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RESEARCH ARTICLE

Clinical significance of cellular and acellular mucin pools in rectal carcinoma following preoperative chemoradiotherapy

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Abstract
Background and objectives The standard treatment for locally advanced rectal cancer (LARC) is neoadjuvant chemoradiotherapy (CRT) followed by surgery. Pathological findings remain the most significant prognostic factor. The presence of mucin pools and their prognostic significance is a controversial issue. The aim of this study was to analyze the incidence of cellular and acellular mucin pools and their clinical significance.
Methods Four-hundred and forty-six consecutive prospectively collected specimens from patients with LARC treated with long-course preoperative CRT and surgery were analyzed. Kaplan–Meier analysis was performed.
Results Mucin pools were present in 182 specimens (40.8 %); 66 (14.7 %) were acellular, and viable tumor cells were identified in 116 (26 %). The complete pathological response rate was 13.5 % (60 of 446). With a median follow-up of 79.0 months, the 5- and 10-year disease-free survivals for patients with acellular and cellular mucin pools were 81.5, 78.1, 63.7 and 61.2 %, respectively ($p \leq 0.026$). The presence of cells in the colloid response to treatment was associated with a 17.8 and 16.9 % decrease in 5- and 10-year disease survival vs. acellular colloid response.
Conclusions Our results suggest that cellular mucin pools are an indicator of an aggressive phenotype and harbingers of a worse prognosis.

Keywords Rectal cancer · Neoadjuvant chemoradiation · Cellular mucin · Pathological response · Outcome

Introduction

The current standard treatment for locally advanced rectal cancer (LARC; stages II/III) is preoperative, combined-modality radiation and chemotherapy (CRT) followed by surgery based on total mesorectum excision surgery (TME) [1–6]. Despite a significant reduction in local recurrence, the overall survival (OS) and disease-free survival (DFS) of patients with LARC have remained unchanged in the last decade, because of systemic relapse. Currently, the most consistent prognostic factors following the CRT schedule are the pathological findings in the surgical specimen [7–11].

Dworak et al. [12] described in 1997 the presence of extracellular mucin "lakes" in patients pretreated with CRT and their association with the tumor response grade (TRG) classification. In 1973, Castro et al. [13] described a high percentage of mucinous carcinomas in patients previously irradiated for gynecological tumors. Mucin production is considered to be a form of tumor response, although the mechanism remains obscure and its clinical significance is controversial [14, 15].

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The present study investigated the incidence of mucin pools, whether they contained tumor cells, and their significance with respect to oncological outcomes. The study included a large cohort of patients with LARC treated with long-term preoperative CRT followed by TME.

Materials and methods

Patient eligibility

This retrospective study of a prospectively collected database was approved by the local ethics committee. The analysis was conducted in accordance with "Strengthening the Reporting of Observational Studies in Epidemiology" (STROBE) [16].

Between May 1990 and December 2012, 500 patients diagnosed with rectal cancer underwent surgery at Clínica Universidad de Navarra. The study comprised a cohort of 446 consecutive patients diagnosed with LARC (cT3–T4 or cN+, Union for International Cancer Control [UICC] TNM classification) [17] staged by endorectal ultrasonography, computed tomography, or magnetic resonance imaging. Adenocarcinoma was confirmed by biopsy and located <15 cm from the anal verge. Patients with distant metastasis ($n = 54$) were excluded.

All patients received neoadjuvant CRT by external beam radiation to the pelvis. CRT consisted of a median radiation dose of 4680 cGy using a three- or four-field technique or a seven-field intensity-modulated technique in 28 fractions at 180 cGy per day, in accordance with previously described techniques [18, 19]. Two different chemotherapy protocols were administered: 5-fluorouracil (5-FU) alone (225 mg/m^2 on days 1–4 and 24–28) or capecitabine (825 mg/m^2 twice daily Monday to Friday) in combination with Oxaliplatin (60 mg/m^2 on days 1, 8, and 15) or Carboplatin [20].

All patients underwent complete surgical resection 6–8 weeks after completion of CRT. Surgery consisted of low anterior resection, abdominoperineal resection, or Hartmann procedure at the surgeon's discretion based on the TME and conditions of each patient. In 13 patients, transanal endoscopic microsurgery (TEM) was carried out.

Pathological assessment

Hematoxylin and eosin sections were assessed by a senior pathologist (JJS) who had specific training in gastrointestinal pathology and was unaware of the patients' outcome clinical data. No additional histochemistry or immunohistochemistry was carried out either for the tumor, lymph nodes, or mucin pools.

Macroscopic assessment

The surgical specimens were opened anteriorly, pinned onto a cork board, and fixed in 4 % formaldehyde overnight; the largest diameter of the tumor was registered after fixation. The mesorectum face was inked to register the circumferential surgical margin. The tumor and the surrounding mesorectum were serially sliced in the transverse plane to identify the areas of deepest invasion. The 2 cm below the tumor was also sliced and investigated to assess the distal intramural spread. The tumor was sagitally sliced every 2 mm; a minimum of six blocks was required for each macroscopic lesion. A meticulous search of the mesorectum was performed to identify as many lymph nodes as possible. Each lymph node was analyzed in its entirety in separate blocks [21].

Tumor response to neoadjuvant therapy

Pathological grading of tumor regression grade was performed in accordance with Ruo and Shia [8, 22]. A simplified four-point scale based on the amount of residual viable tumor versus fibroinflammatory changes within the gross tumor mass was used [8, 23]. The scale consisted of complete regression (100 % response, category 4), near-complete regression (>95 % response: categories TRG3+), intermediate regression (>67 to <94 % response: category TRG3), and poor response (≥0 to ≤66 % response: categories TRG1 to 2).

Mucin pools

Mucin pools constituting more than 10 % of the lesional area of the primary tumor were recorded as "mucin pools present" [23]. Patients were categorized into three groups regarding the pattern of mucin pools: patients without mucin pools, patients with mucin pools containing single or clusters of tumor cells, and patients with acellular mucin pools [8, 24] (Figs. 1, 2). Acellular mucin was considered as a form of tumor response, not as residual/viable tumor [8, 23]. The irradiated tumor was staged according to the American Joint Committee on Cancer (AJCC) TNM classification of malignant tumors [25].

Surveillance

Patients were prospectively followed up every 3 months for 2 years, every 6 months for the next 3 years, and then annually thereafter according to National Comprehensive Cancer Network guidelines [1]. Local recurrence was defined as clinical or radiological tumor regrowth within the pelvic treatment field. Distant recurrence was defined as

tumor growth in any other area. Relapse was diagnosed based on two consecutive CT scans within 4–6 weeks. Histopathological confirmation was performed when feasible.

Statistical analysis

Results were expressed as medians for continuous variables and proportions for qualitative variables. The Mann–Whitney *U* and Kruskal–Wallis tests were used to compare means in two or more groups, and χ^2 test was used to compare proportions. Follow-up data were taken from the time of the last clinic appointment (before end of the study on 30 September 2014) or event (recurrence or death). Deaths from unrelated causes were censored for the purpose of survival analysis. DFS and OS were expressed as percentages and analyzed using the Kaplan–Meier method. Survival curves were compared using the log-rank test. All statistical tests were two sided with a 5 % level of significance and were performed with SPSS/PC v.15 for Windows (SPSS, Chicago, IL, USA).

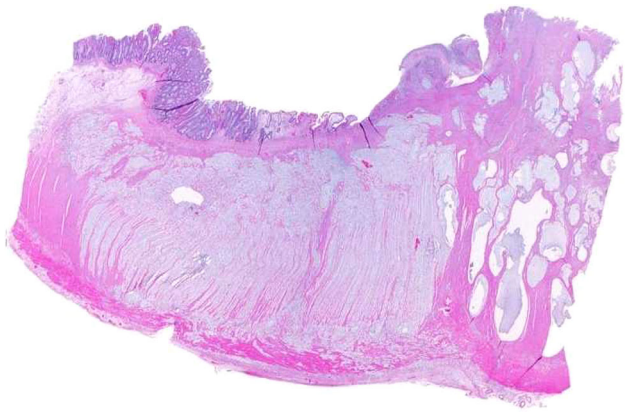

Fig. 1 Colloid response following neoadjuvant treatment in rectal adenocarcinoma showing mucin pools at the primary tumor

Results

Patients, tumor characteristics, and treatment schedule

The clinical data, tumor characteristics, and treatment details of the patients are listed in Table 1. The tumors were evenly located in the middle and lower thirds of the rectum, with a mean distance from the anal verge of 6.5 cm (range 0–15 cm). A sphincter-saving procedure was performed in 72 % of the patients.

At a median follow-up period of 79.0 months, out of the 446 patients, 94 (21.0 %) had died; 48 (10.7 %) because of disease progression, and 46 (10.3 %) due to other causes. A total of 125 patients (28.0 %) developed cancer relapse:

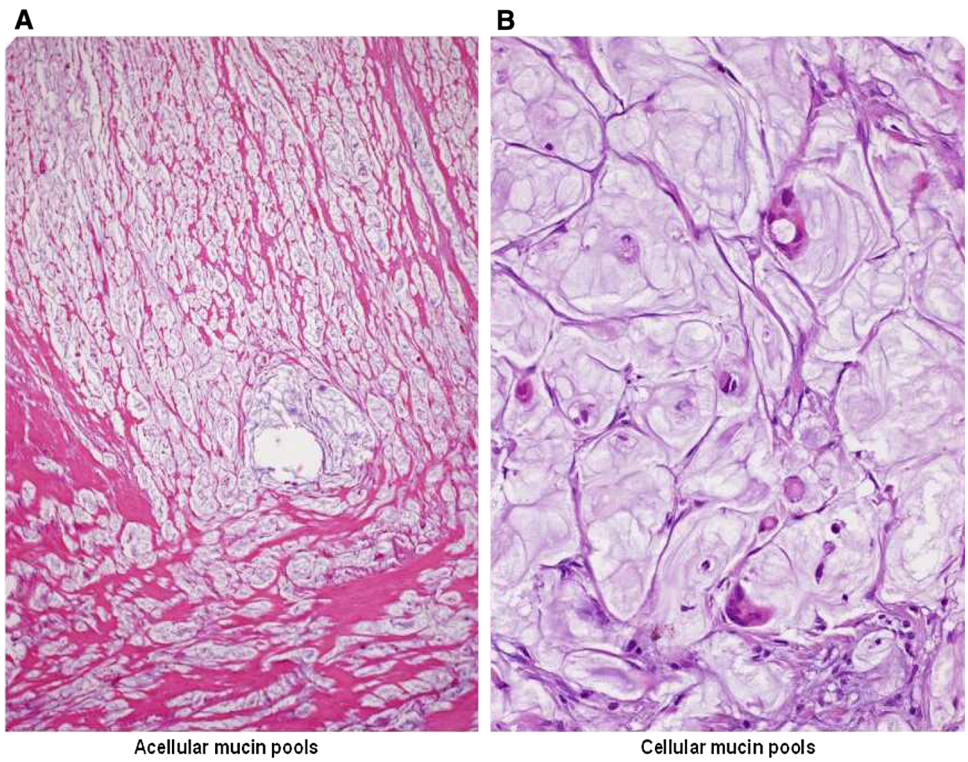

Fig. 2 Colloid response following neoadjuvant CT–RT in rectal adenocarcinomas. **a** Mucin pools without cells (hematoxylin and eosin—H&E—stain ×50). **b** Mucin pools with tumor cells (H&E ×100)

Table 1 Clinical and pathological characteristics of the study participants

Variable	No. (frequency)
Median age	59.1
Sex	
Male	301 (67.5 %)
Distance from the anal verge (cm)	
>11	64 (14.3 %)
6–10	166 (37.2 %)
≤5	216 (48.4 %)
Mean distance from the anal verge (range)	6.5 (0–15)
Time RT-surgery (days)	39.7
Length RT (days)	34.8 (29–38)
Dosage RT (cGy)	4680 (4500–5000)
Chemotherapy schedule	
5FU + leucovorin	30 (6.7 %)
5FU + oxaliplatin	230 (51.5 %)
5FU + carboplatin	186 (41.7 %)
Surgical procedure	
Anterior resection	308 (69.1 %)
Abdominoperineal resection	110 (24.7 %)
Hartmann	15 (3.4 %)
TEM[a]	13 (2.9 %)
Circumferential resection margin (%)	
Positive or <1 mm	22 (4.9 %)
Distal resection margin (%)	
Positive or <1 mm	12 (2.7 %)
Number of examined LNs	
Median (range)	10.7 (0–54)
μTNM classification (UICC)[b]	
Stage 0	60 (13.5 %)
Stage I	139 (31.2 %)
Stage II	119 (26.7 %)
Stage III	128 (28.7 %)
Pathologic response[c]	
1 and 2	122 (27.4 %)
3	181 (40.5 %)
3+	82 (18.4 %)
4	61 (13.8 %)
Venous invasion	77 (17.2 %)
Perineural invasion	84 (18.8 %)

[a] Transanal endoscopic microsurgery

[b] Acellular mucin pools were defined as "no residual tumor"

[c] According to Shia (Ref. [8])

eleven (2.4 %) with local recurrence, 101 (22.6 %) with distant relapse, and 13 (2.9 %) with both local and distant recurrence.

Pathological analysis

The definitive pathological findings, according to the TNM classification and the current College of American Pathologists (CAP) recommendations, and considering acellular mucin as no residual tumor, are shown in Table 2. A total of 61(13.6 %) of the 446 patients had pT0, and 318 (71.3 %) did not show evidence of lymph node invasion (N0). Involvement of the distal edge (≤1 mm) was observed in 12 patients (2.7 %), and the circumferential margin (≤1 mm) was affected in 22 patients (4.9 %). The median distance between the lower edge of the tumor and the section limit was 3 cm. The median number of lymph nodes analyzed was 10.7 (range 0–54).

According to TNM classification and the CAP consensus statement, 61 patients (13.7 %) had no residual tumor confined to the rectal wall (pT0), 139 patients (31.2 %) were stage I, and 119 (26.7 %) and 128 (28.7 %) were stages II and III, respectively.

Mucin pools (or colloid response) were reported in 182 of 446 tumors (40.8 %). In 66 of these (14.7 %), the mucin pools were acellular, whereas viable tumor cells were identified in the mucin pools of 116 patients (26.0 %) (Table 2).

The pathological response to CRT (TRG) and grouping into four main subpopulations was as follows: 122 patients (27.4 %) were assessed as unfavorable or poor response (TRG 1 to 2), 181 patients (40.5 %) had an intermediate response (TRG 3), and 143 patients (32.2 %) had a favorable tumor response (TRG3+ to 4).

The relationship between clinico-pathological factors and colloid response phenotype is shown in Table 2. There were no significant differences regarding the *N* stage tumor staging and the mean radiation dose although a trend toward more radiation fields was observed.

Survival analysis

At a median follow-up of 79.0 months (range 3–250 months), the DFS for the entire group of patients was 71.9 and 69.8 % at 5 and 10 years, respectively (Fig. 3). The actuarial 5- and 10-year disease-free survival for patients without colloid response was 81.8 and 73.6 % respectively (Fig. 4). The 5- and 10-year DFS for the 66 patients with acellular mucin was 81.5 and 78.1 %. Meanwhile, for patients with a colloid response with viable tumor cells, the 5- and 10-year DFS was 62.5 and 61.2 %, respectively ($p = 0.026$).

The DFS for patients presenting a colloid response with or without viable tumor cells is shown in Fig. 4. Patients with a colloid response harboring tumor cells had a 17.8 % decrease in DFS at 5-year and 16.9 % decrease in DFS at

Table 2 Patient's clinical and pathological features regarding different patterns of colloid response

Variable	Mucin (–) $n = 264$ (59.1 %)	Cellular mucin $n = 116$ (26 %)	Acellular mucin $n = 66$ (14.7 %)	p
Median age	58.9	58.2	61.5	0.153
Sex				
Male	183 (68.8 %)	72 (23.9 %)	46 (15.3 %)	0.35
Distance from the AV (cm)				
>11	35 (54.7 %)	19 (29.7 %)	10 (15.6 %)	0.725
6–10	99 (59.6)	39 (23.5 %)	28 (16.9 %)	
<5	130 (60.2 %)	58 (26.9)	28 (13)	
Mean distance from the AV (cm)	6.3	6.6	6.7	0.728
Time RT-surgery (days)	39.6	39.9	39.9	0.9
Length RT (days)	36.1	33	32.3	0.603
Dosage RT (cGy)	5043	5042	4849	0.052
Chemotherapy schedule				
5FU + leucovorin	20 (66 %)	4 (13.3 %)	6 (20 %)	
5FU + oxaliplatin	131 (57 %)	62 (27 %)	37 (16.1 %)	0.577
5FU + carboplatin	113 (60.8 %)	50 (26.9 %)	23 (12.4 %)	0.472
Surgical procedure				
Anterior resection	176 (57.1 %)	81 (26.3 %)	51 (16.6 %)	0.469
Abdominoperineal resection	68 (61.8 %)	29 (26.4 %)	13 (11.8 %)	
Hartmann	9 (60 %)	4 (26.7 %)	2 (13.3 %)	
TEM[a]	11 (84.6 %)	2 (15.4 %)	–	
Number of examined LNs[b]	10.1	11.4	11.8	0.167
μTNM classification (UICC)				
Stage 0	40 (66.7 %)	–	20 (33.3 %)	0.001
Stage I	89 (64 %)	29 (20.9 %)	21 (15.1 %)	
Stage II	68 (57.1 %)	38 (31.9 %)	13 (10.9 %)	
Stage III	67 (52.3 %)	49 (38.3 %)	12 (9.4 %)	
Pathologic response[c]				
1 and 2	69 (56.6 %)	46 (37.7 %)	7 (5.7 %)	0.001
3	108 (59.7 %)	54 (29.8 %)	19 (10.5 %)	
3+	47 (57.3 %)	16 (19.5 %)	19 (23.1 %)	
4	40 (65.5 %)	–	21 (34.4 %)	
Venous invasion	48 (62.3 %)	22 (28.6 %)	7 (9.1 %)	0.295
Perineural invasion	50 (59.5 %)	27 (32.1 %)	7 (8.3 %)	0.11

[a] Transanal endoscopic microsurgery

[b] Lymph nodes "residual tumor"

[c] According to Shia (Ref. [8])

10-year follow-up (78.1 % for acellular mucin vs. 61.2 % for cellular mucin).

Discussion

Since the first description by Dworak in 1997 [12] of mucin pools in rectal tumor specimens after CRT, few clinicians have reported on this topic, although the updated guidelines of the CAP and AJCC recommend that the presence of mucin pools after CRT be investigated [26]. Variability in the incidence of mucin pools has been reported, and their prognostic significance remains controversial [23, 24]. In our series of consecutive 446 LARC patients treated with long-term preoperative CRT and TME, we found a mucin pool incidence of 40.8 %, which is in line with the results of Shia et al. [8, 23, 27] who reported an incidence of 31 % with a similar long-term schedule of CRT. The incidence in our cohort was higher than that found by other authors, who reported that mucin pools occurred exclusively in

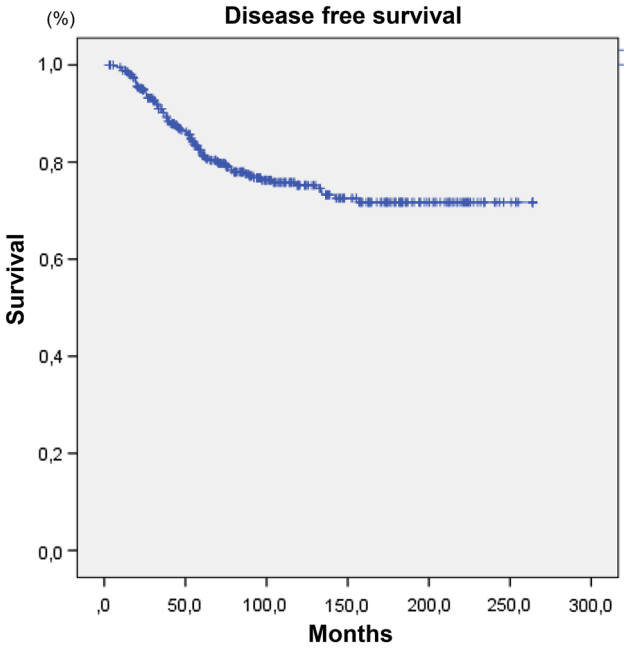

Fig. 3 Kaplan–Meier disease-free survival curve for the entire cohort of 446 patients

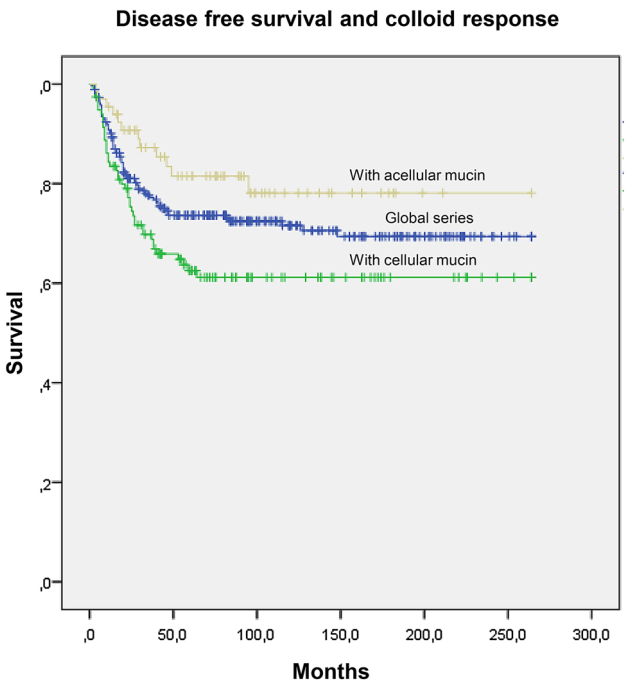

Fig. 4 Kaplan–Meier disease-free survival for patients treated for rectal cancer according to the presence of acellular and cellular mucin response to neoadjuvant treatment

patients with pCR [28, 29]; in this subpopulation, they found that the incidence was around 20–25 %. Not surprisingly, the majority of these authors did not find a significant impact on oncological outcomes, given the excellent prognosis of this subpopulation. By contrast, de Campos-Lobato et al. [29] described a trend toward a worse outcome in patients with simultaneous pCR and acellular mucin, with an increased rate of distant relapse and a decreased DFS. Rullier described an intermediate behavior regarding survival in patients with mucin pools that fell between a favorable response ("downstaging") and an unfavorable response ("no response") [27], with a more-aggressive phenotype and a worse prognosis.

The present study took place in a single institution in which 446 consecutive patients were enrolled and uniformly treated with long-term CRT and surgery performed by surgeons experienced in TME. To obtain the most accurate assessment of TRG (i.e., colloid response), pathological examinations were performed by a single pathologist; the examinations were performed expressly for this study, and the pathologist was unaware of patient outcome. The oncological outcomes with respect to the TRG response are in line with our previous reports [19, 20, 30] and with those of other authors [7, 8, 23, 31].

We differentiated the mucinous phenotype according to the presence or absence of viable tumor cells. We found acellular mucin pools and mucin pools with viable tumor cells in 36.2 and 63.7 %, respectively, of tumors with mucin pools. These findings are in line with those recently reported by Shia, who found an incidence of acellular and cellular mucin pools of 48 and 52 %, respectively, in treated tumors [23].

In contrast with the findings of authors who did not report on the presence of tumor cells in mucin pools, we observed a significant difference in the 5- and 10-year DFS of the acellular and cellular mucin pool groups: 5- and 10-year DFS of 81.5 and 78.1 % for acellular mucin vs. 5- and 10-year DFS of 62.5 and 61.2 %, respectively, for cellular mucin ($p < 0.0225$). These findings are in agreement with those reported by Shia and Lim [23, 24] of a 3-year DFS of 100 % vs. 65 % for acellular and cellular mucin, respectively. Shia et al. [23] showed that when acellular mucin pools were considered to be part of the tumor, the correlation between pathological response and 3-year DFS became insignificant, although tumor and nodal staging were predictive of outcome. They concluded that the presence of mucin pools without associated tumor cells does not have a significant negative impact on clinical outcomes, and that mucin pools should not be regarded as residual tumor, in agreement with the current CAP guidelines [17, 23, 24, 26].

The tumor response to CRT is a complex and dynamic process in which different factors are involved (e.g., tumor–host interrelationship, radiotherapy dose, long-course vs. short-course schedule, neoadjuvant chemotherapy, and interval between preoperative CRT and surgery) [15]. Despite intense investigations of tumor parameters that may be predictive of clinical outcomes, pathological findings remain the most reliable prognostic factors,

particularly pN [9, 32]. In line with our findings, several authors have described three well-differentiated subpopulations with respect to the prognostic significance of TRG to CRT: favorable, intermediate, and unfavorable or poor response [8, 11, 31–33]. Fokas et al. [7] recently reported a strong association between the local tumor response to CRT and the absence of distal relapse with improved 5- and 10-year DFS. Although the mechanism of this phenomenon is a topic of intense discussion, it seems reasonable to investigate the tumor phenotype profile to discriminate between good and poor response to determine whether changes need to be made in therapeutic or surveillance schedules.

According to our results and in line with those of other authors, differentiating between acellular and cellular mucin pools appears to be of critical importance. Patients in the latter group could benefit from treatment intensification to prevent a future relapse [23, 24, 27].

The present study has some limitations that warrant discussion. First, it is a retrospective study; nevertheless, clinical, surgical, and outcome data were collected prospectively, and the pathological examinations were performed expressly for the present study. Second, there were some variations in the CRT regime, due to the advances made in chemotherapy and radiotherapy over the last decade.

In summary, our results provide further evidence that mucin pools following CRT should be investigated with respect to the presence of viable tumor cells. Until the results of prospective, larger-scale studies become available, our findings support the current CAP consensus statement that acellular mucin should not be regarded as residual tumor in the pTNM classification, although a thorough search for tumor cells remains mandatory.

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Compliance with ethical standards

Disclosure statement All authors have read and approved the manuscript and it is not under consideration elsewhere. The authors are not aware of any affiliations, memberships, funding, or financial holdings that might perceived as affecting the objectivity of the manuscript.

Informed consent Patients were identified from a prospective institutional database and the study was approved by the local Research Ethics Committee.

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