate analysis including localisation of the primary tumour, odds for RFS were better for primary tumours situated in the left in comparison to the right colon (HR = 0.64, 95 % CI 0.45–0.93, p = 0.017). Right colon and rectal cancer localisation had identical risk of recurrence (HR = 1.00, 95 % CI 0.68–1.48, p = 0.988). In a subsequent multivariate analysis, both left colon and rectal cancer localisations had significantly better OS in comparison to right colon. A significantly increased recurrence risk was observed for patients resected for node positive

compared to node negative primary tumour (HR = 1.53, 95 % CI 1.12–2.11, p = 0.008). No significant differences were found in RFS or OS when testing T stage, vessel invasion, tumour differentiation grade, microsatellite instability, *KRAS* mutational status or tumour budding (Table 3).

Characteristics of liver metastases

All 506 liver metastases were assessed. A total of 432 liver metastases were assessable for GP (85.0 %) and 74 were NA (15.0 %). The GP distribution was: desmoplastic GP in 127 CLM (25.0 %), pushing GP in 154 CLM (30.3 %), replacement GP in 69 CLM (13.6 %) and mixed GP in 82 CLM (16.1 %). From each of the 254 patients, the GP of the largest liver metastasis was chosen for further analysis (possible in 224 of the 254 patients). The distribution of GP in the largest liver metastasis was: desmoplastic GP for 63 patients (28.1 %), pushing GP for 77 patients (34.4 %), replacement GP for 28 patients (12.5 %) and mixed GP for 56 patients (25.0 %) (Tables 1, 2; Fig. 1). In cases of non-assessability of largest liver metastasis (n = 30), this was because of no viable tumour cells on the slide (n = 17), lacking tumour-liver interface on the slide (n = 9) and missing tissue blocks of largest metastasis (n = 4). An inter-observer evaluation of the assessments was carried out between two investigators (RE and MI), demonstrating a kappa value for all five GP categories (desmoplastic, pushing, replacement, mixed and NA) of 0.50 (0.44–0.55), with $\kappa = 0.69$ (0.63–0.75) when comparison was reduced to the dichotomic statement: desmoplastic versus non-desmoplastic.

Size of the largest CLM (i.e., largest diameter) varied significantly between GP: desmoplastic (median: 25 mm), pushing (median: 35 mm), replacement (median: 28 mm) and mixed GP (median: 35 mm), p = 0.001. The metastasis 'diameter' was significantly correlated with OS (p = 0.023), but not with RFS, in the univariate analysis. In the multivariate analysis, no differences were observed for RFS and OS based on the size of the largest liver metastasis (Table 3). Based on timing of the appearance of liver metastases, patients were diagnosed with synchronous (n = 159) or metachronous disease (n = 95). Of the patients with synchronous disease, 94 patients (59.1 %) had multiple liver metastases while 65 (40.9 %) had a solitary liver metastasis. Furthermore, for patients with metachronous disease (n = 95), multiple liver metastases were diagnosed in 44 patients (46.3 %) while a solitary liver metastasis was diagnosed in 51 patients (53.7 %). A significantly higher frequency of multiple CLM appeared for patients with synchronous disease versus metachronous disease, p = 0.047. The GP distribution of CLM between synchronous and metachronous disease did not differ significantly.

Recurrence pattern after hepatic resection

Site of relapse after hepatic resection was registered for all patients and correlated to GP. Liver-only relapse occurred in 115 out of 193 (59.6 %) patients with a registered relapse. Lung-only relapse was only registered in 32 patients (16.6 %), whereas relapse simultaneously to the lungs and the liver was registered in seven patients (3.6 %). Metastasis at other sites, including lymph nodes, peritoneum, brain or bone, was observed in 36 patients (18.7 %). In three cases (1.6 %), no information of recurrence site was available. No interaction was found between GP and recurrence site (Fig. 2; p = 0.87).

Growth pattern and neo-adjuvant therapy

Excluding patients with NA GPs (n = 26) or cases where relevant blocks were missing from the archives (n = 4), the GP of largest liver metastasis was assessed from 224 patients. No previous chemotherapy was given to 139 patients (62.1 %), while 85 patients received neo-adjuvant therapy with either 5FU ± oxaliplatin (n = 47) or 5FU ± oxaliplatin + bevacizumab (n = 29), respectively (Table 2). The remaining 9 patients received other regimens. Desmoplastic GP (n = 63) was observed in 34 of the chemo naive patients (54.0 %) and in 30 of the patients (46.0 %) who received neo-adjuvant treatment (n = 16 for '5FU ± oxaliplatin', n = 12 for '5FU ± oxaliplatin + bevacizumab' and n = 3 for 'other'; p = 0.22). Comparing the fraction of desmoplastic in 'untreated' *versus* the fraction of desmoplastic GP in patients who received neo-adjuvant treatment, the difference was still not significant (p = 0.12).

Patients with multiple CLMs (n = 138) were evaluated for similarity of GPs of the metastastic lesions. We found that 53 patients (38.4 %) with more than two assessable liver metastases had identical morphology in all their metastases. In the remaining 62.6 %, the GP differed between concurrent metastases.

Survival analysis

Median OS was 50.2 months (95 % CI 41.5–59.7 months). The 1 year OS estimate was 94 % (95 % CI 91.0–97.0 %), the 3 year OS estimate was 63.8 % (95 % CI 57.8–69.8 %) and the 5 year OS estimate was 41.8 % (95 % CI 33.8–49.8 %). No interaction was observed between growth pattern and "treatment groups": p = 0.16 for RFS and p = 0.91 for OS. In the univariate Cox regression analysis, we therefore stratified the patients (n = 254)

Table 3 Univariate and multivariate Cox proportional hazards on recurrence free (RFS) and overall survival (OS)

Characteristics	RFS						OS					
	Univariate			Multivariate			Univariate			Multivariate		
	HR	95 % CI	p	HR	95 % CI	p	HR	95 % CI	p	HR	95 % CI	p
Age												
per.10 year	0.95	0.83–1.10	0.501				1.15	0.96–1.37	0.136			
Gender												
Women	1						1					
Men	1.06	0.80–1.41	0.686				1.34	0.92–1.94	0.124			
Primary tumour												
Localisation												
Right colon	1			1			1			1		
Left colon	0.64	0.45–0.93	**0.017**	0.60	0.41–0.87	**0.008**	0.63	0.40–0.98	**0.039**	0.63	0.40–0.99	**0.045**
Rectum	1	0.68–1.48	0.988	0.92	0.62–1.37	0.695	0.57	0.35–0.94	**0.028**	0.57	0.34–0.95	**0.030**
Tumour depth												
T1	1						1					
T2	1	0.32–3.10	0.996				3.18	0.40–25.62	0.277			
T3	0.96	0.35–2.60	0.929				3.04	0.42–21.99	0.270			
T4	0.99	0.35–2.75	0.981				2.82	0.38–20.71	0.309			
Node status												
Node negative	1			1			1			1		
Node positive	1.53	1.12–2.11	**0.008**	1.22	1.04–1.44	**0.016**	1.20	0.81–1.77	0.364	1.05	0.86–1.28	0.664
Vessel status												
Vessel negative	1						1					
Vessel positive	1.02	0.76–1.38	0.882				1.03	0.71–1.48	0.881			
Differentiation grade												
Good	1						1					
Moderate	1.14	0.76–1.72	0.536				1.16	0.67–2.00	0.605			
Poor	0.97	0.51–1.87	0.931					0.80–3.81	0.165			
Microsatellite instability												
MSS	1						1					
MSI	1.24	0.50–3.07	0.634				2.11	0.76–5.84	0.152			
KRAS status												
KRAS wt	1						1					
KRAS mut	1.22	0.79–1.87	0.368				1.49	0.84–2.64	0.171			
Tumour budding												
No	1						1					
Yes	1.35	0.92–1.98	0.130				1.01	0.60–1.71	0.960			
CLM number												
Single	1			1			1			1		
Multiple	1.37	1.03–1.82	**0.029**	1.45	1.07–1.97	**0.017**	1.46	1.02–2.11	**0.040**	1.43	0.97–2.11	0.074
CLM timing												
Metachronous	1						1					
Synchronous	0.91	0.67–1.22	0.510				0.82	0.57–1.20	0.312			
CLM distribution												
Unilobar	1						1					
Bilobar	1.23	0.90–1.69	0.185				1.30	0.89–1.92	0.176			
CLM surgical margin												
R0	1						1					
R1	1.03	0.78–1.36	0.850				1.11	0.77–1.59	0.576			

Table 3 continued

Characteristics	RFS						OS					
	Univariate			Multivariate			Univariate			Multivariate		
	HR	95 % CI	p	HR	95 % CI	p	HR	95 % CI	p	HR	95 % CI	p
CLM size												
Max diameter	1.10	0.97–1.24	0.131	1.00	1.00–1.00	0.900	1.20	1.02–1.40	**0.023**	1.00	1.00–1.00	0.440
Extrahepatic disease												
No	1						1					
Yes	1.34	0.76–2.38	0.316				1.14	0.55–2.36	0.723			
CLM growth pattern												
Desmoplastic	1			1			1			1		
Pushing	1.44	0.98–2.12	0.064	1.37	0.93–2.02	0.116	1.35	0.82–2.24	0.239	1.35	0.81–2.25	0.252
Replacement	1.93	1.17–3.18	**0.010**	2.16	1.29–3.62	**0.003**	1.90	0.99–3.65	0.054	2.26	1.16–4.38	**0.016**
Mixed	1.78	1.18–2.68	**0.006**	1.70	1.12–2.59	**0.013**	1.66	0.98–2.81	0.058	1.72	1.01–2.94	**0.047**

Values in bold are significant at $p < 0.5$

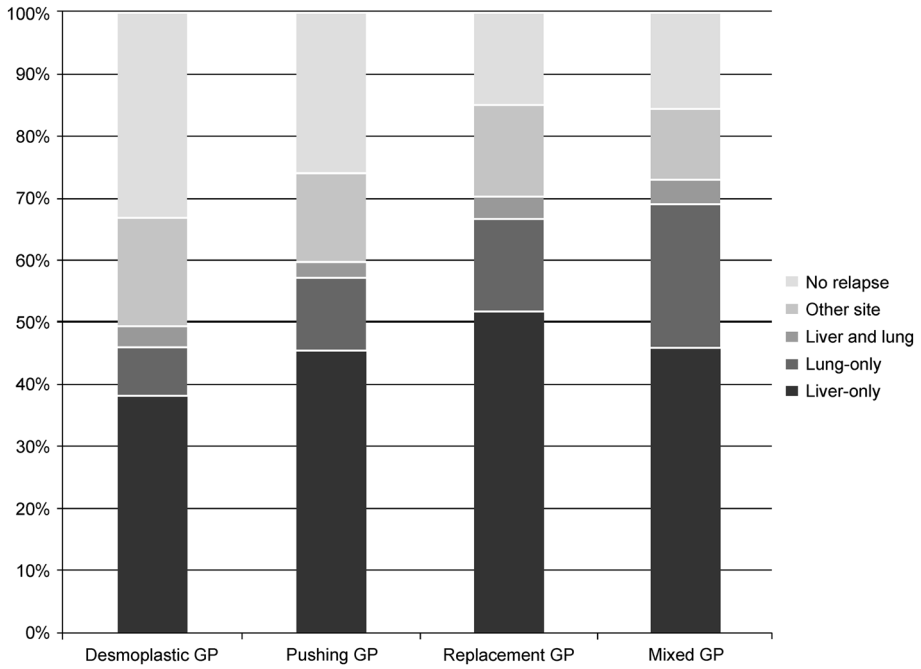

Fig. 2 Distribution of recurrence sites according to different growth patterns of the liver metastases. Liver-only recurrence is highlighted with *dark grey boxes*. Lung-only relapse is shown with *grey boxes*, while liver and lung relapse is demonstrated with a *light grey boxes*. The very *light grey coloured boxes* indicate relapse at other sites, whereas the *white boxes* demonstrate the percentage of patients without a relapse. Recurrence site according to growth pattern is shown for the desmoplastic, the pushing, the replacement and the mixed growth pattern

according to neo-adjuvant treatment status ('untreated', 'neo-adjuvant 5FU ± oxaliplatin', 'neo-adjuvant 5FU ± oxaliplatin + bevacizumab' and 'other'). Using desmoplastic GP as a reference, increased recurrence risk was observed for replacement (HR = 2.16, 95 % CI 1.29–3.62, p = 0.003) and mixed GP (HR = 1.78, 95 % CI 1.18–2.68, p = 0.006), but not pushing growth pattern (HR = 1.44, 95 % CI 0.98–2.12, p = 0.064).

The multivariate Cox analysis included variables with $p \leq 0.10$ obtained in the univariate analyses. The tested variables were localisation of primary tumour, node status, single versus multiple CLM and CLM size. Also in this analysis, RFS was found to be inferior for patients resected for liver metastases with replacement GP (HR = 2.16, 95 % CI 1.29–3.62, p = 0.003) or mixed GP (HR = 1.70, 95 % CI 1.12–2.59, p = 0.013), but not pushing GP (HR = 1.37, 95 % CI 0.93–2.02, p = 0.116) in comparison to desmoplastic GP. In the multivariate analysis, patients resected for liver metastases with a replacement GP had a significantly worse OS (HR = 2.26, 95 % CI

1.16–4.38, p = 0.016). An inferior OS was also observed for patients resected for liver metastases with a mixed GP (HR = 1.72, 95 % CI 1.01–2.94, p = 0.047) No statistically significant differences in outcome was observed between synchronous and metachronous, unilobar and bilobar or between large (>1 mm) and minimal (<1 mm) surgical margins. Results from the univariate and multivariate analyses are listed in Table 3. The Kaplan–Meier model was used to estimate RFS and OS according to GP (Fig. 3a–d). A significant difference of RFS (p = 0.03) was estimated based all GPs (Fig. 3a), while the estimate of OS was non-significant (p = 0.23; Fig. 3c).

In order to evaluate the homogeneity of the HR values, pushing, replacement and mixed GP were grouped into a 'non-desmoplastic GP' and the impact of non-desmoplastic *versus* desmoplastic GP on RFS and OS, respectively, were analysed by Cox analysis. Recurrence risk after resection of non-desmoplastic metastases was significantly higher than resection of a desmoplastic metastasis (HR = 1.59, 95 % CI 1.13–2.24, p = 0.008; univariate analysis). For OS, the difference was non-significant (HR = 1.53, 95 % CI 0.98–2.40, p = 0.064). Multivariate analysis provided almost the same HR (for RFS: HR = 1.43, 95 % CI 1.00–2.24, p = 0.051 and for OS: HR = 1.45, 95 % CI

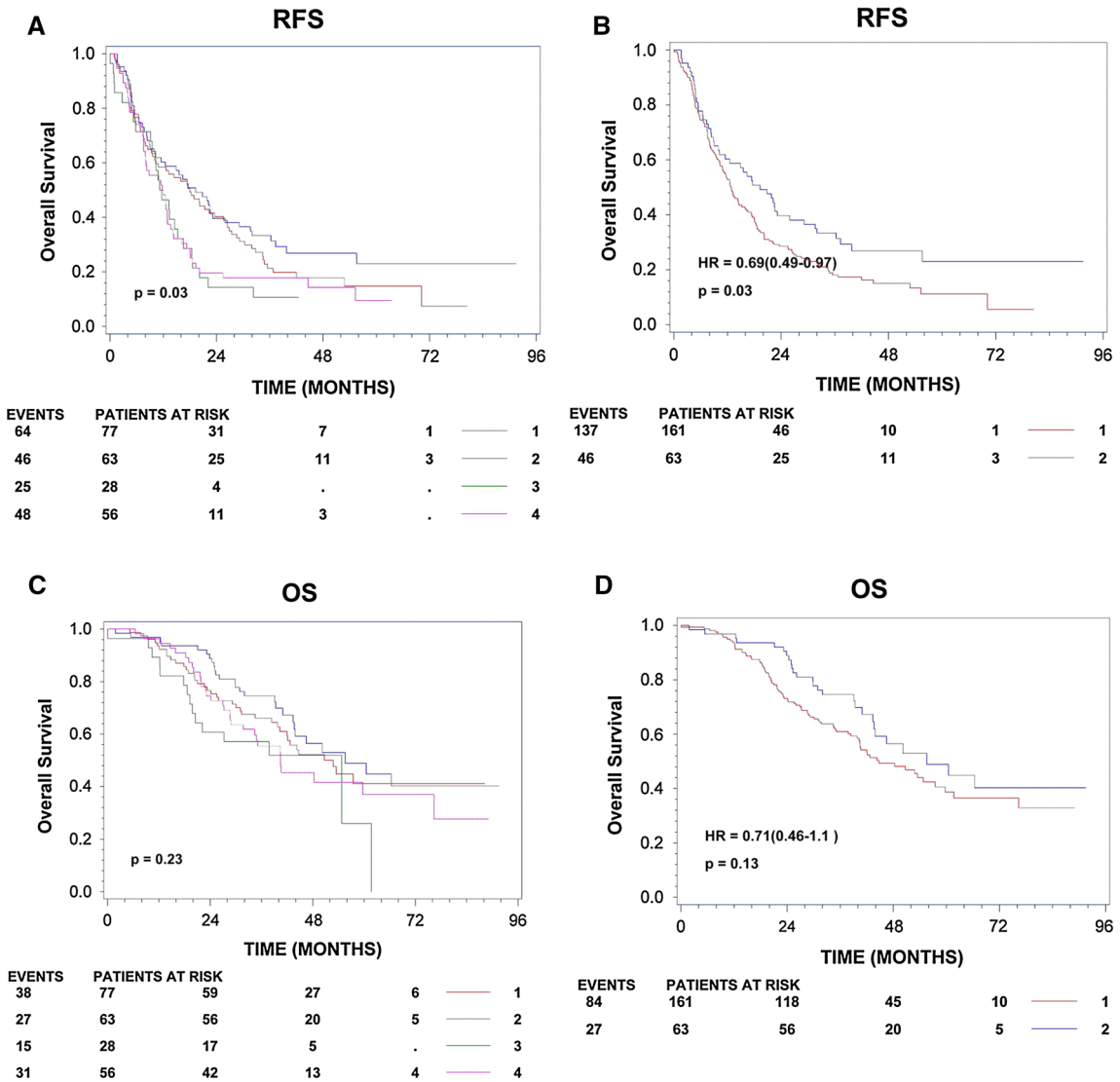

Fig. 3 Kaplan–Meier curves for recurrence free (**a**–**b**) and overall (**c**-**d**) survival. RFS (**a**) and OS (**c**) according to all growth patterns are estimated. The *blue line* represents patients resected for liver metastasis with a desmoplastic growth pattern (n = 63). The *red line* represents patients resected for liver metastases with pushing growth pattern (n = 77). The *green line* represent patients resected for liver metastases with a replacement pattern (n = 28). The *purple line* represents patients resected for liver metastases with a mixed pattern (n = 56). The Kaplan–Meier model was also applied for the estimate of RFS (**b**) and OS (**d**) according to desmoplastic *versus* non-desmoplastic. The *blue* line represents patients resected for a desmoplastic growth pattern, whereas the *red* line represents patients resected for a non-desmoplastic growth pattern

0.91–2.31, p = 0.120). The Kaplan–Meier model was applied for the estimate of RFS (p = 0.03) (Fig. 1b) and OS (p = 0.16; Fig. 1d), when patients were subdivided into desmoplastic *versus* non-desmoplastic GP.

Discussion

This study confirmed that histological GPs of resected liver metastases carry important prognostic information for post-operative clinical care. Mixed and replacement GP signal significantly poorer prognosis than desmoplastic GP, while the patients resected for pushing metastases tended to have earlier recurrence.

Several previous studies have demonstrated a prognostic impact of liver metastasis GP [20, 22–24, 26], although GP classification criteria tend to vary with time and study group. We applied the GP categories described by Vermeulen et al. [19], since these criteria appears to be the best validated. Our study is the first to demonstrate that the prognostic impact of GP is independent of neo-adjuvant treatment. A Japanese study including 152 patients [25] demonstrated superior RFS for patients resected for a liver metastasis with a collagenous encapsulation of the tumour compared to patients without this encapsulation. The study included patients who underwent liver resection in the period from 1992 to 1996, which was before the currently applied neo-adjuvant therapy of mCRC was available. Encapsulation of metastatic tumour cells, which most likely corresponds to the desmoplastic GP applied in our study, was additionally reported as a good prognostic marker in another Japanese study that included 122 patients [38]. In that study, the 5-year post-hepatectomy survival was 71 % in contrast to 19 % for patients with *versus* without encapsulation of tumour cells. Ueno and colleagues observed that encapsulation of tumour cells, by a collagen rim broader than 500 μm, was significantly correlated to fewer extrahepatic recurrences during a three year follow-up [39]. In a Swedish study by Nyström and colleagues, a superior OS was observed for patients resected for liver metastases surrounded by a rim of collagen [27]. In the study, a small cohort resected for CLMs was evaluated (n = 48) and only the GPs desmoplastic and pushing GPs were assessed. Additionally, in a recent Belgian study (n = 205) superior OS was observed for patients resected for liver metastases with a desmoplastic GP [20]. As in our study, the Belgian study evaluated the largest liver metastasis from patients with multiple metastases.

In a previous study by our group, a superior OS for patients resected for liver metastases with desmoplastic GP was found [26]. It should be mentioned that this observation was obtained despite differences in the study design. The current cohort was based on patients from the Department of Surgery who underwent a liver resection with no former surgical treatment of liver metastases (i.e., radiofrequency ablation), while the previous cohort was identified based on pathology records. Furthermore, the current study consequently chose the liver metastasis with the largest diameter from patients with multiple liver metastases, as this was suggested to represent the fastest growing or oldest tumour, thereby theoretically being a major determinant for prognosis. In our former study, the liver metastasis with the most representative tumour-liver interface was chosen. But, most importantly, the survival analyses in the current study included impact of neo-adjuvant treatment status.

In a small cohort of chemonaive mCRC patients (n = 24), we previously showed that the GP was identical in multiple liver metastases in 12 patients (50 %) [21]. This agrees with the results of the present study, showing that 38.4 % of the patients had similar GP in their metastases, independent of neo-adjuvant treatment. Identical morphology of liver metastases in the individual patient has also been described in other studies [19, 25]. It has been acceptable to pick only one metastasis for analysis, often the largest, considering it to be the worst. However, genetic and proteomic findings suggest that clonal selection and resistance to therapy can be a major challenge in the treatment of patients with metastatic disease, and our results indicate that analysis of only one metastases may not suffice.

Besides CRC, liver metastasis GP has previously been described in studies including patients with other primary cancer types such as melanoma [40] and hepatocellular carcinoma [41] as well as breast [42] and lung [43]. Assessment of histopathological characteristics of liver metastases has also been widely discussed and recently reviewed concluding that with an increasing number of hepatectomies, there will be an increased need for well-defined prognostic histopathological markers [44, 45].

Several studies have addressed the prognostic impact of synchronous *versus* metachronous CLM [46–52]. We did not observe a significant difference in RFS or OS of synchronous *versus* metachronous liver metastases albeit late metastases (emerging >1 year after resection of primary tumour) tend to have a better prognosis than early and synchronous metastases [53]. However, there were significantly more patients with multiple synchronous CLM than multiple metachronous CLM.

In conclusion, desmoplastic GP in resected liver metastases predicts a reduced risk of recurrence in comparison to other GPs. The observation needs to be validated in an independent patient cohort. No difference of OS was observed for patients resected for liver metastasis of desmoplastic *versus* non-desmoplastic GP. The prevalence and impact of desmoplastic GP was independent of whether or not neo-adjuvant chemotherapy had been given.

This GP therefore seems to be clinically relevant for post-operative patient management, such as the role of adjuvant chemotherapy and intensity of the follow-up programme for these "lower risk" patients.

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Conflict of interest None.

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