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Cancer Imaging

Positron Emission Tomography/Computed Tomography and Biomarkers for Early Treatment Response Evaluation in Metastatic Colon Cancer

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Metastatic colon cancer • Treatment response evaluation • Positron-emission tomography • Tissue inhibitor of metalloproteinases-1 • Carcinoembryonic antigen • Urokinase plasminogen activator receptor domain I

Abstract

Background. Treatment options for metastatic colon cancer (mCC) are widening. We prospectively evaluated serial 2-deoxy-2-[18F]fluoro-D-glucose positron-emission tomography/computed tomography (PET/CT) and measurements of tissue inhibitor of metalloproteinases-1 (TIMP-1), carcinoembryonic antigen (CEA), and liberated domain I of urokinase plasminogen activator receptor (uPAR(I)) for early assessment of treatment response in mCC patients.

Methods. Thirty-three mCC patients scheduled for first-line chemotherapy with capecitabine, oxaliplatin (CAPOX), and bevacizumab participated; 27 were evaluated by PET/CT before treatment, after one and four treatment series. Morphological and metabolic response was independently assessed according to Response Evaluation Criteria in Solid Tumors and European Organization for Research and Treatment of Cancer PET criteria. Plasma TIMP-1, plasma uPAR(I), and serum CEA were determined.

Results. Metabolic response after one treatment course predicted the ability of CAPOX and bevacizumab to induce morphological response after four treatment series with a sensitivity of 80%, specificity of 69%, and odds ratio of 13.9 (95% confidence interval [CI] 1.9; 182). Early metabolically stable or progressive disease was associated with shorter progression-free survival (hazard ratio [HR] = 3.2 [CI 1.3; 7.8]). Biomarker levels at early evaluation were associated with shorter OS (TIMP-1 per unit increase on a log-2-transformed ng/mL scale: HR = 2.6 [CI 1.4; 4.9]; uPAR(I) per 25 fmol/mL increase: HR = 1.5 [CI 1.1; 2.1]).

Conclusion. This monocentric study demonstrated predictive value of early metabolic PET response and prognostic value of TIMP-1 and uPAR(I) levels in mCC treated with CAPOX and bevacizumab. Results support investigation of PET/CT, TIMP-1, and uPAR(I) guided early treatment adaptation in mCC. ***The Oncologist*** 2014;19:1–9

Implications for Practice: This study investigated early response evaluation in a well-characterized cohort of patients with chemotherapy-naïve metastatic colon cancer receiving first-line combination chemotherapy with capecitabine, oxaliplatin (CAPOX), and bevacizumab. Our results demonstrated the ability of early 2-deoxy-2-[18F]fluoro-D-glucose-positron-emission tomography/computed tomography (PET/CT) after only one treatment series to predict radiologic response after four treatment series. Biomarkers tissue inhibitor of metalloproteinases-1 (TIMP-1) and urokinase plasminogen activator receptor (uPAR(I)) measured after one treatment series, furthermore, could be associated with the overall survival of the patients. These results support investigation of a combination of PET/CT, TIMP-1, and uPAR(I) for early evaluation of response to treatment with CAPOX and bevacizumab, with a perspective of PET/CT-guided early, personalized treatment adaptation in metastatic colon cancer.

Introduction

Colorectal cancer (CRC) is one of the three malignancies most commonly causing cancer deaths in developed countries [1]. Colon cancer (CC) accounts for two thirds of all CRC cases and differs from rectal cancer in gender distribution and sites of metastases [2–4].

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About 25% of all patients with newly diagnosed CRC have metastatic disease [3]. In addition, 40% of patients undergoing intended curative resection for CRC will eventually develop local recurrence or metastatic disease [5].

Treatment options for metastatic CRC (mCRC) have widened over the last decades and now include surgical or ablative treatment of metastases and an increasing number of options in combination chemotherapy [6]. The antimetabolite 5-fluorouracil (5-FU) and the oral 5-FU prodrug capecitabine remain central chemotherapy agents. They are partnered with the DNA-damaging agents irinotecan or oxaliplatin, leading to response rates of 40%–50% and prolonging overall survival (OS) [7, 8]. Biological agents such as the monoclonal antibodies cetuximab, targeting the epidermal growth factor receptor (EGFR), and bevacizumab, targeting vascular endothelial growth factor A, have demonstrated additional benefits for selected patients [9–11]. Still, patients diagnosed with mCRC face 5-year survival rates of less than 10% [12]. Given the reasonably wide range of treatment options available, reliable early chemotherapy response evaluation and predictive biomarkers that could help to switch patients with unresponsive tumors to more effective treatments in time are much needed.

For assessment of disease response to chemotherapy, tumor size-based radiologic criteria using the Response Evaluation Criteria in Solid Tumors (RECIST) [13] and the revised RECIST 1.1 [14] are the gold standard in clinical trials. However, these criteria have limitations evaluating the effects of cytostatic targeted treatments that do not necessarily lead to tumor shrinkage. Reliable computed tomography (CT)-based evaluation of therapy response is not possible before at least 6–8 weeks and often up to 3 months after the initiation of costly and often cumbersome treatment.

There is evidence that functional imaging of tumor metabolism using positron-emission tomography (PET) and the radiotracer 2-deoxy-2-[18F]fluoro-D-glucose (FDG) is useful for early metabolic response assessment in various malignancies, including CRC [15]. A study in patients with locally advanced rectal cancer receiving neoadjuvant 5-FU, leukovorin, and oxaliplatin (FOLFOX) showed that metabolic response was able to predict pathological treatment response and had prognostic value [16].

Early changes in tumor metabolism measured as standardized uptake values (SUV) in serial FDG PET scans have been shown to predict radiological response and survival in mCRC patients receiving different types of palliative chemotherapy [17, 18].

Recommendations for PET criteria for response evaluation of anticancer treatment were proposed by the European Organization for Research and Treatment of Cancer (EORTC PET criteria) in 1999 [19] and in the PET response criteria (PERCIST) in 2009 [20].

Mutations in the KRAS or BRAF gene predict unresponsiveness of mCRC to EGFR-targeted treatments [21, 22]. Other than that, the glycoprotein carcinoembryonic antigen (CEA) is currently the only biomarker routinely used in therapy monitoring in mCRC [23, 24]. Changes in serum CEA levels mainly guide clinical decisions in situations with conflicting imaging results and clinical treatment response [24]. Tissue inhibitor of metalloproteinases-1 (TIMP-1) is a glycoprotein that inhibits the proteolytic, invasive activity of metalloproteinases [25], but it is also involved in inhibition of apoptosis [25] and promotion of angiogenesis [26]. Plasma TIMP-1 has shown prognostic value in mCRC treated with capecitabine and oxaliplatin (CAPOX) [27] and both predictive and prognostic value in mCRC treated with irinotecan-based chemotherapy regimens [28].

The urokinase plasminogen activator receptor (uPAR) is present in stromal cells at the invasive front of CC [29]. The liberated domain I of uPAR (uPAR(I)) is an independent prognostic marker in CRC [30, 31].

The aim of this study was to prospectively evaluate the value of FDG PET/CT and biomarkers TIMP-1, CEA, and uPAR(I) in early response assessment and for prediction of outcome in a cohort of metastatic CC (mCC) patients treated with first-line chemotherapy with CAPOX and bevacizumab.

Patients and Methods

Patients

Patients diagnosed with mCC and scheduled for palliative first-line chemotherapy with CAPOX and bevacizumab at the Department of Oncology and Hematology, Næstved Hospital, between July 2009 and September 2011 were eligible. Patients with underlying inflammatory bowel disease, diabetes, manifest kidney disease, or a history of malignant neoplasm other than CC or nonmelanoma skin cancer were not included in the study. Patients suffering from claustrophobia, weighing more than 150 kg or with a history of allergic reactions to intravenous (iv.) iodinated contrast agents, were excluded. The study was approved by the Research Ethics Committee of Region Zealand (SJ129) and complied with the Helsinki Declaration. Written informed consent was obtained.

Pretreatment PET/CT was conducted in 33 patients. Measurable lesions according to RECIST 1.1 could not be identified in two patients, which were excluded. Three patients died before initiation of the planned chemotherapy. One patient never received all three components of the planned combination therapy, leaving 27 patients for treatment response evaluation (Table 1). One patient withdrew from further treatment after one treatment cycle because of rapidly declining performance status. In total, 26 patients underwent all scheduled PET/CT scans (Fig. 1).

Treatment and Imaging Schedule

Treatment was administered according to guidelines and consisted of oxaliplatin (Hospira, Lake Forest, IL, http://www.hospira.com), 130 mg/m^2 iv. per 30 minutes; bevacizumab (Avastin; Roche Pharmaceuticals, Basel, Switzerland, http://www.roche-applied-science.com), 7.5 mg/kg iv. per 15 minutes; and orally administered capecitabine (Xeloda; Roche Pharmaceuticals), 1,000 mg/m^2, twice daily for 2 weeks, followed by a 1-week interval, in repeat cycles. Doses were reduced and/or treatment intervals were extended in case of severe toxicity.

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Table 1. Characteristics of patients undergoing response evaluation

Number of patients		27 (100 %)
Gender: male/female		13 (42 %)/18 (58 %)
Age: years, median (range)		66 (42–81)
Primary tumor resected: Yes/No		5 (19 %)/22 (81 %)
Prior adjuvant chemotherapy: Yes/No		0 (0 %)/27 (100 %)
KRAS/BRAF status: WT/MUT		13 (48 %)/14 (52 %)
MMR: deficient/normal/NA		3 (11 %)/19 (70 %)/5 (19 %)
TIMP-1 (ng/mL): median (range)	Pretreatment	331 (126–1,805) ($n = 26^a$)
	Early evaluation	240 (118–588) ($n = 27$)
	Late evaluation	189 (81–1,452) ($n = 24^a$)
CEA (ng/mL): median (range)	Pretreatment	47 (1–5,376) ($n = 26^a$)
	Early evaluation	37 (2–4,926) ($n = 26^a$)
	Late evaluation	13 (1–753) ($n = 24^a$)
uPAR(I) (fmol/mL): median (range)	Pretreatment	33 (12–162) ($n = 27$)
	Early evaluation	32 (11–135) ($n = 22^a$)
	Late evaluation	31 (12–115) ($n = 25^a$)

[a]Missing numbers due to technical problems at blood sampling/ processing.
Abbreviations: CEA, carcinoembryonic antigen; MMR, mismatch repair system; MUT, mutation detected; *n*, number of patients; NA, not assessed; TIMP-1, tissue inhibitor of metalloproteinases-1; uPAR(I), urokinase plasminogen activator receptor domain I; WT, wild type.

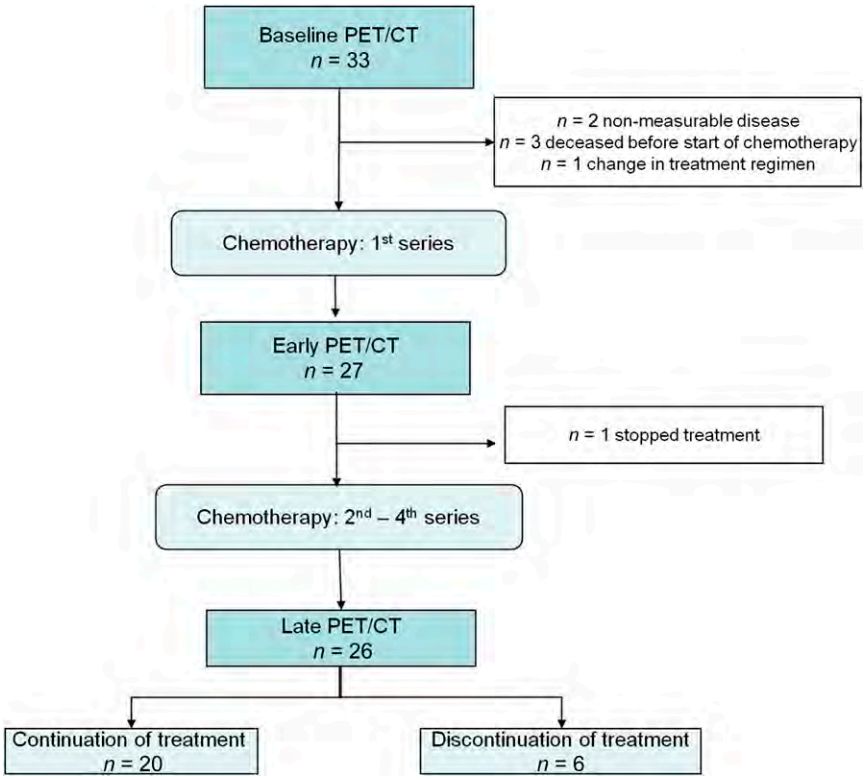

Figure 1. Flow chart of study. "Early" refers to evaluation after one chemotherapy treatment series; "late" refers to evaluation after four chemotherapy treatment series. Abbreviations: *n*, number of patients; PET/CT, positron emission tomography/computed tomography.

Pretreatment PET/CT was performed a mean of 12 days (range 0–44) before treatment start, early evaluation a mean of 20 days (range 15–48) after start of first treatment cycle, and late evaluation a mean of 20 days (range 14–72) after start of the fourth treatment cycle.

Three patients had treatment delays because of surgical procedures. Treatment-associated toxicity requiring dose reduction occurred in 10 patients; additional 5 patients had initial dosage reduced as a result of age or performance status.

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Table 2. RECIST 1.1 categories and therapy response evaluation PET categories according to adapted EORTC PET criteria

Response category	Adapted EORTC PET criteria	RECIST 1.1
Responders		
CR	Resolution of FDG uptake in all lesions to background	Disappearance of measurable disease
PR	Reduction >25% in sum of SUV_{max} of up to 5 target lesions	≥30% decline in sum of largest diameters of up to 5 target lesions
Nonresponders		
SD	No PD or PR/CR	No PD or PR/CR
PD	New FDG uptake in metastatic lesions (new lesions and/or progression in existing nontarget lesions); increase >25% in sum of SUV_{max} of up to 5 target lesions; visible increase in extent of FDG uptake (20% in largest diameter)	New lesions; increase ≥20% in the sum of the largest diameters of up to 5 target lesions and absolute increase of ≥5 mm

Abbreviations: CR, complete response; EORTC, European Organization for Research and Treatment of Cancer; FDG, 2-deoxy-2-[18F]fluoro-D-glucose; PD, progressive disease; PET, positron emission tomography; PR, partial remission; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; SUV_{max}, maximal standardized uptake value.

Peripheral blood samples for biomarker analyses were collected according to a validated standard operating procedure [32] immediately before injection of the FDG tracer.

PET/CT Imaging

Acquisition

Patients fasted for at least 6 hours before examination, resulting in median serum glucose level of 5.5 mmol/L (range 4.2–14.4) at tracer injection. A median FDG dose of 408 MBq (target dose: 400 MBq; range 184–444) was administered iv., followed by a median resting uptake period of 71 minutes (intended: 60 minutes; range 57–123).

All PET/CT scans were performed on the same PET/CT scanner (Siemens Biograph 40; Siemens, Erlangen, Germany, http://www.healthcare.siemens.com) from the base of the skull to the upper thighs. The CT examination was enhanced by iodinated contrast agent given orally (Optiray [Covidien, Hazelwood, MO, www.covidien.com], 300 mg iodine/mL, 20 mL in 500 mL water 30 minutes before start) and i.v. (100 mL, 5 mL/s immediately before start).

CT parameters were tube potential 120 kV, 2 mm slices with a collimation of 1.2 mm × 24, pitch 0.8, CareDose4D on, quality reference mAs 170, and varying tube current for dose reduction.

PET emission data were acquired for 3 minutes at each of six or seven axial bed positions immediately after acquisition of the diagnostic CT images, and low-dose CT data were used for attenuation correction. Patients were instructed to breathe normally and immobilized using cushions.

Pretreatment, early, and late PET/CT were carried out under identical conditions in terms of patient preparation and positioning, image reconstruction, and processing. The time between tracer administration and start of imaging differed by a maximum of 19 minutes in all but three patients; in these, the time differences were up to 23 minutes, 30 minutes, and 53 minutes, respectively. Serum glucose levels differed by a maximum of 1.5 mmol/L in all but one patient, who had serum glucose levels of 6.3 mmol/L, 8.8 mmol/L, and 14.4 mmol/L.

Image Reconstruction

PET data were reconstructed using ordered-subset expectation maximization iterative reconstruction with four iterations, eight subsets. Parameters were 5 mm full width at half-maximum Gaussian filter, pixel size 4.07 mm × 4.07 mm, 3-mm slices. PET data were corrected for decay, scatter, and random events, and attenuation corrected using low-dose CT data.

CT data were reconstructed using filtered back projection with a B40f medium kernel, slice increment 1.0 mm, 2-mm slices. Studies were archived on a picture archiving and communications system (GE Healthcare, Little Chalfont, U.K., http://www.gehealthcare.com) and displayed on Siemens Leonardo workstations for analysis.

Radiation Dose

The effective radiation dose for the PET/CT scan was approximately 20 mSv with 400 MBq × 0.02 mSv = 8 mSv from the FDG dose [33] and 12 mSv from the CT scan [34]. Routinely, patients would have undergone two CT scans, replaced by a total of three PET/CT scans in the study, with an equivalent of 36 mSv additional radiation dose. Considering the advanced malignant nature of the disease studied, the additional lifetime cancer risk was regarded acceptable.

Image Analysis

A clinical description of late evaluation PET/CT guided attending clinicians in treatment decisions. They had access to a clinical description of early response assessment but agreed not to make any treatment changes accordingly.

PET/CT data were analyzed in two independent readings by experienced certified radiologists and nuclear medicine physicians working in pairs. All readers were unaware of treatment outcome and blinded to results of other readings. Attenuation-corrected PET images, CT images, and coregistered PET/CT images were displayed together. Semiquantitative analysis of PET images was performed by scanner-specific software calculating SUV using the ratio of the tissue radioactivity concentration in the tumor volume (in MBq/kg) at time t, $c(t)$, and the injected activity dose (in MBq) at time of injection ($t = 0$) divided by the patient body weight (in kg):

$$\mathrm{SUV}(t) = \frac{c(t)}{\text{Injected activity}/\text{Body weight}}$$

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Response Assessment

Radiologists and nuclear medicine physicians jointly recorded all organs or sites with metastatic involvement in every patient, including sites of nonmeasurable disease. Tumor diameters and maximum SUV (SUV_{max}) of up to three lesions at each site were assessed.

For anatomical response assessment, up to five target lesions per patients were defined according to RECIST 1.1 [14]. Changes in the sum of tumor diameters of target lesions before and after treatment were assessed in percentages, and patients were categorized into anatomical response groups: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) [14] (Table 2).

Metabolic response categories were delimited according to adapted EORTC PET criteria [19], independently defining as target lesions up to five lesions per patient displaying the highest SUV_{max} values at pretreatment PET/CT. Changes in the sum of SUV_{max} of target lesions before and after treatment were assessed in percentages, and patients were categorized into metabolic response groups (Table 2).

Response at early PET/CT compared with pretreatment PET/CT was labeled early response; response at late PET/CT compared with pretreatment PET/CT was labeled late response.

In case of differences in metabolic or anatomical response category assessments, a third experienced radiologist or nuclear medicine physician decided on the final response category.

Tissue Biomarkers

Resected tumors or biopsy material underwent immunohistochemical staining for mismatch repair system deficiencies using mouse anti-human MLH-1 (clone ES05; Novocastra, Newcastle upon Tyne, U.K., http://www.novocastra.co.uk), MSH-2 (clone FE11; Biocare Medical, Concord, CA, http://www.biocare.net), PMS-2 (clone A16-4; BD Biosciences, San Diego, CA, http://www.bdbiosciences.com), and rabbit anti-human MSH-6 (clone EP49; Epitomics, Bulingame, CA, http://www.epitomics.com).

KRAS mutational analysis for 11 different mutations in codons 12, 13, and 61, and, in KRAS wild-type patients, BRAF mutational analysis for seven different mutations in codon 600, 464, 466, and 469 was performed by pyrosequencing on a PyroMark Q24 system (Qiagen, Düsseldorf, Germany, http://www.qiagen.com) using the therascreen KRAS Pyro Kit and therascreen BRAF Pyro Kit (Qiagen). All samples were measured in duplicate.

Circulating Biomarkers

Blood samples were collected in endotoxin-free tubes (Venosafe; Terumo, Leuven, Belgium, http://www.terumo-europe.com). All samples were kept at room temperature for a maximum of 1 hour. After centrifugation, serum and plasma supernatants and cell pellets were transferred separately to cryo-tubes and stored at −80°C. TIMP-1 protein levels were determined in EDTA plasma using a validated kinetic-rate enzyme-linked immunosorbent assay platform [35]. uPAR(I) levels were determined in citrate plasma using a validated time-resolved fluorescence assay [31]. Serum CEA concentrations were analyzed using an automated ADVIA Centaur analyzer (Siemens). All analyses were performed at the end of follow-up by technicians blinded to clinical outcomes.

Statistical Analysis

To assess the ability of early metabolic response data for predicting late radiologic response, sensitivity and specificity with binomial confidence limits were calculated, as well as age- and sex-adjusted odds ratio (OR) based on logistic regression. The Kaplan-Meier method and Cox regression were used to analyze progression-free survival (PFS) and OS. The earliest date of progression was defined as the date of late PET/CT. OS was evaluated March 4, 2013.

Two landmark time points were used for survival analysis: the first day of the first series of chemotherapy treatment (baseline), using pretreatment information of PET/CT data and biomarkers, and the date of early PET/CT evaluation, using updated information of PET/CT data and biomarkers. Only the patients alive and event-free were included at the respective landmark time points. Cox regression was adjusted for patient age and sex and applied to log-2-transformed TIMP-1 and CEA levels and untransformed uPAR(I) levels. Results were presented as hazard ratios (HR) with 95% confidence intervals (CI). Levels of soluble biomarkers at different evaluation time points were compared using paired Wilcoxon signed rank test. The level of statistical significance was set at 5%. All data analyses were performed using R [36].

Results

Prognostic and Predictive Value of PET/CT Imaging

Response Assessment

No patient showed CR. When dichotomizing patients into responders (PR) and nonresponders (PD + SD), early PET response category correctly predicted late CT response category in 19 of 26 cases (73%) with a sensitivity of 80% [CI 44; 98] and specificity of 69% [CI 41; 89]. Adjusted for age and gender, this translated into an OR of 13.9 [CI 1.9; 182] for early metabolic prediction of late radiologic response to first-line treatment with CAPOX and bevacizumab for mCC ($p = .02$).

Survival

Median PFS (Kaplan-Meier method) of patients treated with CAPOX and bevacizumab was 182 days (interquartile range [IQR] 85; 272] calculated from the start of the first treatment series. Metabolic nonresponse assessed at the early evaluation landmark significantly raised the risk of disease progression (HR = 3.2 [CI 1.3; 7.8]; $p = .01$; Fig. 2).

Median OS (Kaplan-Meier method) was 357 days (IQR 161; 763), calculated from the start of the first treatment series. The risk of death was increased (but not significantly) for metabolic nonresponders compared with responders assessed at the early evaluation landmark time point (HR = 1.5 [CI 0.6; 3.7]; $p = .38$; Fig. 2).

At baseline, Cox regression adjusted for age and gender could not show a significant change of OS for changes in SUV_{max} in the most FDG-avid lesion at pretreatment PET/CT (HR = 1.03 [CI 0.96; 1.11]; $p = .36$). A 10-mm increase in diameter of the largest lesion measured on pretreatment PET/CT was

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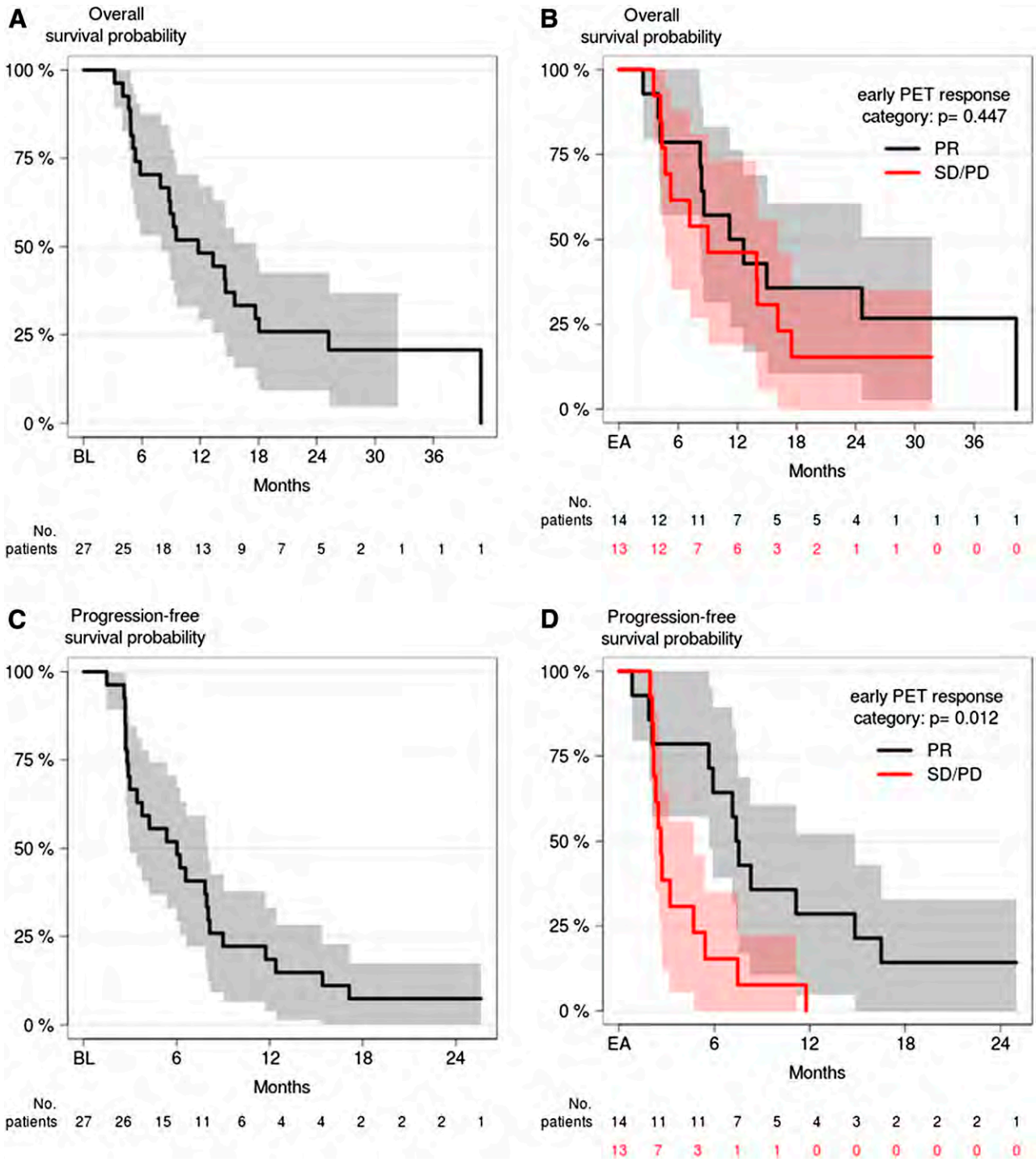

Figure 2. Kaplan-Meier curves of overall survival **(A, B)** and progression-free survival **(C, D)** grouped according to response category. Abbreviations: BL, baseline (start of chemotherapy); CT, computed tomography; EA, early evaluation; PD, progressive disease; PET, positron emission tomography; PR, partial remission; SD, stable disease.

significantly associated with a higher risk of death (HR = 1.08 [CI 1.02; 1.14]; $p = .006$). Also, the number of metastatic sites at pretreatment PET/CT increased the risk of death (HR = 1.24 [CI 1.03; 1.50]; $p = .023$) (Table 3).

Prognostic Value of Biomarkers

KRAS/BRAF mutational status at baseline was not significantly associated with OS (HR = 1.06 [CI 0.42; 2.72]; $p = .897$; Table 3).

TIMP-1

High pretreatment and early posttreatment levels of TIMP-1 levels were significantly associated with OS (see Table 3).

TIMP-1 levels (see Table 1) decreased after treatment (paired Wilcoxon rank test $p = .00013$ at early evaluation). Taking biological intrasubject variation [37] into account, 10 of 27 patients at early evaluation were identified who individually displayed a significant decrease (>31%) in

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Table 3. Association between explanatory variables and survival, modeled in Cox regression models adjusted for age and gender

Explanatory variable	Measured at	Effect on	HR	95% CI	*p* value
SUV_{max}	Pretreatment	OS since baseline	1.03	0.96; 1.11	.363
Tumor diameter (10-mm increase)	Pretreatment	OS since baseline	1.08	1.02; 1.14	.006
Number of metastatic sites	Pretreatment	OS since baseline	1.24	1.03; 1.5	.023
KRAS/BRAF (WT)	Pretreatment	OS since baseline	1.06	0.42; 2.72	.897
TIMP-1 (1 unit increase on a log-2 transformed ng/mL scale)	Pretreatment	OS since baseline	1.68	1.15; 2.44	.007
	Early evaluation	OS since early evaluation	2.58	1.35; 4.94	.004
CEA (1 unit increase on a log-2 transformed ng/mL scale)	Pretreatment	OS since baseline	1.09	0.94; 1.28	.246
	Early evaluation	OS since early evaluation	1.12	0.96; 1.30	.165
uPAR(I) (25 fmol/mL increase)	Pretreatment	OS since baseline	1.40	1.12; 1.75	.003
	Early evaluation	OS since early evaluation	1.51	1.11; 2.06	.008

Abbreviations: CEA, carcinoembryonic antigen; CI, confidence interval; HR, hazard ratio; OS, overall survival; SUVmax, maximal standardized uptake value; TIMP-1, tissue inhibitor of metalloproteinases 1; uPAR(I), urokinase plasminogen activator receptor domain I; WT, wild type.

plasma TIMP-1, but no patients displayed a significant increase (>45%).

CEA

CEA measured before treatment or at early evaluation was not significantly associated with survival outcomes (Table 3).

Pretreatment CEA levels (Table 1) were increased above the cutoff used in diagnosis of CRC [24] at 5 ng/mL in all but three patients. The CEA levels decreased after treatment (paired Wilcoxon rank test $p = .056$ at early evaluation).

uPAR(I)

High uPAR(I) levels were associated with a significant worsening of survival outcomes in mCC patients treated with CAPOX and bevacizumab both before treatment and at early response evaluation (Table 3).

The uPAR(I) levels (Table 1) decreased after treatment (paired Wilcoxon rank test $p = .0048$ at early evaluation).

Rater Agreement

Discrepancies in early metabolic response category assessment between the two independent PET/CT readers were found in 1 of 27 cases (4%) and in late radiologic response category assessment in 2 of 26 cases (7%).

Discussion

This prospective, monocentric PET/CT study in mCC patients showed that metabolic response on FDG PET after a single course of CAPOX and bevacizumab could predict the ability of this treatment to induce morphological response after four treatment series.

Early metabolic response on FDG PET was of significant prognostic value for PFS: patients experiencing SD or PD at metabolic response assessment after one treatment course had a three times higher hazard toward shorter PFS. At the same time, high TIMP-1 and high uPAR(I) levels at baseline and after a single course of treatment conveyed an increase of hazard toward both shorter PFS and OS.

This is important because early predictors of unfavorable outcome in a specific chemotherapy regimen could guide treatment modifications.

Early Metabolic Response Assessment

The present results regarding prediction of PFS are similar to findings in a retrospective study in patients receiving neoadjuvant chemotherapy with FOLFOX or 5-FU, leukovorin, and irinotecan for CRC liver metastases [38]. In this study, metabolic SD or PD was found to carry a multivariate HR of 3.6 for shorter PFS [38].

Hendlisz et al. [18] reported results of a prospective study of 40 mCRC patients receiving different types of chemotherapy as first- or second-line treatment. A dominant metabolic response was assessed based on changes in SUV_{max} of up to 10 individual lesions. Early metabolic response identified radiologic response with an accuracy of 67.5%, which is similar to the one found in the present study, but with a higher sensitivity (100%) [18]. The negative predictive value (NPV) was reported as 100% [18], comparing with 85% in the present study. Hendlisz et al. [18] further found that early metabolic response was significantly associated with OS, but not PFS. De Bruyne et al. [39] report that high SUV_{max} at follow-up correlated with shorter PFS in a group of mCRC patients receiving neoadjuvant chemotherapy, including bevacizumab.

Five of 27 patients in the present study showed heterogeneous response patterns that resulted in nontarget evaluation defining PD at early metabolic evaluation. The EORTC PET recommendations used in this study address the issue of heterogeneous therapy response both by choosing several target lesions and by having nontarget response influence response classification. Classification of heterogeneous response is approached in different ways by other groups, most prominently in the PERCIST recommendations, which advocate that at every time point one single most metabolic active tumor lesion is representative of the patient´s disease [20], and in the work of Hendlisz et al. [18], in which a dominant response pattern is identified after assessment of response individually for up to 10 metastatic lesions.

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Most studies in the field use different definitions of metabolic response, look at heterogeneous study populations receiving various different types of chemotherapy, and as such arrive at varying cutoff values for prediction or prognosis, as reviewed by Vriens et al. [40]. In the present study, all patients in a well-characterized CC-only cohort received the exact same treatment combination and were otherwise chemotherapy-naive. This is unique in the literature and a major force of this study. Metabolic response was assessed according to EORTC PET criteria using up to five target lesions in serial contrast-enhanced PET/CT scans with a standard diagnostic protocol. A 25% cutoff delimiting stable metabolic disease was chosen and used both after one and four treatment series. The EORTC definition of PR suggests a "reduction of a minimum of 15%–25% in tumor [18F]-FDG SUV after one cycle of chemotherapy, and greater than 25% after more than one treatment cycle" [13]. The 15% threshold has been criticized as too modest [20]. None of the participating patients in this cohort had a SUV_{max} reduction of between 15% and 25% after one treatment series.

To maximize benefits of the hybrid technology, nuclear medicine physicians and radiologists collaborated in identifying sites of metastatic lesions and disease burden on PET/CT scans. However, choice of target lesions for evaluation of metabolic response was independent of anatomical criteria. Overall, our data demonstrate this evaluation method to be easily reproducible in the clinical setting.

There are methodological flaws that should be corrected in further studies: clinicians were not blinded to results of the early PET/CT evaluation. However, no patient was taken off the planned treatment as a result of findings at the early PET/CT evaluation; one patient who discontinued treatment after early evaluation did so in spite of signs of metabolic PR.

In three patients a prolonged interval between tracer injection and start of PET scan could have affected the validity of SUV_{max}-based response evaluation. One patient had a prolonged uptake period before the pretreatment scan; in this patient, both early and late responses were classified as PD as a result of a new metastatic lesion. Late evaluation scans in two patients had prolonged uptake periods; response was in both classified as metabolic PR anyway. Prolonged uptake therefore had no influence on response classification in this cohort.

In eight patients, time from pretreatment scan to start of chemotherapy was more than 16 days; this could lead to underestimation of therapy response and NPV. The EORTC recommends an interval of no more than 2 weeks between pretreatment scan and start of therapy [13].

Value of Biomarkers in Early Response Assessment

In mCRC patients receiving CAPOX without the addition of bevacizumab, prognostic value toward shorter OS of high pretreatment TIMP-1 plasma levels and plasma TIMP-1 increase after treatment has been reported [27]. Data in the present study show prognostic value of both pre- and early posttreatment TIMP-1 and uPAR(I) levels. These results were obtained in multivariable regression models adjusted for age and gender. The choice of these covariates was based on findings of age- and gender-related differences in TIMP-1 levels in CC patients [41]. Because of the small sample size, no other covariates were included in the analyses.

This study was not conceived to explore cutoff values for prediction of radiological response or survival for the different markers. Prognostic value of early posttreatment TIMP-1 and uPAR(I) must be confirmed in larger studies allowing for multivariable regression analyses, including other known predictors as covariates. If appropriate cutoff values can be established, TIMP-1 and uPAR(I) levels after only one treatment series could potentially help identify the mCC patients who are less likely to benefit from further treatment with CAPOX and bevacizumab.

Conclusion

This monocentric study demonstrated predictive value of early metabolic response assessment in mCC receiving first-line treatment with CAPOX and bevacizumab, both regarding prediction of later anatomical response and PFS. Furthermore, prognostic value of pre- and early posttreatment values of TIMP-1 and uPAR(I) was demonstrated. Data from this well-characterized cohort support further investigation of FDG PET/CT, TIMP-1, and uPAR(I) guided early treatment modifications in mCC.

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Disclosures

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