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Mucinous adenocarcinomas: Poor prognosis in metastatic colorectal cancer

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Abstract *Purpose:* Mucinous histology of metastatic colorectal cancer (CRC) has been associated with poor prognosis, however this has never been assessed in large well-defined study populations treated with the current used systemic agents. We investigated the prognostic value of mucinous histology in two large phase III studies in metastatic CRC.
Patients and methods: The study population included 1010 metastatic CRC patients who were treated with chemotherapy and targeted therapies in two phase III studies. Patients were classified according to the histology of the primary tumour in mucinous adenocarcinomas (MC) and non-mucinous adenocarcinomas (AC).
Results: Patients with MC ($n = 99$) were older, had more often a normal serum lactate dehydrogenase (LDH), extrahepatic localisation of metastases, larger primary tumour diameter and a higher T classification compared to patients with AC ($n = 911$). A deficient mismatch repair system and *BRAF* mutations were observed in 17% and 22% of patients with MC, compared to 3% and 7% in patients with AC, respectively. Clinical outcome was investigated in both studies separately, showing a worse overall survival (OS), progression free survival and overall response rate in patients with MC compared to patients with AC. Patients with MC received less cycles of treatment compared to AC, but did not suffer from a higher incidence of grade 3/4 toxicity. In multivariate analysis, mucinous histology was as an independent negative prognostic factor for OS, resulting in a combined hazard ratio of 1.78 (95% confidence interval (CI) 1.35–2.35).

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Conclusions: Patients with metastatic mucinous CRC have distinct clinicopathological features and poor response to chemotherapy and targeted agents. The strong negative prognostic value of MC warrants the use of this pathological feature as a stratification factor for clinical trials in metastatic CRC.

1. Introduction

Mucinous adenocarcinomas (MC) are a histological subtype of colorectal cancer (CRC), in which the tumour cells secrete abundant extracellular mucin involving more than 50% of the tumour volume. They account for 5–15% of all primary colorectal carcinomas,[1] and MC are clinically, morphologically and molecularly different from adenocarcinomas (AC). MC are more often correlated with an advanced stage at presentation,[2,3] a larger diameter,[4] localisation in the proximal colon,[2,4] and peritoneal dissemination.[5–7] Furthermore, MC affect younger patients and are associated with the Lynch syndrome.[8,9] Mucinous tumours exhibit a distinct molecular profile compared to AC, involving a higher incidence of *KRAS* and *BRAF* mutations,[10,11] diploidy,[12] deficient mismatch repair system (dMMR),[13] and CpG island hypermethylation.[14]

Approximately 50% of CRC patients develop metastatic disease and in case of irresectable metastases there is no curative treatment available. However, survival can be improved by using cytotoxic regimens (fluoropyrimidines, oxaliplatin, irinotecan) in combination with targeted therapy (vascular endothelial growth factor (VEGF)- and epidermal growth factor receptor (EGFR) antibodies). Two studies investigated the response to chemotherapy in advanced CRC patients with MC versus AC. Mucinous tumours appeared to be less responsive to fluoropyrimidines,[6] irinotecan and oxaliplatin-based chemotherapy.[7] The response of tumours with mucinous histology to chemotherapy plus targeted agents has not been reported.

The prognostic value of mucinous histology remains controversial. Several studies report that MC are associated with poor prognosis,[6,7,15] which however is not confirmed by others.[2,16] This may be attributed to the small number of patients with MC in these studies. It should be noted that several known prognostic factors, like tumour node metastasis (TNM) classification and *BRAF* mutation status,[17] were not included in most of these studies.

Therefore, the aim of this retrospective analysis was to assess the prognostic value of MC in metastatic CRC in more detail.

2. Patients and methods

2.1. Study population

All patients included in our analysis participated in two phase III randomised clinical trials from the Dutch Colorectal Cancer Group (DCCG). In the CAIRO study[18] (CKTO 2002–07, ClinicalTrials.gov; NCT00312000) 820 metastatic CRC patients were randomised between first-line sequential or combination treatment with capecitabine, irinotecan and oxaliplatin. The primary end-point was overall survival (OS), and secondary end-points included progression free survival (PFS) and response rate. Median OS was 16.3 months (95% confidence interval (CI) 14.3–18.1) for the sequential treatment group and 17.4 months (95% CI 15.2–19.2) for the combination group ($p = 0.33$). As expected, combination therapy was associated with a prolonged first-line PFS compared with sequential therapy (7.8 versus 5.8 months; $p = 0.0002$). The CAIRO2 study[19] (CKTO 2005–02, ClinicalTrials.gov; NCT00208546) included 755 metastatic CRC patients, who were randomly assigned to receive first-line treatment with capecitabine, oxaliplatin and bevacizumab, or the same schedule with the addition of cetuximab. The primary end-point of this study was PFS, and secondary end-points were OS and response rate. The addition of cetuximab significantly decreased the median PFS (9.6 months (95% CI 8.4–10.5) versus 10.7 months (95% CI 9.7–12.3); $p = 0.01$). The median OS was 20.3 months (95% CI 17.8–24.7) in the patients treated without cetuximab and 19.4 months (95% CI 17.5–21.4) in the patients treated with cetuximab ($p = 0.16$). In both studies, treatment cycles were given every 3 weeks. Assessment of the tumour response was performed every 3 cycles (9 weeks) using computerized tomography (CT) imaging and evaluated according to the Response Evaluation Criteria for Solid Tumours (RECIST).[20] The written informed consent required for all patients before study entry also included translational research on tumour tissue.

In our analysis, we included eligible patients with a resection of the primary tumour of which formalin-fixed paraffin-embedded (FFPE) tissue was available. The patients were classified based on the histology of the primary tumour according to the guidelines of the World Health Organization (WHO).[21] Tumours were considered MC if more than 50% of their volume consisted of mucin. AC were defined as tumours without extracellular mucin. Other histological subtypes were excluded from our analysis.

2.2. Clinicopathological features

The following clinical parameters were collected for each patient: age, gender, Eastern Cooperative Oncology Group (ECOG) performance status, serum lactate dehydrogenase (LDH), site of the primary tumour, prior

adjuvant therapy, number and sites of metastatic disease, metachronous (>6 months after initial diagnosis) or synchronous (⩽6 months of initial diagnosis) presentation of metastases, and regimen used as first-line treatment.

Histopathological review was performed on haematoxylin and eosin-stained coupes by two independent observers, including an experienced pathologist (I.D.N). The TNM classification of malignant tumours was used to describe the extent of cancer spread in terms of invasion depth and lymph node stage.[22] The maximum diameter, median number of detected and involved lymph nodes were recorded.

2.3. Microsatellite instability

Immunohistochemistry (IHC) was performed on tissue microarrays (TMA's) of FFPE material of the primary tumour. To determine the expression of the four mismatch repair (MMR) proteins (MLH1, MSH2, MSH6 and PMS2) the slides were stained with antibodies against these proteins, as described before.[23] Tumours were considered positive for MMR expression if nuclear staining was present in at least one tumour cell and negative if there was complete absence of nuclear staining.

Analysis of microsatellite instability (MSI) was performed in all tumours of which at least one staining for the MMR proteins was negative or the IHC staining was not interpretable, using a validated protocol.[23] Two microsatellite markers (BAT 25 en BAT 26) were used, and if only one of these markers showed instability, the analysis was extended with four other markers (BAT 40, D2S123, D5S346, D17S250). A tumour was defined MSI if at least two of the six markers showed instability.

2.4. Hypermethylation of MLH1 promoter

The DNA methylation status of the *MLH1* promoter regions was determined after bisulphite treatment of the DNA using the EZ DNA methylation KIT, ZYMO Research (Orange, CA, United States of America (USA)) as described before.[24]

2.5. KRAS and BRAF mutation analysis

Genomic DNA was isolated from 4–8 microdissected 50 μm sections of FFPE primary tumour tissue as previously described.[25] The *KRAS* and *BRAF* mutation status[25,26] were assessed in duplicate by sequencing analysis in patients of the CAIRO2 study.

2.6. Statistical analysis

The comparison in baseline clinicopathological features between MC and AC was done regardless of study treatment, using the χ^2-test, Fisher's Exact Test or Wilcoxon rank sum test where appropriate. Clinical outcome of patients with MC and AC was investigated in both studies separate, due to an improvement of clinical outcome caused by the addition of bevacizumab to first-line chemotherapy in the CAIRO2 study. OS was calculated as the interval from the date of randomisation until death from any cause or until the date of last follow-up. PFS for first-line treatment was calculated from the date of randomisation to the first observation of disease progression, death from any cause or last follow-up date. OS and PFS curves were estimated using the Kaplan–Meier method and compared with the log-rank test. Overall response was defined as partial response or complete response. Disease control was defined by stable disease with a duration of more than four months or partial response or complete response. Differences in response and disease control rates were tested with a χ^2-test. Multivariate analysis of OS was performed by means of a Cox proportional hazard model to determine if mucinous histology was an independent prognostic factor for survival after correction for; age, gender, localisation of metastases, performance status, serum LDH, site of primary tumour, prior adjuvant therapy, metastatic sites involved, onset of metastases, treatment arm, invasion depth and lymph node status. In the CAIRO2 study, *KRAS* and *BRAF* mutation status were also included in the multivariate analysis. The adjusted hazard ratios of mucinous histology in the CAIRO and CAIRO2 study were checked for heterogeneity. If no heterogeneity was present, the hazard ratios were combined on a log scale, using a DerSimonian-Laird random effects meta-analysis with inverse variance weighting. p values below 0.05 were considered as statistically significant. The analyses were performed using SAS 8.2 software.

3. Results

3.1. Study population

A total of 1099 eligible patients, 552 from the CAIRO and 547 from the CAIRO2 study were available for our analysis. In the CAIRO study, 50 patients (9%) were classified with MC, 435 patients (79%) with AC and 67 patients (12%) with other histological subtypes, including adenocarcinomas with a mucinous component of less than 50%. In the CAIRO2 study, 49 patients (9%) were diagnosed with MC, 476 patients (87%) with AC and 22 patients (4%) with other histological subtypes of CRC. Patients with other histological subtypes were excluded from our analysis.

3.2. Patient characteristics

The median age of patients with MC ($n = 99$) was 67 years compared to 63 years for patients with AC ($n = 911$) ($p = 0.005$). Patients with MC more often had a normal serum LDH ($p < 0.0001$) and extrahepatic

Table 1
Baseline characteristics of patients with MC and AC treated in the CAIRO and CAIRO2 study.

		MC ($n = 99$)	AC ($n = 911$)	p-Value
Age	Median (range)	67 (36–84)	63 (28–81)	0.005
Gender	Male	58 (59%)	548 (60%)	0.83
	Female	41 (41%)	363 (40%)	
Localisation metastases	Hepatic	19 (20%)	303 (34%)	0.02
	Extrahepatic	40 (41%)	281 (31%)	
	Hepatic + extrahepatic	38 (39%)	312 (34%)	
	Locally advanced	0	7 (1%)	
	Unknown	2	8	
WHO performance status	0	66 (67%)	594 (65%)	0.61
	1	30 (30%)	301 (33%)	
	2	3 (3%)	16 (2%)	
Serum LDH	Normal	80 (82%)	557 (62%)	<0.0001
	>ULN	18 (18%)	347 (38%)	
	Unknown	1	7	
Site of primary tumour	Colon	52 (53%)	418 (46%)	0.08
	Rectosigmoid	30 (30%)	240 (26%)	
	Rectum	17 (17%)	252 (28%)	
	Unknown	0	1	
Prior adjuvant therapy	No	84 (85%)	758 (83%)	0.69
	Yes	15 (15%)	152 (17%)	
	Unknown	0	1	
Metastatic sites involved	1	41 (42%)	420 (47%)	0.47
	2	34 (35%)	316 (35%)	
	>2	22 (23%)	160 (18%)	
	Unknown	2	15	
Metastases onset	Metachronous	46 (46%)	411 (45%)	1.00
	Synchronous	53 (54%)	500 (55%)	
Treatment arm CAIRO study	Sequential	26 (52%)	212 (49%)	0.77
	Combination	24 (48%)	223 (51%)	
Treatment arm CAIRO2 study	CAPOX + Bev	32 (65%)	231 (49%)	0.03
	CAPOX + Bev + Cet	17 (35%)	245 (51%)	

MC, mucinous adenocarcinomas; AC, adenocarcinomas; ULN, upper limit of normal; CAPOX, capecitabine and oxaliplatin; Bev, bevacizumab; Cet, cetuximab.

localisation of metastases ($p = 0.02$) compared to patients with AC. A trend was observed for a decreased incidence of MC versus AC in rectum carcinomas ($p = 0.08$). In the CAIRO2 study, a lower percentage of patients with MC was treated with cetuximab ($p = 0.03$) (Table 1).

3.3. Primary tumour characteristics

The primary tumour diameter of patients with MC ($n = 99$) was significantly higher compared to primary tumours of AC patients ($n = 911$) ($p < 0.0001$). Tumours with mucinous histology had a higher T classification in comparison with AC ($p = 0.03$). Furthermore, a trend was observed towards a higher median number of positive lymph nodes in patients with MC compared to AC ($p = 0.06$). dMMR was observed in 17% of the mucinous primary tumours compared to 3% in AC ($p < 0.0001$). Hypermethylation of the *MLH1* promoter was the cause of dMMR in 83% of the tumours with mucinous histology. In the CAIRO2 study, *BRAF* mutations were demonstrated in 22% of the MC patients compared to 7% in AC patients ($p = 0.002$) (Table 2).

3.4. Outcome of treatment with chemotherapy (CAIRO study)

Patients in the CAIRO study were treated with first-line sequential or combination chemotherapy containing capecitabine, irinotecan and oxaliplatin. In univariate analysis, the median OS for patients with MC was 13.2 (95% CI 10.2–17.9) compared to 19.2 months (95% CI 17.9–20.6) in the group with AC ($p = 0.03$). A trend of decreased PFS was observed in patients with MC versus AC ($p = 0.09$). These differences in OS and PFS were observed in both treatment arms. The overall response rate in the 437 patients who were evaluated was 12% in the MC group and 37% in the group patients

Table 2
Primary tumour characteristics of patients with MC and AC treated in the CAIRO and CAIRO2 study.

		MC ($n = 99$)	AC ($n = 911$)	p-Value
Diameter (mm)	Median (range)	50 (20–140)	40 (3–135)	<0.0001
Invasion depth	T 1–2	3 (3%)	71 (8%)	0.03
	T 3	64 (67%)	632 (72%)	
	T 4	28 (30%)	172 (20%)	
	Unknown	4	36	
Lymph node status	N 0	24 (26%)	246 (29%)	0.20
	N 1	27 (30%)	307 (36%)	
	N 2	40 (44%)	294 (35%)	
	Unknown	8	64	
Number lymph nodes	Median (range)	9 (1–55)	8 (0–44)	0.23
Number positive lymph nodes	Median (range)	3 (0–54)	2 (0–41)	0.06
MMR status	pMMR	82 (83%)	884 (97%)	<0.0001
	dMMR	17 (17%)	27 (3%)	
KRAS mutation status (CAIRO2 study)	Wild type	27 (59%)	275 (61%)	0.75
	Mutation	19 (41%)	176 (39%)	
	Unknown	3	25	
BRAF mutation status (CAIRO2 study)	Wild type	35 (78%)	421 (93%)	0.002
	Mutation	10 (22%)	30 (7%)	
	Unknown	4	25	

MC, mucinous adenocarcinomas; AC, adenocarcinomas; MMR, mismatch repair system; pMMR, proficient MMR; dMMR, deficient MMR.

Table 3
Efficacy of the CAIRO study treatment (capecitabine, oxaliplatin and irinotecan) in patients with MC and AC.

		MC ($n = 50$)	AC ($n = 435$)	p-Value
Median OS	Months (95% CI)	13.2 (10.2–17.9)	19.2 (17.9–20.6)	0.03
Median PFS1	Months (95% CI)	5.3 (3.9–6.6)	7.2 (6.6–8.1)	0.09
Overall response rate 1	CR + PR	5/43 (12%)	144/394 (37%)	0.0006
Disease control rate 1	CR + PR + SD	34/4 (79%)	341/394 (87%)	0.17
Median cycle number	Median (range)	6 (1–42)	12 (0–55)	0.004
Overall toxicity	Grade >3	36 (72%)	287 (66%)	0.43

MC, mucinous adenocarcinomas; AC, adenocarcinomas; OS, overall survival; PFS, progression free survival; CR, complete response; PR, partial response; SD, stable disease; CI, confidence interval.

with AC ($p = 0.0006$). Disease control was observed in 79% of the patients in the MC group and 87% of those in the AC group ($p = 0.17$). The median number of the overall treatment cycles was significantly different between patients with MC versus AC (6 versus 12, respectively, $p = 0.004$). The reasons for treatment discontinuation were not significantly different between both patients groups. The incidence of any grade 3 or 4 adverse event was 72% in the patients with mucinous histology and 66% in the AC patients ($p = 0.43$) (Table 3).

3.5. Outcome of treatment with chemotherapy plus targeted agents (CAIRO2 study)

In the CAIRO2 study patients received first-line capecitabine, oxaliplatin and bevacizumab with or without cetuximab. The median OS was significantly lower for patients with MC compared to patients with AC (median 13.1 (95% CI 9.9–20.2) versus 21.5 months (95% CI 19.8–22.9); $p = 0.009$). Furthermore, the median PFS in the MC group was 7.2 months (95% CI 5.7–8.6) compared to 10.6 months (95% CI 10.1–11.4) in patients with AC ($p < 0.0001$). These differences in OS and PFS were observed in both treatment arms. The overall response rate for the patients with MC was 10% and 54% for the group with AC ($p < 0.0001$). In 85% of the MC patients disease control was observed compared to 94% of the patients with AC ($p = 0.003$). Patients with MC received less cycles of treatment compared to patients with AC (6 versus 9 cycles, $p = 0.006$). No significant differences were observed in the causes for discontinuation of treatment between the group with MC and the group with AC. The incidence of any grade 3 or 4 adverse event was 71% versus 79% in the MC and AC patients ($p = 0.27$) (Table 4).

Table 4
Efficacy of the CAIRO2 study treatment (capecitabine, oxaliplatin, bevacizumab with or without cetuximab) in patients with MC and AC.

		MC ($n = 49$)	AC ($n = 476$)	p-Value
Median OS	Months (95% CI)	13.1 (9.9–20.2)	21.5 (19.8–22.9)	0.009
Median PFS	Months (95% CI)	7.2 (5.7–8.6)	10.6 (10.1–11.4)	<0.0001
Overall response rate	CR + PR	4/41 (10%)	222/411 (54%)	<0.0001
Disease control rate	CR + PR + SD	35/41 (85%)	387/411 (94%)	0.03
Median cycle number	Median (range)	6 (1–36)	9 (0–48)	0.006
Overall toxicity	Grade >3	35 (71%)	376 (79%)	0.27

MC, mucinous adenocarcinomas; AC, adenocarcinomas; OS, overall survival; PFS, progression free survival; CR, complete response; PR, partial response; SD, stable disease; CI, confidence interval.

3.6. The independent prognostic role of mucinous histology

In multivariate Cox regression analysis, mucinous histology was a strong predictor of OS in the CAIRO study (hazard ratio (HR) 1.80; 95% CI 1.24–2.62; $p = 0.003$). Other independent negative predictors for OS in metastatic CRC patients were male gender (HR 1.32; 95% CI 1.05–1.64; $p = 0.01$), primary tumour localisation in the colon (HR 1.55; 95% CI 1.19–2.01; $p = 0.01$), WHO performance status 2 (HR 1.92; 95% CI 1.16–3.23; $p = 0.01$), abnormal serum LDH (HR 1.85; 95% CI 1.48–2.31; $p < 0.0001$), more than 2 metastatic sites involved (HR 2.53; 95% CI 1.89–3.38; $p < 0.0001$), T4 classification (HR 1.59; 95% CI 0.99–2.56; $p = 0.04$) and a poor differentiation grade (HR 1.53; 95% CI 0.89–2.64; $p = 0.006$).

In the CAIRO2 study, mucinous histology remained also strongly associated with OS in multivariate analysis (HR 1.76; 95% CI 1.16–2.67; $p = 0.008$). Other clinicopathological features associated with worse survival in this multivariate model were; WHO performance status 1 (HR 1.36; 95% CI 1.06–1.75; $p = 0.02$), abnormal serum LDH (HR 1.65; 95% CI 1.28–2.12; $p = 0.0001$), N2 classification (HR 1.45; 95% CI 1.05–2.01; $p = 0.05$) and *BRAF* mutations (HR 2.61; 95% CI 1.57–4.32; $p = 0.0002$).

The test for heterogeneity ($p = 0.93$) showed that the adjusted hazard ratios for MC versus AC were similar in the CAIRO (1.80; 95% CI 1.24–2.62) and CAIRO2 study (1.76; 95% CI 1.16–2.67). Therefore these hazard ratios were combined in a meta-analysis which resulted in an overall hazard ratio of 1.78 (95% CI 1.35–2.35) for mucinous histology in advanced CRC patients treated with systemic therapy (Fig. 1).

4. Discussion

This is the largest retrospective analysis in mucinous metastatic CRC patients treated with the current standard systemic treatments. We demonstrated that MC are less responsive to fluoropyrimidine-based chemotherapy and targeted agents compared to AC. Furthermore, in a multivariate analysis mucinous histology was shown to be a strong negative prognostic factor for survival.

It is recognised that MC are a different entity in patients with CRC. Specific clinical and pathological features are associated with mucinous histology, of which a larger diameter, higher T classification and extrahepatic localisation of metastases were confirmed in our analysis. Patients with MC treated in the CAIRO studies were older compared to the patients with AC, which has not been previously described. In stage II and III CRC, patients with MC were significantly younger. Mucinous histology is associated with Lynch syndrome,[27] however, the incidence of MSI in metastatic CRC is low.[23] Therefore, it is likely that the association between mucinous histology and age depends on the presence and cause of a dMMR system.

Our data confirm previous results on differences in molecular signatures between MC and AC in respect to MMR[10,28,29] and *BRAF* mutation status,[14,30] supporting the hypothesis that MC are a distinct biologic entity. In previous studies, the incidence of *KRAS* mutations between MC and AC showed opposite results.[30,31] However, these data were derived from a small subset of patients and could not be confirmed in our larger patient cohort. Further identification of specific genetic changes in the mucinous phenotype, i.e. using gene expression

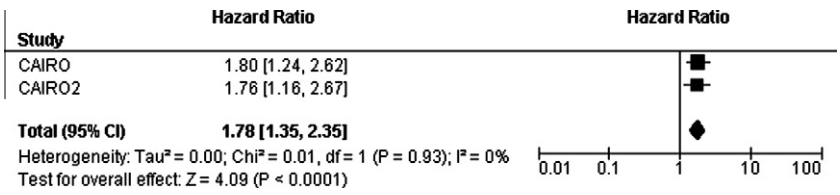

Fig. 1. Meta-analysis of both studies estimating the prognostic value of mucinous histology in metastatic colorectal cancer (CRC) treated with systemic therapy.

profiling, are necessary and useful to understand the molecular aetiology of these tumours.

Fluoropyrimidines, irinotecan and oxaliplatin are the effective chemotherapeutic agents used in metastatic CRC. Negri et al.[6] first reported about the reduced response to 5-FU based chemotherapy of metastatic mucinous CRC. These results were confirmed in a similar subset of patients that received irinotecan and oxaliplatin in addition to fluoropyrimidines as first-line chemotherapy.[7] Our analysis also showed a highly significant worse response to these chemotherapeutic agents for MC compared to AC. Bevacizumab, a humanised monoclonal antibody against VEGF, combined with 5-FU based chemotherapy is nowadays the standard first-line treatment for metastatic CRC. Our study is first reporting the outcome of chemotherapy plus targeted agents in MC versus AC. Since the CAIRO2 study[19] had a negative outcome, possibly due to a negative interaction between the study drugs,[32] the responsiveness of metastatic mucinous CRC to cetuximab cannot be assessed in our series.

The mechanisms for the difference in treatment sensitivity of MC compared to AC remains unclear, but there are several possible explanations. First, differences in molecular features may partly explain the unresponsiveness of tumours with mucinous histology. dMMR is more frequently observed in MC compared to AC, and associated with a reduced response to adjuvant 5-FU treatment in stage II and III CRC patients.[33,34] However, the predictive value of dMMR in response to systemic therapy in metastatic CRC is difficult to assess due to its low incidence in metastatic CRC patients.[23] Other molecular markers, such as thymidylate synthase (*TYMS*) and *GSTP1*, are also overexpressed in mucinous CRC[35] and possibly correlated with resistance to chemotherapy. We did not investigate these or other chemotherapy pathway markers in our analysis, because they have not been validated for clinical use in metastatic CRC.[36] No predictive markers for response to bevacizumab have yet been identified.

Secondly, the mucins themselves may play a role in the ability of tumour cells to escape the effect of systemic therapy. It has been established that mucins play a role in the processes of tumour progression, invasion, survival and protection against the host immune response.[37] MUC2 is a colonic mucin that is overexpressed in the mucinous subtype of CRC[38] and correlated with resistance to 5-FU *in vitro*.[39] The mucin lakes may also be a physical barrier for the delivery of targeted therapy to the tumours cells, however this has not been investigated.

A third explanation could be that the evaluation of the response to treatment is inadequate in MC patients. In the CAIRO studies we used CT scanning and evaluated response according to RECIST criteria. If neoplastic cells in MC respond to systemic therapy, the total tumour volume is probably more affected by the unresponsive mucin lakes, which could result in false negative conclusions. Our observation that the difference in disease control rate between MC and AC was less compared to the difference in objective response rate supports this hypothesis. If confirmed, the RECIST criteria may not be the optimal instrument to evaluate the objective response rate to treatment in the mucinous subtype of CRC.

The prognostic value of MC is highly controversial, which may be attributed to a large amount of heterogeneous studies with small subsets of patients. Most studies suggested that MC are associated with poor prognosis,[6,7,15] while others found no correlation between histological subtype and clinical outcome.[2,16] Our study differs from the published literature in one important aspect, in that only patients with a previous resection of the primary tumour were included, for the obvious reason of tissue availability. Our group has shown that patients with a resection of the primary tumour may have a better prognosis compared to patients without resection.[40] Since the prognostic value of resection of the primary tumour in metastatic CRC patients has not been clearly established, the possible influence of this parameter cannot be assessed. Therefore we assessed the prognostic value of mucinous histology in metastatic patients with a resection of the primary tumour and we observed a significant shorter OS in patients with MC compared to AC. As mentioned before, MC present with distinct clinicopathological features and most of these features are correlated with a worse prognosis. For example, *BRAF* mutations were more frequently observed in MC compared to AC, which is a strong negative prognostic marker in metastatic CRC.[17] However, even with *BRAF* mutations included in a multivariate analysis, mucinous histology remained a strong independent negative prognostic factor. The hazard ratio for mucinous histology is equal or even higher compared to well known stratification factors, such as serum LDH, WHO performance status and number of metastatic sites involved. Appropriate stratification facilitates the interpretation of study results and prevents heterogeneity in response and survival rates. Our findings suggest that mucinous histology should be one of the stratification factors in metastatic CRC trials. Due to the considerable inconsistency in reporting clinicopathological features and use of stratification factors, we believe there is an urgent need for re-establishing the most important prognostic factors in metastatic CRC.

In conclusion, MC are a distinct entity of CRC with specific clinicopathological and genetic features. Mucinous histology is an independent negative prognostic factor for OS in 1010 metastatic CRC patients treated with chemotherapy and targeted agents, which may be explained by resistance to this systemic treatment. We recommend including mucinous histology as a prognostic factor for patients with metastatic CRC.

Conflict of interest statement

None declared.

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