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Pathological complete response may underestimate distant metastasis in locally advanced rectal cancer following neoadjuvant chemoradiotherapy and radical surgery: Incidence, metastatic pattern, and risk factors

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ABSTRACT

Aim: To evaluate the pattern of tumor relapse of pathological complete response (pCR) patients with locally advanced rectal cancer (LARC) following neoadjuvant chemoradiotherapy (nCRT) and total mesorectal excision (TME), and to identify predictive factors of distant metastasis in pCR patients after nCRT.

Method: This was a retrospective analysis of 118 LARC patients who achieved a pCR following nCRT and TME from 2008 to 2015. Clinicopathological and therapeutic parameters were evaluated as possible predictors of distant metastasis-free survival (DMFS), and COX regression analysis was performed.

Results: After a median follow-up of 57 months, the 5-year overall and disease-free survival rates were 94.7% and 88.1%, respectively. Overall, 6 patients (5.1%) died, no local recurrence occurred, 13 patients (11%) developed distant metastases, including lung (n = 5), liver (n = 2), bone (n = 3), lung and brain (n = 1), peritoneal (n = 1), and spleen (n = 1) metastasis. On univariate analysis, tumor distance from the anal verge (HR = 0.706, $P = 0.039$), acellular mucin pools (HR = 6.687, $P = 0.002$), and MUC1 expression (HR = 8.280, $P < 0.001$) were independently associated with DMFS. COX regression demonstrated that MUC1 expression (HR = 3.812, $P = 0.041$) remained to be an independent predictor of DMFS in pCR patients.

Conclusion: Distant metastasis still remained a major concern in pCR patients following nCRT and TME. Tumor distance from the anal verge, acellular mucin pools, and MUC1 expression were associated with distant metastasis in patients with pCR. MUC1 staining remained to be an independent risk factor for DMFS. Such information could facilitate treatment decision in these patients, such as adjuvant chemotherapy and follow-up.

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Introduction

Neoadjuvant chemoradiotherapy (nCRT) followed by total mesorectal excision (TME) surgery has been established as the standard care for patients with locally advanced rectal cancer (LARC). This multimodality is associated with well-established benefits, such as tumor down-sizing and down-staging, and better local control [1–3]. Approximately 15%–30% of patients will develop a pathological complete response (pCR), and show excellent oncological outcome [4,5]. In addition, there has been an ever-increasing interest in advocating the organ preservation strategy for such patients in an attempt to avoid radical surgery and post-operative complications [6–9].

The ultimate goal of organ preservation in rectal cancer is to guarantee a better quality of life without compromising the oncological outcome. Nevertheless, is it ready for prime time now? There are still several issues remained unanswered, including

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accurate identification of pCR after nCRT, the risk of tumor regrowths and systemic dissemination, and the overall survival of patients [10]. Until now, most published studies were focused on accurate identification of cCR, little attention has been paid to systemic metastases in such patients (i.e., ypCR) [11–14]. A pooled analysis of long-term outcome in rectal cancer patients with pCR after CRT has demonstrated that the 5-year risk for local recurrence and distant metastasis were 2.8% and 11.2%, respectively [4]. Similarly, according to the German CAO/ARO/AIO-94 phase 3 trial, the 10-year cumulative incidence of distant metastases was 11.8% in pCR patients after nCRT [15]. In other words, pCR largely reduces the risk of local recurrence, whereas the reduction of systemic relapse is not that dramatic and it remains a major concern in the treatment of rectal cancer.

In this context, we hypothesize that pCR might not actually represent the initial metastatic burden of the primary tumor. Since the long-term results of organ preservation are still awaited, identification of pCR patients at high risk of tumor relapse is also of great clinical importance. Therefore, we conducted the present study to evaluate the pattern of tumor relapse of pCR patients with LARC following nCRT and TME, and to identify predictive factors of systemic relapse after nCRT, which might be helpful in counseling patients with decision making about organ preservation.

Patients and methods

Patient selection

LARC patients who were treated in the Department of Colorectal Surgery of Fujian Medical University Union Hospital (FMUUH, Fuzhou, PR China) from 2008 to 2015 were identified from our prospective database. Eligibility criteria included the following: (1) histologically proven rectal adenocarcinomas, (2) rectal tumors located < 12 cm from the anal verge, (3) clinically staged as cT3-4 and/or N+ rectal tumors, (4) patients treated with nCRT and radical surgery, and (5) pathologically staged as T0N0M0 (ypCR). Exclusion criteria were distant metastasis at diagnosis, synchronous malignancy or a history of other malignant tumors, emergency surgery or palliative surgical resection, local excision or a "Watch-and-Wait" strategy. This study was approved by the FMUUH institutional review board (IRB).

Treatment

The pre- and post-nCRT oncological assessment and tumor staging were performed using digital rectal examination, serum carcinoembryonic antigen (CEA) test, serum carbohydrate antigen 199 (CA199) test, chest X-ray or computed tomography (CT) scan, abdominopelvic magnetic resonance imaging (MRI), and transrectal ultrasonography (ERUS). Preoperative radiotherapy was delivered at a dose of 45Gy in 25 fractions followed by a primary tumor boost of 5.4Gy over a period of 5–6 weeks. Chemotherapy was administered concurrently using one of two regimens [capecitabine plus oxaliplatin (CapeOX) or 5-fluorouracil/folinic acid plus oxaliplatin (FOLFOX)]. Surgery was scheduled 6–8 weeks after completing radiotherapy. Patients underwent radical surgery according to the TME principle, and high ligation of the inferior mesenteric artery was routinely performed. About 3–4 weeks after surgery, patients received adjuvant chemotherapy (CapeOX or FOLFOX) for 6 months.

Pathological evaluation

Surgical specimens were assessed by at least two experienced pathologists following a standardized protocol. A pCR was defined as the absence of viable adenocarcinoma cells in the surgical specimen, both in the tumor and regional lymph nodes (ypT0N0). The presence of mucinous components in the tumor was evaluated. Acellular mucin pools were defined as mucin pools constituting more than 10% of the lesion area of the tumor area as evaluated by hematoxylin-eosin (H&E) staining [16,17], as shown in Fig. 1A and B. To exclude remnant tumor cells in acellular mucin pools, at least 10 multilevel sections were performed in at least three tumor blocks per case [18]. Mucin 1 (MUC1) immunohistochemical (IHC) staining in the tumor was performed with *anti*-MUC1 antibodies (MXB,biotechnologies, China) using an avidin-biotin complex immunoperoxidase method [19]. CEA immunohistochemical staining in acellular mucin pools was performed with *anti*-CEA antibodies (MXB, biotechnologies, China) using an avidin-biotin complex immunoperoxidase method. Results of MUC1 and CEA staining in the surgical specimen were classified as positive (≥10%) or negative (<10%), as shown in Fig. 1C, D, E, and F.

Follow-up

Patients were regularly followed up every 3 months for the first 3 years, then every 6 months for the next 2 years, and annually thereafter. Follow-up included physical examination (including digital rectal examination), serum CEA and CA199 test, chest X-ray or CT scan, abdominopelvic MRI or CT scan, and an annual colonoscopy. Positron emission tomography (PET) scan was used to better clarify recurrence and/or metastasis, when necessary. Patients were followed up until death or the cut-off date (July 31, 2018).

Statistical analysis

Statistical analyses were performed using IBM SPSS statistics 20.0 software (IBM SPSS INC., Chicago, USA) and graphs were created by GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA). Categorical variables were assessed using Chi-square or Fisher's exact test, where applicable. Local recurrence (LR) was defined as radiological or pathological evidence of any tumor relapse within the pelvis, perineum, or anastomosis. Distant metastasis (DM) was identified as recurrent disease outside the pelvis as diagnosed by imagining or pathological examinations. Survival outcomes were calculated using the Kaplan-Meier method and compared by the log-rank test. A Cox regression model was utilized to determine risk factors for metastasis-free survival (DMFS). A two-tailed $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 118 LARC patients underwent nCRT and TME and achieved a pCR were identified. Among them, 53.4% (63/118) were male and 46.6% (55/105) were female, with a mean age of 54.3 years. The majority of the tumors were clinically staged as cT4 (65.3%) and cN+ (89.8%) disease. The median radiation dose was 4955.7 Gy, and the interval from radiation to surgery was 8.3 ± 1.7 months. Postoperative adjuvant chemotherapy was administered to 93.2% of patients. The other clinical features and treatment modalities were presented in Table 1.

Oncological outcomes

After a median follow-up of 57 (range, 9–118) months, the 5-year overall and disease-free survival rate was 94.7% and 88.1%,

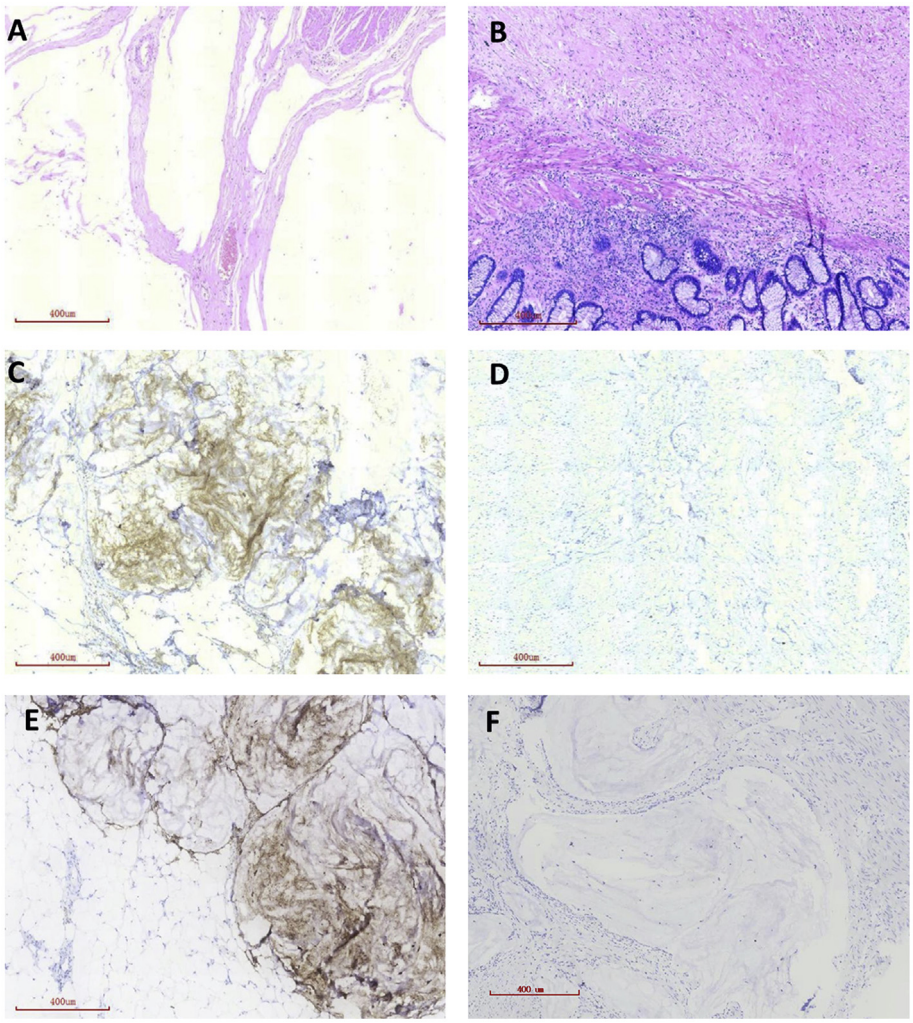

Fig. 1. Pathologic images of the resected specimen from patients with pCR.
(A) Example of specimen with acellular mucin lakes.
(B) Example of specimen without acellular mucin lakes.
(C) Example of positive MUC1 staining in acellular mucin lakes.
(D) Example of negative MUC1 staining in acellular mucin lakes.
(E) Example of positive CEA staining in acellular mucin lakes.
(F) Example of negative CEA staining in acellular mucin lakes. **pCR**, pathological complete response; **MUC1**, mucin 1; **CEA**,carcinoembryonic antigen.

respectively. During the study period, 6 patients (5.1%) died and 13 patients (11%) developed tumor recurrence. Among the 6 patients who were died during the follow-up period, all died of metastatic diseases except one died of a heart attack. The mean time to death was 20 ± 11 months. The mean time to recurrence was 34 ± 20 months. Among the patients with tumor relapse, no local recurrence occurred, distant recurrence occurred in 13 patients, including lung ($n = 5$), liver ($n = 2$), bone ($n = 3$), lung and brain ($n = 1$), peritoneal ($n = 1$), and spleen ($n = 1$) metastasis, as demonstrated in Table 2.

Acellular mucin pools and MUC1 immunostaining

Thirty-two patients (27.1%) had acellular mucin pools in the surgical specimen. Patients with acellular mucin pools had a similar 5-year OS rate (89.8% vs. 96.5%, $P = 0.210$), but a significantly decreased DFS rate (67.4% vs. 96.5%, $P = 0.003$), when compared with patients without acellular mucin pools. Seventeen patients (14.4%) had positive MUC1 expression in the resected specimen. The rate of positive staining for MUC1 was significantly higher in pCR patient with recurrence (53.8% vs. 9.5%, $P = 0.003$). The 5-year OS rate was 94.1% for patients with positive MUC1 staining and 94.8% for patients with negative MUC1 staining ($P = 0.851$), and positive MUC1 staining was associated with a lower DFS rate (50.3% vs. 93.5%, $P < 0.001$), as shown in Fig. 2.

We performed CEA immunohistochemical staining in 32 patients with acellular mucin pools. A total of 14 patients had CEA positivity in the surgical specimen with acellular mucin pools. However, we found no statistical correlation between CEA positivity and MUC1 positivity ($\chi^2 = 0.008$, $P = 0.928$). Additionally, the median serum post-CRT CEA level was similar between MUC1-positive and -negative groups (2.7 ± 1.3 ng/ml vs. 2.1 ± 1.1 ng/ml, $P = 0.168$). Likewise, no significant difference in the serum post-CRT CEA level was found between CEA-positive and -negative groups (2.4 ± 1.1 ng/ml vs. 2.4 ± 1.3 ng/ml, $P = 0.981$).

Risk factors for DMFS

On univariate analysis, tumor distance from the anal verge (HR = 0.706, $P = 0.039$), acellular mucin pools (HR = 6.687,

Table 1
Clinical features and treatment modalities of pCR patients after nCRT.

Variables	n (%)
Sex	
Male	63 (53.4)
Female	55 (46.6)
Age (years)	54.3 ± 12.3
Distance from the anal verge (cm)	5.6 ± 1.9
Histopathology	
Adenocarcinoma	115 (97.5)
Mucinous/signet ring adenocarcinoma	3 (2.5)
Pre-nCRT cT stage	
3	41 (34.7)
4	77 (65.3)
Pre-nCRT cN stage	
0	12 (10.2)
+	106 (89.8)
Pre-nCRT CEA level (ng/ml)	2.5 (1.4–4.7) *
Pre-nCRT CA199 level (U/ml)	11.2 (5.8–19.1) *
Radiation dose (Gy)	4955.7 ± 179.8
Preoperative chemotherapy regimen	
Oxaliplatin-based	53 (44.9)
Fluoropyrimidine only	65 (55.1)
Interval from radiation to surgery (months)	8.3 ± 1.7
Post-nCRT CEA level (ng/ml)	1.8 (1.3–2.6)*
Post-nCRT CA199 level (U/ml)	11.7 (6.9–18.1)*
Surgical approach	
Open	32 (27.1)
Laparoscopy	86 (72.9)
Type of surgery	
LAR	109 (92.4)
APR	99 (7.6)
Postoperative complications	25 (21.2)
Adjuvant chemotherapy	110 (93.2)
Acellular mucin pools	32 (27.1)
MUC1 expression	17 (14.4)

Data are expressed as number (%), mean ± standard deviation, or median (interquartile range), when appropriate.
* Median (interquartile range).
nCRT, neoadjuvant chemoradiotherapy; **CEA**, carcinoembryonic antigen; **CA199,** carbohydrate antigen 199; **LAR**: low anterior resection; **APR**, abdominoperineal resection; **MUC1**, mucin 1.

$P = 0.002$), and MUC1 expression (HR = 8.280, $P < 0.001$) were independently associated with DMFS in patients with pCR following nCRT and TME (Table 3). All significant variables in the univariate analysis were entered into a Cox regression model. The results demonstrated that MUC1 expression (HR = 3.812, 95%CI: 1.054–13.782, $P = 0.041$) remained to be an independent predictor of DMFS in pCR patients, as shown in Table 3.

Association between MUC1 expression and clinicopathological features

We further evaluated the correlation between positive MUC1 expression and clinicopathological features, and the results demonstrated that positive MUC1 expression was not associated with age, sex, tumor distance from the anal verge, histology, pre-nCRT CEA and CA199 level, post-nCRT CEA and CA199 level, radiation dose, preoperative chemotherapy regimen and interval from radiation to surgery (all $P > 0.05$).

Discussion

Unlike previous studies focused on the identification of CR after nCRT, the present study aimed to address issues of systemic tumor control in order to maximize the oncological benefits of pCR patients. We demonstrated that pCR almost eradicated tumor local recurrence, but distant metastasis still remained a major concern in rectal cancer patients with pCR. Moreover, we found that tumor distance from the anal verge, acellular mucin pools, and MUC1 expression were associated with distant metastasis in patients with pCR. After adjusting for confounding factors, MUC1 staining remained to be an independent risk factor for distant metastasis. Such information could be helpful in counseling patients of organ preservation.

In our series, pCR almost eradicated the risk of local recurrence; it might be attributed to both nCRT and the strict adherence to the oncological principle of TME in our specialized unit. However, the impact of pCR on systemic relapse was not that impressive, 13 patients (11%) experienced systemic recurrence after a median follow-up of 57 months, which was comparable with results published previously [4,15]. It is possible that the current chemoradiotherapy regimen (45–50.4Gy dose radiation and fluoropyrimidine-based chemotherapy) is sufficient for the reduction of local recurrence but insufficient to decrease significant rates of distant metastasis. Theoretically, radiation is a local treatment aimed directly at the primary tumor, while neoadjuvant chemotherapy is intended as a radiosensitizer to reduce local recurrence, any pre-existing micrometastatic disease and distant tumor spread would, therefore, remain untreated. Additionally, given that the molecular and genetic biology between the primary tumor and metastatic cells are quite different, metastatic cells might remain viable and cause systemic tumor relapse even in patients achieving pCR [20]. In a recently-published pooled analysis [21] comprising 692 rectal cancer patients with cCR after nCRT who received “watch

Table 2
Clinicopathological details of 13 pCR patients with tumor recurrence.

No	Age (yrs)	Sex	Initial staging	Tumor DAV (cm)	Radiation dose (Gy)	Concurrent CTx	Interval to surgery (w)	Surgery	LNs harvested	Adjuvant CTx	Pattern of recurrence	DFS (mo)	Outcome	OS (mo)
1	39	F	T3N+	5	5000	CapeOX	6.9	ULAR	6	Yes	Lung	38	Dead	41
2	65	F	T3N+	5	4500	Capecitabine	7	ULAR	7	No	Lung	88	Alive	91
3	57	M	T4N+	5	5040	CapeOX + FOLFOX	6.7	LAR	11	Yes	Bone	7	Dead	10
4	64	F	T4N+	5	5000	CapeOX + FOLFOX	7	ULAR	10	Yes	Lung, brain	20	Dead*	23
5	74	M	T4N0	3	4500	FOLFOX	13.3	APR	14	Yes	Liver	25	Dead	14
6	63	M	T4N+	3	5040	CapeOX + FOLFOX	6.4	LAR	9	Yes	Liver	49	Alive	52
7	68	M	T4N+	3	4500	CapeOX + FOLFOX	5.3	ISR	5	Yes	Bone	36	Alive	39
8	47	F	T4N+	4	5040	CapeOX	8	LAR	12	Yes	Lung	37	Alive	66
9	46	F	T4N+	4	5040	CapeOX	7.3	LAR	4	Yes	Peritoneal	20	Dead	23
10	54	M	T3N+	5	5000	Capecitabine	7.6	ULAR	14	Yes	Spleen	52	Alive	55
11	58	F	T4N0	7	5000	CapeOX	7.6	ULAR	11	Yes	Bone	21	Alive	38
12	68	F	T3N+	5	5000	Capecitabine	10.1	ULAR	10	No	Lung	30	Alive	33
13	58	M	T3N+	4	5000	Capecitabine	9.1	LAR	9	Yes	Lung	23	Alive	26

M, male; **F**, female; **DAV**, distance from the anal verge; **CTx**, chemotherapy; **CapeOX**, capecitabine plus oxaliplatin; **FOLFOX**, 5-fluorouracil/folinic acid plus oxaliplatin; **LAR**, low anterior resection; **ULAR**, ultra-low anterior resection; **ISR**, intersphincteric resection; **APR**, abdominoperineal resection; **LNs**, lymph nodes; **DFS**, disease-free survival; **OS**, overall survival.
* Among the 6 patients who died during the follow-up period, all died of metastatic diseases except patient No.4 died of a heart attack.

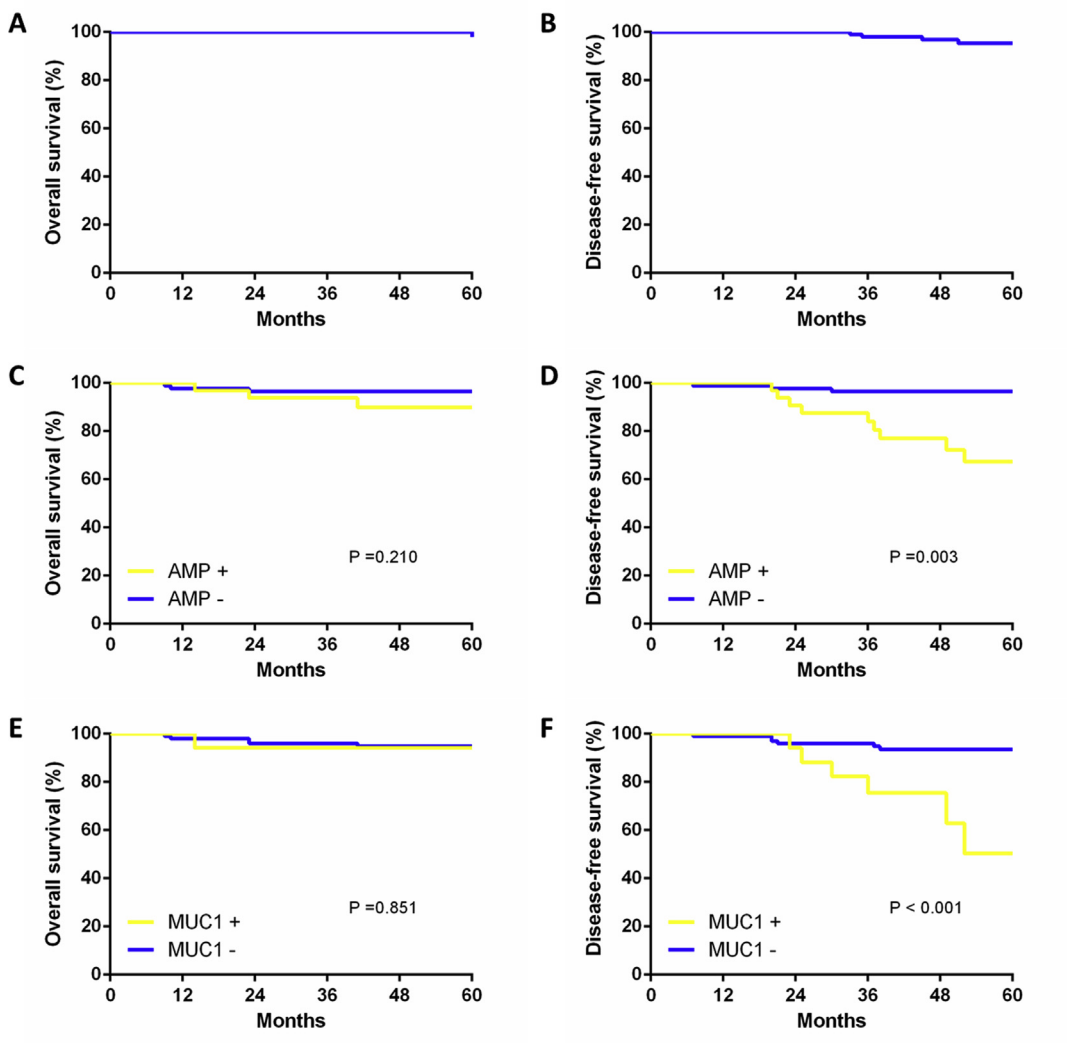

Fig. 2. Kaplan–Meier estimates for oncological outcomes in patients with pCR.
(A) Overall survival for patients with pCR.
(B) Disease-free survival for patients with pCR.
(C) Overall survival for patients with pCR stratified by acellular mucin pools.
(D) Disease-free survival for patients with pCR stratified by acellular mucin pools.
(E) Overall survival for patients with pCR stratified by MUC1 expression.
(F) Disease-free survival for patients with pCR stratified by MUC1 expression. **pCR**, pathological complete response; **MUC1**, mucin 1.

and wait" policy, 60% of systemic metastases occurred without evidence of local tumor regrowths, suggesting that systemic relapse might stem from pre-existing micrometastases, which could not be eradicated by nCRT and TME surgery. In this setting, one could expect local and systemic recurrence rates to be higher or at least similar if organ preservation is performed for these patients.

In line with previous studies [13,22,23], we also noted a metastatic pattern of a predominance of non-liver metastases in pCR patients. In our series, only 2 patients developed liver metastasis, while non-liver metastases included 5 lung metastases, 1 brain metastasis, 1 peritoneal dissemination, 1 spleen metastasis, and 3 bone metastases. It is possible that nCRT might impact the pattern of recurrence due to site-dependent differences in response, which alters tumor biology in ways that limit liver metastasis or favor non-liver metastasis.

These observations suggested that the risk of tumor systemic relapse should also be taken into account when considering organ preservation policy in CR patients. In this sense, the identification of high-risk patients for systemic relapse after pCR is of great clinical importance. Our study took one step further and identified predictive factors for systemic relapse in LARC patients achieving pCR. We found that tumor distance from the anal verge, acellular mucin pools, and MUC1 expression were associated with systemic relapse in patients with pCR. MUC1 staining remained to be an independent risk factor for DFS after adjustment for confounding factors.

Acellular mucin pools have been reported to occur in 16%–66% of rectal cancer patients after nCRT [24–28]. While some authors believed acellular mucin pools to be a marker of tumor response to neoadjuvant treatment [24,26,29], others suggested acellular mucin pools have a negative impact on oncological outcome [25,30]. Campos-Lobato et al. [25] have reported a trend that pCR patients with acellular mucin pools had an increased distant relapse and a decreased DFS and OS rate. Recently, Kaneko et al. [30] demonstrated that metachronous systemic metastasis was markedly higher in pCR patients with acellular mucin pools, and suggested intensive postoperative adjuvant treatment and close surveillance of recurrence in this subset of patients. In our series, pCR patients with acellular mucin pools have increased disease

Table 3
Cox regression analysis of risk factors for DMFS in pCR patients after nCRT.

Factors	Univariate analysis			Cox regression analysis		
	HR	95% CI	P value	HR	95% CI	P value
Sex (male vs. Female)	1.067	0.355–3.204	0.908			
Age	1.037	0.987–1.089	0.145			
BMI	1.010	0.852–1.199	0.906			
Distance from the anal verge	0.706	0.508–0.982	0.039	0.784	0.567–1.084	0.142
Histopathology (mucinous or signet ring adenocarcinoma vs. adenocarcinoma)	0.480	0.172–1.337	0.160			
Pre-nCRT cT stage	1.216	0.374–3.951	0.745			
Pre-nCRT cN stage	0.591	0.131–2.668	0.494			
Pre-nCRT CEA level	0.983	0.903–1.070	0.697			
Post-nCRT CA199 level	0.997	0.984–1.009	0.608			
Radiation dose	0.999	0.996–1.001	0.261			
Preoperative chemotherapy regimen (oxaliplatin-based vs. fluoropyrimidine only)	0.875	0.476–1.608	0.666			
Interval from radiation to surgery	0.930	0.668–1.295	0.667			
Post-nCRT CEA level	0.718	0.407–1.266	0.253			
Post-nCRT CA199 level	1.005	0.963–1.049	0.809			
Surgical approach (laparoscopic vs. open)	0.938	0.287–3.068	0.916			
Type of surgery (LAR vs. APR)	0.958	0.125–7.374	0.967			
Postoperative complication	0.938	0.287–3.068	0.916			
Lymph nodes harvested	0.949	0.853–1.055	0.333			
Acellular mucin pools	6.687	2.048–21.833	0.002	3.409	0.846–13.732	0.084
MUC1 expression	8.280	2.756–24.873	<0.001	3.812	1.054–13.782	0.041

DMFS, distant metastasis-free survival; **pCR,** pathological complete response; **nCRT,** neoadjuvant chemoradiotherapy; **HR**, hazard ratio; **CI**, confidential interval; **BMI**, body mass index; **CEA**, carcinoembryonic antigen; **CA199**, carbohydrate antigen 199; **LAR**: low anterior resection; **APR**, abdominoperineal resection; **MUC1**, mucin 1.

recurrence, indicating that acellular mucin pools may be an indicator of aggressive tumor biology, which was in accordance with previous findings [25,30]. However, acellular mucin pools were not identified as an independent risk factor of DMFS in multivariate analysis.

Although there is insufficient data to support the prognostic impact of acellular mucin pools in patients with rectal cancer after nCRT [31], we believe clinicians should be cautious towards acellular mucin pools. To exclude remnant tumor cells in acellular mucin pools, at least 10 multilevel sections were performed in at least three tumor blocks per case. Since CRT could induce qualitative changes in mucinous components in the surgical specimen, identifying whether acellular mucin pools are induced by nCRT or originally existing would be of great importance. We performed immunohistochemical analysis of MUC1 expression in the surgical specimen to test our hypothesis [32].

MUC1 is a transmembrane mucin glycoprotein that plays a role in tumorigenesis, progression, and metastasis in colorectal cancer, and has been reported to be related to poor oncological outcomes [33–35]. Additionally, the prognostic value of MUC1 has been proven in many epithelial cancers, especially in nonsmall cell lung cancer (NSCLC) and gastrointestinal cancers [35]. There are several potential mechanisms to explain the poor prognostic implication of MUC1 expression. MUC1 plays a role in several processes involved in tumor invasion and metastasis, including cell adhesion, immune suppression, and epithelial-mesenchymal transition (EMT) to promote the invasiveness of tumor [33]. Additionally, MUC1 positivity has been reported to be significantly correlated with tumor differentiation [36] and metastasis [37] in colorectal cancer. Noteworthy, MUC1 could be induced by irradiation [32]. In our cohort, the rate of positive MUC1 staining for acellular mucin pools was higher than that in patients without acellular mucin pools. Multivariate analysis demonstrated MUC1 staining to be an independent risk factor for DFS, which was in accordance with previous findings [37]. We performed CEA immunohistochemical staining in 32 patients with acellular mucin pools, but found no statistical correlation between CEA positivity and MUC1 positivity. Additionally, we didn't find any association between MUC1 expression and clinicopathological parameters. Thus, the reason for the prognostic impact of MUC1 expression in pCR patients remains unclear, and the potential role of MUC1 in pCR patients deserves further investigations.

In line with a previous study [4], our findings demonstrated that the effect of pCR on long-term outcome was not affected by clinical T or N stage, administration of adjuvant chemotherapy, distance from the anal verge, or type of surgery. The National Comprehensive Cancer Network (NCCN) guidelines recommend adjuvant chemotherapy in patients receiving nCRT regardless of the final stage; however, solid evidence to support the prognostic benefit of adjuvant chemotherapy in pCR patients is still lacking [38]. In our series, most pCR patients received chemotherapy after surgery, and adjuvant chemotherapy was not identified as a risk factor of DFS in our analysis.

Despite the limited evidence from our study, we suggest organ preservation which should be well balanced between the quality of life and the risk of both local and systemic recurrence. More intensive neoadjuvant treatment, including radiation dose escalation or induction/consolidation chemotherapy, could be offered to patients at high risk of relapse before considering the organ preservation strategy without compromising the oncological outcome. Another implication of our finding is that such high-risk patients with pCR may warrant more intensive adjuvant chemotherapy and active postoperative surveillance.

There were some limitations in our study. First, the present study was a single institutional retrospective analysis with a relatively small sample size. To minimize selection bias, we included patients receiving the consistent pretreatment workup, therapeutic, and follow-up strategies. Second, due to the relatively small sample of our study, the number of local recurrence was zero, which could be a limitation to our study. Thirdly, correlations between pretreatment biopsy and oncological outcomes could not be evaluated in our study, because a large proportion of patients included in the present study were referred to our institution from elsewhere and thus pretreatment biopsy could not be obtained in most cases. Fourth, due to the retrospective nature, certain clinicopathological factors, such as fixed tumors, the circumferential extent of tumors were not available for all patients, and thus were not evaluated in this study. Finally, the risk factors of systemic relapse were clinicopathological variables obtained from the postoperative surgical specimen. New biomarkers based on molecular and genetic profiles from pre-treatment biopsy specimen

could be more useful in counseling patients with treatment decision.

In our study, pCR almost eradicated tumor local recurrence, but systemic relapse still remained a major concern in rectal cancer patients with pCR. Tumor distance from the anal verge, acellular mucin pools, and MUC1 expression were associated with systemic relapse in patients with pCR. MUC1 staining remained to be an independent risk factor for DFS. Such information could facilitate treatment decision in these patients, such as adjuvant chemotherapy and follow-up.

Conflict of interest statement

None declared.

Role of the funding source

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