ORIGINAL ARTICLE

The effectiveness of neoadjuvant chemoradiotherapy in oesophageal adenocarcinoma with presence of extracellular mucin, signet-ring cells, and/or poorly cohesive cells

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Abstract

Oesophageal adenocarcinomas may show different histopathological patterns, including excessive acellular mucin pools, signet-ring cells (SRCs), and poorly cohesive cells (PCCs). These components have been suggested to correlate with poor outcomes after neoadjuvant chemoradiotherapy (nCRT), which might influence patient management. However, these factors have not been studied independently of each other with adjustment for tumour differentiation grade (i.e. the presence of well-formed glands), which is a possible confounder. We studied the pre- and post-treatment presence of extracellular mucin, SRCs, and/or PCCs in relation to pathological response and prognosis after nCRT in patients with oesophageal or oesophagogastric junction adenocarcinoma. A total of 325 patients were retrospectively identified from institutional databases of two university hospitals. All patients were scheduled for ChemoRadiotherapy for Oesophageal cancer followed by Surgery Study (CROSS) nCRT and oesophagectomy between 2001 and 2019. Percentages of well-formed glands, extracellular mucin, SRCs, and PCCs were scored in pre-treatment biopsies and post-treatment resection specimens. The association between histopathological factors (≥1 and >10%) and tumour regression grade 3–4 (i.e. >10% residual tumour), overall survival, and disease-free survival (DFS) was evaluated, adjusted for tumour differentiation grade amongst other clinicopathological variables. In pre-treatment biopsies, ≥1% extracellular mucin was present in 66 of 325 patients (20%); ≥1% SRCs in 43 of 325 (13%), and ≥1% PCCs in 126 of 325 (39%). We show that pre-treatment histopathological factors were unrelated to tumour regression grade. Pre-treatment presence of >10% PCCs was associated with lower DFS (hazard ratio [HR] 1.73, 95% CI 1.19–2.53). Patients with post-treatment presence of ≥1% SRCs had higher risk of death (HR 1.81, 95% CI 1.10–2.99). In conclusion, pre-treatment presence of extracellular mucin, SRCs, and/or PCCs is unrelated to pathological response. The presence of these factors should not be an argument to refrain from CROSS. At least 10% PCCs pre-treatment and any SRCs post-treatment, irrespective of the tumour differentiation grade, seem indicative of inferior prognosis, but require further validation in larger cohorts.

Keywords: mucinous adenocarcinoma; signet-ring cell carcinoma; poorly cohesive cells; oesophageal adenocarcinoma; chemoradiotherapy; prognosis; tumour regression

Introduction

Oesophageal adenocarcinomas (OACs) account for approximately 70% of locally advanced oesophageal cancers in high-income Western countries and the incidence is increasing [1]. Ten-year overall survival (OS) of adenocarcinoma patients treated with neoadjuvant chemoradiotherapy (nCRT) and surgery according to the ChemoRadiotherapy for Oesophageal cancer followed by Surgery Study (CROSS [2]) regimen is 36% [3]. Better understanding of the association between histopathologic features and therapy response may guide different therapeutic strategies, such as primary surgery and/or alternative (neo)adjuvant regimens [4,5].

The 2019 World Health Organization Classification of Tumours recognises different histological patterns of OAC [6]. Patterns include, but are not limited to, excessive acellular mucin pools, signet-ring cells (SRCs), and poorly cohesive cells (PCCs), which can occur in conjunction. Such patterns, reflecting mucinous and poorly cohesive adenocarcinoma subtypes, are currently reported histologically, but the clinical relevance is unclear. Extracellular mucin pools have been mostly attributed to therapy effects after nCRT, but their relevance as a separate factor before and after nCRT has not been extensively investigated [6,7]. SRCs are typically non-cohesive and exhibit one or multiple intracellular mucin vacuoles pushing the cell nucleus to the periphery of the cell. They occur in up to 16% of OAC [8]. PCCs are non-cohesive cells without characteristic SRC morphology. They have been described as uncommon in OAC [6]. Most of the available literature on the clinical relevance of these patterns concerns SRCs. In OAC, junctional and gastric adenocarcinoma, the presence of SRCs has been recognised as an adverse pathological feature regarding tumour regression and prognosis after nCRT [9–12]. For gastric SRC carcinoma, direct surgery plus adjuvant chemotherapy as an alternative treatment strategy to perioperative chemotherapy is being investigated [13]. However, important shortcomings apply to the aforementioned studies. Patients were excluded who did not undergo surgery after nCRT, and the tumour differentiation grade was not considered in multivariable analysis models.

Especially regarding the poorly cohesive cellular components, a correlation can be expected with tumour differentiation grade. The differentiation grade is based on the proportion of well-formed glands within the entire tumour area [6]. It describes the extent to which the tumour resembles the tissue from which it originates, and is used in cancer staging [14]. It is yet unknown what the relevance is of SRCs and/or PCCs irrespective of tumour differentiation grade. For example, it is unclear whether a well-differentiated tumour (i.e. having >95% well-formed glands) with any SRCs or PCCs shows a different response to nCRT and prognosis compared to a well-differentiated tumour without SRCs or PCCs. Conversely, the same holds for poorly differentiated tumours with versus without SRCs or PCCs. Poor tumour differentiation grade, rather than the presence of any of the aforementioned histopathological factors, could thus well be the dominating factor relating to inferior therapy response and prognosis instead of SRCs or PCCs.

The aim of the present study was to investigate the association between the presence of pre- and post-treatment extracellular mucin, SRCs, and/or PCCs on the one hand, and response to nCRT on the other hand, independent of tumour differentiation grade.

Materials and methods

Study design

This was a retrospective dual centre cohort study, which was approved by the Medical Ethical Committee of the Erasmus MC (MEC-2021-0410). The requirement for informed consent was waived.

Patients

Patients with locally advanced OAC who underwent nCRT according to the CROSS regimen [2] were eligible regardless of the number of cycles of nCRT completed. Patients treated between 2001 and 2019 at the Erasmus MC were identified from the CROSS trial database and the post-CROSS cohorts [15–17]. Patients treated between 2017 and 2019 at the Radboudumc were identified from an institutional database. Patients were included when the pathology slides of pre-treatment biopsies and the resection specimen were available at the university hospital. Patients who were treated at the Erasmus MC but had undergone diagnostic staging at a referring centre were included when the pre-treatment biopsy slides were available at the regional collaborative pathology laboratory Pathan B.V. in Rotterdam. Patients who did not undergo surgery, e.g. because of detected interval distant metastasis or deterioration of physical condition after nCRT, were only included for survival analysis.

Study endpoints

The primary endpoint was response to nCRT according to Chirieac's tumour regression grade (TRG), including [18]: TRG 1, 0% residual vital tumour; TRG 2, 1–10% tumour; TRG 3, 11–50% tumour; TRG 4, 51–100% tumour.

Secondary endpoints included OS and disease-free survival (DFS). OS was calculated from date of diagnosis to date of death or last follow-up and DFS until date of tumour recurrence (locoregional and/or distant), death or last follow-up, as collected from the electronic patient records.

Histopathological assessment

Five pathologists (AMV, RSvdP, AHAGO, LO, and MD), who were blinded for study outcomes, each reviewed a partition of the pathology slides. Three histopathologic factors were scored on the available haematoxylin and eosin (H&E) slides from biopsies and resection specimens: (1) excessive extracellular mucin, (2) SRCs, and (3) PCCs (Figure 1). The factors were scored relative to the entire tumour area. The following scoring categories were decided during joint discussion between pathologists, similarly to available categories in the literature [12,18]: <1%, 1–10%, 11–50%, and 51–100%. The distinction between the <1 and ≥1% categories was made to ensure that at least 1% of a factor was present for factors scored as ≥1%. A median score was derived based on all available slides. Approximately four tumour-containing biopsies and six to eight tumour (bed)-associated resection specimen slides per patient were available for review. For the most recently treated patients, the tumour bed was embedded completely according to recent guidelines [19]. Histopathological factors in the resection specimen were analysed only for patients with residual tumour in the resection specimen (TRG 2–3–4), since in patients with TRG 1, the estimation of the histopathological factors relative to the tumour area was not possible. Regarding PCCs, some cohesiveness was accepted (Figure 1C,D). Typical radiotherapy effects such as degradation of cells were not scored as PCCs. For exploratory analyses, two additional variables were scored: (1) the sum of SRCs plus PCCs; (2) the highest category of any of the three histopathological factors (e.g. see supplementary material, Table S1). Furthermore, in patients with SRCs after nCRT it was explored whether a Barrett segment was present in the resection specimen, to examine the possible relation with the location of the primary tumour.

Independently from the previously described factors, the percentage of well-formed glands was scored in biopsy and resection specimen H&E slides according to the eighth edition of the American Joint Committee on Cancer (AJCC) cancer staging manual: >95% (good tumour differentiation), 50–95% (moderate), and <50% (poor) [20]. Tumour differentiation was also scored relative to the entire tumour area. For instance, a tumour with 80% well-formed glands and 20% SRCs was scored moderate for tumour differentiation with 11–50% SRCs.

The following histopathological parameters were extracted from the pathology reports if available, and were (re-)assessed if unavailable: TRG, ypT stage, ypN stage, pre-treatment pathological tumour (prepT) stage, and pre-treatment pathological nodal (prepN) stage. prepT and prepN stages reflect pre-treatment tumour involvement, as assessed using the extent of treatment associated changes in the resection specimen. Since these variables are of prognostic importance, they were included [21]. Radical resection (R0) was defined as a tumour-free margin (<1 mm distance included). Pathologically complete response (pCR) was defined as ypT0N0.

Consensus meetings

Consensus on histopathological assessment, including the scoring of tumour differentiation grade, was established during four meetings. The first two meetings were used to formulate criteria for scoring histopathological factors based on scoring several cases individually, followed by a joint discussion and consensus. The third meeting was organised to evaluate whether five challenging cases were scored with an agreement of at least >60% (i.e. at least three of the five pathologists agreed on each score), which was successful. After scoring the entire dataset, remaining ambiguous cases were discussed in a final consensus meeting in which all pathologists participated.

Sample size

A proportion of 10% of patients with any component of SRCs was expected in the study cohort based on a recently reported prevalence of 13% [12]. A minimum sample size of 300 patients allowed to detect approximately 30 cases with SRCs, which was considered sufficient for a covariate in multivariable models.

Statistical analysis

A chi-squared test was used to evaluate baseline and post-treatment clinicopathological characteristics, including the tumour differentiation grade, in relation to presence of histopathological factors (statistical significance at $p < 0.05$).
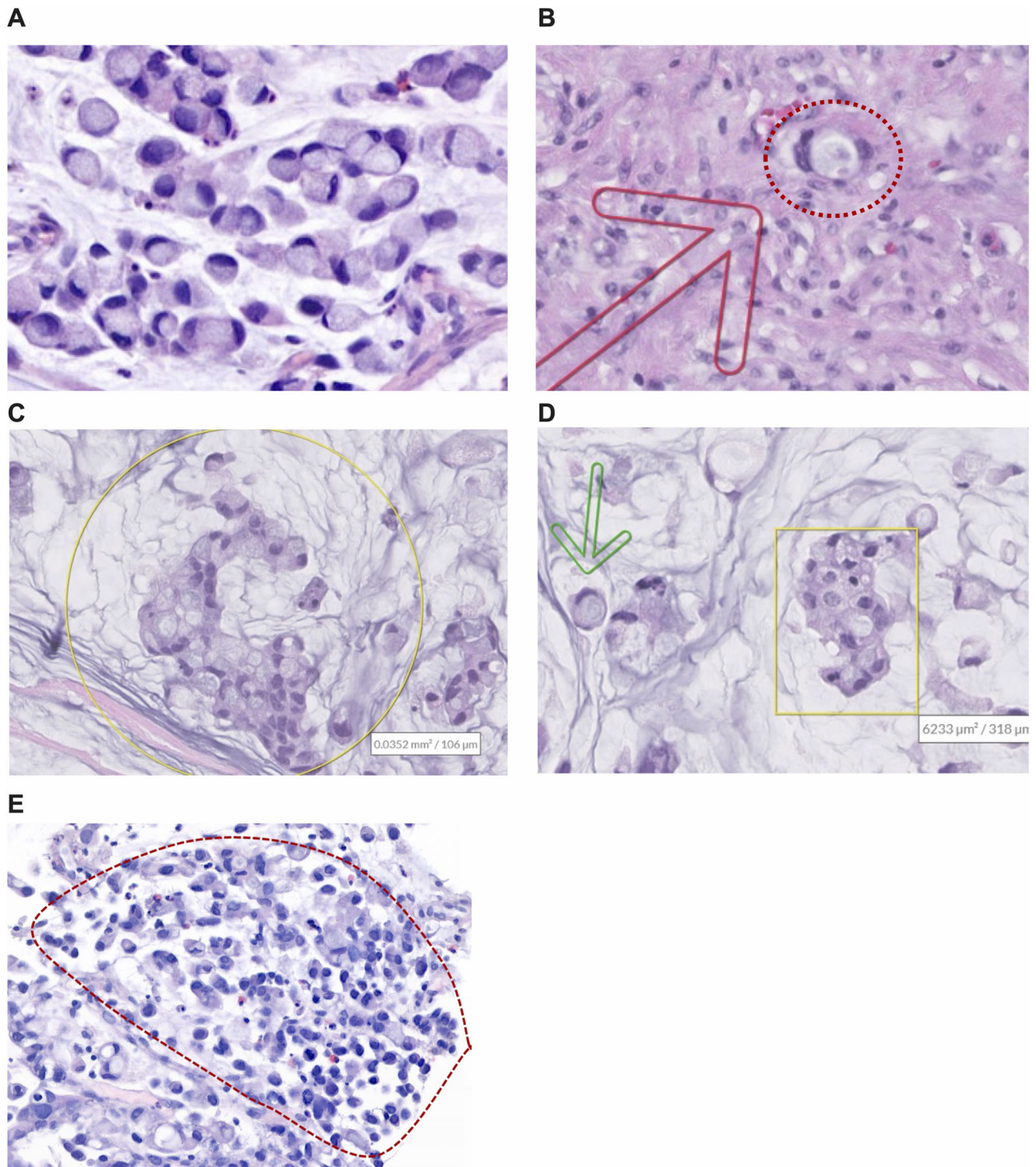
Figure 1. Examples of the evaluated histopathological factors in patients treated with nCRT. (A) SRCs in a biopsy before nCRT. (B) Pseudo SRC in a resection specimen after nCRT (dotted red circle). A small atrophic gland, composed of atrophic epithelial cells mimicking a signet-ring morphology (notice the presence of multiple nuclei at the periphery). (C) Group of PCCs (yellow circle) surrounded by extracellular mucin in the resection specimen after nCRT. Some cohesiveness in PCCs was accepted. (D) SRC (green arrow) and PCCs (yellow box) and surrounding extracellular mucin in a resection specimen after nCRT. (E) Group of predominantly PCCs (red contour) in a pre-treatment biopsy (note the absence of a clear mucin-vacuole in the cytoplasm).

The primary endpoint was dichotomised into major response (TRG 1–2) and minor response (TRG 3–4).

For each pre-treatment histopathological factor, a multivariable logistic regression model was fitted, adjusted for pre-treatment variables including age, gender, cT stage (cT3–4 versus cT1–2) and cN stage (cN+ versus cN0).

For analysis of OS and DFS, all patients who underwent nCRT were included, regardless of subsequent resection. OS and DFS for each histopathological factor were analysed with the Kaplan–Meier estimator with log-rank testing and compared with multivariable Cox proportional-hazards models. Hazard ratios (HRs) in models based on pre-treatment histopathological factors were adjusted for variables as known pre-treatment: age, gender, cT stage (cT3–4 versus cT1–2) and cN stage (cN+ versus cN0). HRs in models based on post-treatment histopathological factors were adjusted for variables as available post-treatment: age, gender, prepT stage (prepT3–4 versus prepT1–2), prepN stage (prepN+ versus prepN0) [21,22], ypT stage (ypT3–4 versus ypT1–2), ypN stage (ypN+ versus ypN0), TRG (TRG 3–4 versus TRG 2), and resection margin status (R1 versus R0).

All models were fitted with and without pre-treatment tumour differentiation grade to examine its effect on the primary and secondary outcomes. Histopathological factors were evaluated as presence of any component ($\geq 1\%$ versus $< 1\%$) and as presence of a considerable component ($> 10\%$ versus 0–10%), since this was considered (after consensus meetings) as an easily reproducible cut-off. To correct for multiple testing, a p value of 0.025 was considered statistically significant using Bonferroni correction (i.e. $p = 0.05/2$ for two tests per factor).

Data were analysed with R version 4.0.4 (R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). The code has been made publicly available on github.com/mjvalkema/CROSS-PA-factors.

Results

Patients

A total of 325 patients were included in the study (Figure 2). Patients received neoadjuvant treatment between 7 March 2001 and 7 May 2019 at the Erasmus MC and between 26 January 2016 and 20 February 2019 at the Radboudumc. Patient and tumour characteristics are shown in Table 1.

A total of 290 of 325 (89%) patients underwent surgery at a median of 9 weeks (interquartile range [IQR] 7–12)

after completion of nCRT. In 35 patients, surgery was not performed because of active surveillance at own request (5), distant metastases (20), died before surgery (5), physically unfit for surgery (4), and refused surgery (1). Resection was performed in 284 of 290 (98%) patients; 4 patients had distant metastases detected perioperatively and 2 had unresectable tumour (T4b).

Histopathological assessment

In pre-treatment biopsies, $\geq 1\%$ extracellular mucin was present in 66 of 325 (20%) patients, $\geq 1\%$ SRCs in 43 of 325 (13%) patients, and PCCs in 126 of 325 (39%) patients. In the resection specimens of patients with residual tumour, $\geq 1\%$ extracellular mucin was present in 78 of 231 (34%) patients, $\geq 1\%$ SRCs in 30 of 231 (13%) patients, and PCCs in 89 of 231 (39%) patients (Table 1).

The association between clinicopathological characteristics and the histopathological factors in biopsies and resection specimens is shown in Table 2 (for $< 1\%$ versus $\geq 1\%$ categories) and in supplementary material, Table S2 (for 0–10% versus >10%). All three histopathological factors were correlated with poor tumour differentiation in biopsies (pre-treatment $p < 0.001$; post-treatment $p < 0.05$).

The proportion of the presence of a histopathological factor could differ pre-treatment and post-treatment (Figure 3). For instance, 20 of 284 (7%) patients had $\geq 1\%$ SRCs in biopsies but none ($< 1\%$) in the resection specimen (Figure 3C). In patients with $\geq 1\%$ SRCs in the resection specimen, a Barrett segment was seen in 6 of 30 cases (20%).

Histopathological factors and response to nCRT

None of the pre-treatment histopathological factors were associated with TRG 3–4 (Table 3) or with pCR (Table 2). Pre-treatment poor tumour differentiation grade was also not correlated with TRG 3–4 (adjusted odds ratio [OR] 0.69, 95% CI 0.42–1.15, $p = 0.16$) (supplementary material, Table S3). Poorly differentiated tumours had higher probability of reaching pCR compared with tumours with good–moderate differentiation (adjusted OR 2.12, 95% CI 1.09–4.15, $p = 0.03$).

Histopathological factors and survival

Survival dates were collected until 15 July 2022. Median potential follow-up time (i.e. time from diagnosis to end of data collection without an event) was 81 months (IQR 52–138). For the entire study cohort, the median follow-up was 49 months for OS (IQR 31–67) and 57 months for DFS (IQR 33–70). Median OS and DFS were

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┌─────────────────────────────────┐          ┌─────────────────────────────────┐
│ Erasmus MC: assessed for        │          │ Radboudumc: assessed for        │
│ eligibility (n = 330)           │          │ eligibility (n = 114)           │
└─────────────────────────────────┘          └─────────────────────────────────┘
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Erasmus MC: assessed for eligibility (n = 330)

Radboudumc: assessed for eligibility (n = 114)

Excluded (n = 105)
biopsy slides outside region Erasmus MC: n = 57
biopsy slides not available: n = 32
resection slides not available: n = 12
no malignancy in pre-treatment biopsy: n = 1
objection for use of data for research: n = 3

Excluded (n = 14)
biopsy slides not available: n = 9
resection slides not available: n = 1
too little tumour in biopsy: n = 1
objection for use of data for research: n = 2
during nCRT switch to palliative therapy: n = 1

Included Erasmus MC (n = 225)

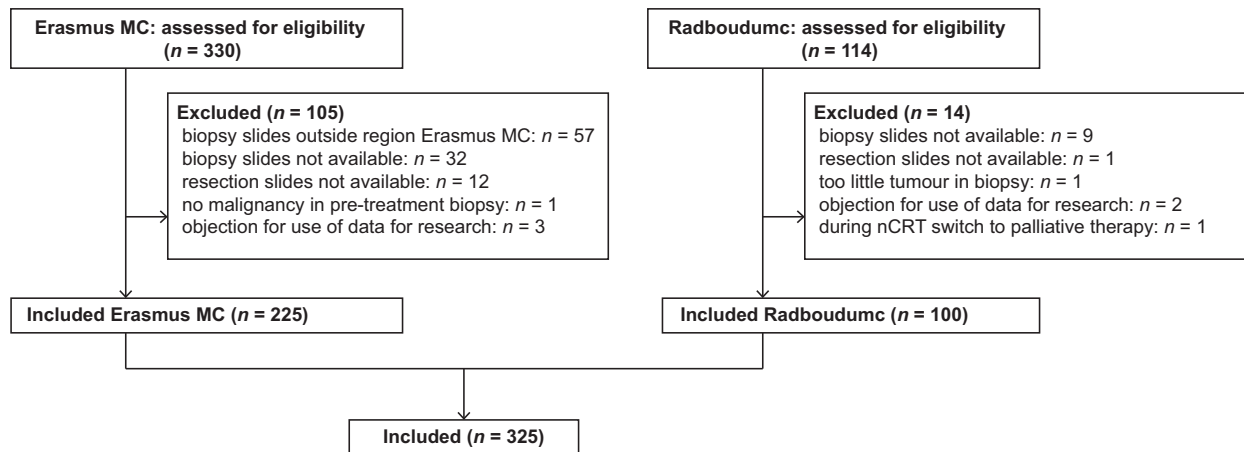**Included Radboudumc (n = 100)**

Included (n = 325)

Figure 2. Study flowchart.

44 months (IQR 36–62) and 31 months (IQR 26–45), respectively. Poor tumour differentiation grade on pre-treatment biopsies was associated with worse OS and DFS compared with good–moderate differentiation (adjusted HR for death 1.40, 95% CI 1.03–1.89, $p = 0.03$; supplementary material, Table S3). Patients with >10% PCCs in pre-treatment biopsies had inferior DFS compared with patients with 0–10% PCCs (adjusted HR 1.73, 95% CI 1.19–2.53, $p = 0.005$) (Table 4 and Figure 4). Pre-treatment extracellular mucin and SRCs were not associated with survival. Of the post-treatment histopathological factors, presence of ≥1% or >10% SRCs in the resection specimen was associated with inferior OS and DFS (≥1% SRCs: adjusted HR for death 1.81, 95% CI 1.10–2.99, $p = 0.02$; >10% SRCs: HR 2.55, 95% CI 1.30–5.04, $p = 0.007$) (Table 4 and Figure 4). The discrepancy between the non-statistically significant association with presence of SRCs pre-treatment and the statistically significant association post-treatment is illustrated in supplementary material, Figure S1.

The effect of pre-treatment tumour differentiation on the HRs is shown in supplementary material, Tables S3 and S4. The survival curves for all histopathological factors, pre- and post-treatment, and the corresponding median survival times, are presented in supplementary material, Figures S2 and S3, and Table S5, respectively.

Discussion

This study demonstrates that presence of extracellular mucin, SRCs, and/or PCCs in pre-treatment biopsies is unrelated to pathological response to nCRT. Therefore, the pre-treatment presence of these factors should not be an argument to refrain from nCRT. The presence of >10% PCCs in pre-treatment biopsies was shown to correspond to inferior DFS, regardless of the tumour differentiation grade. Poor tumour differentiation grade was indeed shown to correspond to worse prognosis and its correlation with any of the histopathological factors pre-treatment was confirmed. An extracellular mucinous component or SRCs in pre-treatment biopsies was not associated with survival. However, after nCRT, presence of SRCs in the resection specimen corresponded to poor OS and DFS.

The prevalence of SRCs in 13% of tumours at diagnosis confirms previous findings of 13 and 16% in two other cohorts of OAC patients treated with CROSS nCRT [8,12]. Our finding that SRCs in biopsies pre-treatment are unrelated to pathological outcome and prognosis after nCRT is in line with one study examining both extracellular mucin plus SRCs [7], but is contradictory to other studies [12,23,24]. The best comparable publication to the present study is the paper by Corsini et al, describing 819 patients treated with CROSS nCRT and resection [12]. Any presence of SRCs before treatment was associated with a decreased pCR rate and with decreased OS in a multivariable model (HR 1.39, 95% CI 1.02–1.89). However, the model did not correct for tumour differentiation grade as was done in the present study. Furthermore, the presence of SRCs was extracted automatically from the pathology reports. This was also done differently than in the present study in which definitions were pre-defined and all pathology slides were revised accordingly by experienced gastrointestinal pathologists. In

Table 1. Patient and tumour characteristics

All patients (n = 325) Pre-treatment characteristic		Patients who underwent resection (n = 284)			
		Post-treatment histopathological characteristic in the resection specimen		Post-treatment histopathological characteristic in the resection specimen	
Sex, male	280 (86.2)	TRG			
Age	65 (59–71)	TRG 1	53 (18.7)		
Tumour location		TRG 2	81 (28.5)		
Distal/GEJ	317 (97.8)	TRG 3	96 (33.8)		
Mid	7 (2.2)	TRG 4	54 (19.0)		
cT stage		ypT stage		prepT stage	
cT1b	4 (1.2)	ypT0	53 (18.7)	prepT1	16 (5.6)
cT2	72 (22.2)	ypT1	56 (19.7)	prepT2	53 (18.7)
cT3	234 (72.0)	ypT2	61 (21.5)	prepT3	213 (75.0)
cT4a	13 (4.0)	ypT3	113 (39.8)	prepT4	2 (0.7)
cTx	2 (0.6)	ypT4	1 (0.4)		
cN stage		ypN stage		prepN stage	
cN0	120 (36.9)	ypN0	175 (61.6)	prepN0	132 (46.6)
cN1	145 (44.6)	ypN1	78 (27.5)	prepN1	89 (31.4)
cN2	56 (17.2)	ypN2	23 (8.1)	prepN2	47 (16.6)
cN3	4 (1.2)	ypN3	8 (2.8)	prepN3	15 (5.3)
Completion of chemoradiotherapy		pCR	46 (16.2)		
Yes	242 (74.5)				
Reduction*	13 (4.0)	Resection margin			
Missing	70 (21.5)	R0	267 (94.0)		
		R1 proximal	2 (0.7)		
		R1 distal	1 (0.4)		
		R1 circumferential†	14 (4.9)		

All patients (n = 325) Pre-treatment histopathological characteristic in biopsies		Patients with residual tumour in the resection specimen (n = 231) Post-treatment histopathological characteristic in the resection specimen	
Differentiation grade		Differentiation grade	
Good	89 (27.4)	Good	38 (16.5)
Moderate	124 (38.2)	Moderate	95 (41.1)
Poor	112 (34.5)	Poor	98 (42.4)
Extracellular mucin		Extracellular mucin	
<1%	259 (79.7)	<1%	153 (66.2)
1–10%	20 (6.2)	1–10%	29 (12.6)
11–50%	23 (7.1)	11–50%	17 (7.4)
51–100%	23 (7.1)	51–100%	32 (13.9)
Signet-ring cells		Signet-ring cells	
<1%	282 (86.8)	<1%	201 (87.0)
1–10%	14 (4.3)	1–10%	14 (6.1)
11–50%	14 (4.3)	11–50%	6 (2.6)
51–100%	15 (4.6)	51–100%	10 (4.3)
Poorly cohesive cells		Poorly cohesive cells	
<1%	199 (61.2)	<1%	142 (61.5)
1–10%	49 (15.1)	1–10%	25 (10.8)
11–50%	31 (9.5)	11–50%	33 (14.3)
51–100%	46 (14.2)	51–100%	31 (13.4)
Signet-ring cells + poorly cohesive cells		Signet-ring cells + poorly cohesive cells	
<1%	197 (60.6)	<1%	134 (58.0)
1–10%	41 (12.6)	1–10%	23 (10.0)
11–50%	20 (6.2)	11–50%	30 (13.0)
51–100%	67 (20.6)	51–100%	44 (19.0)
Highest category of all factors		Highest category of all factors	
<1%	186 (57.2)	<1%	101 (43.7)
1–10%	44 (13.5)	1–10%	36 (15.6)
11–50%	30 (9.2)	11–50%	37 (16.0)
51–100%	65 (20.0)	51–100%	57 (24.7)

Data are n (%), or median (IQR).
GEJ, gastroesophageal junction.
*Three or four cycles chemotherapy or less fractions of radiotherapy.
†Three of 14 patients with R1 margin had poorly differentiated tumours; 11 of 14 had good-moderate tumour differentiation.

Table 2. Association between presence of histopathological factors (<1% versus ≥1%) in biopsies and resection specimens and clinicopathological characteristics

All patients (n = 325), presence of histopathological factors in biopsies (<1% versus ≥1%)

	Extracellular mucin			Signet-ring cells			Poorly cohesive cells		
	<1% n = 259	≥1% n = 66	P	<1% n = 282	≥1% n = 43	P	<1% n = 199	≥1% n = 126	P*
Age	66 (59–71)	64 (57–70)	0.66	66 (59–71)	63 (56–69)	0.29	66 (59–71)	65 (57–70)	0.45
Male	225 (86.9)	55 (83.3)	0.59	244 (86.5)	36 (83.7)	0.80	178 (89.4)	102 (81.0)	0.05
Poor differentiation (versus good–moderate)	**72 (27.8)**	**40 (60.6)**	**<0.001**	**75 (26.6)**	**37 (86.0)**	**<0.001**	**32 (16.1)**	**80 (63.5)**	**<0.001**
cT3–4a (versus cT1–2)	201 (77.6)	48 (72.7)	0.50	218 (77.3)	31 (72.1)	0.58	154 (77.4)	95 (75.4)	0.78
cN+ (versus cN0)	166 (64.1)	39 (59.1)	0.54	181 (64.2)	24 (55.8)	0.37	128 (64.3)	77 (61.1)	0.64
prepT3–4 (versus prepT1–2)	174 (76.3)	41 (73.2)	0.76	187 (75.1)	28 (80.0)	0.67	127 (72.2)	88 (81.5)	0.10
prepN+ (versus prepN0)	117 (51.5)	34 (60.7)	0.28	128 (51.6)	23 (65.7)	0.17	85 (48.6)	66 (61.1)	0.05
Resection, yes	228 (98.3)	56 (96.6)	0.76	249 (98.4)	35 (94.6)	0.36	176 (98.3)	108 (97.3)	0.86
pCR	35 (15.4)	11 (19.6)	0.56	39 (15.7)	7 (20.0)	0.68	28 (15.9)	18 (16.7)	1.00
TRG			0.37			0.69			0.85
TRG 1	40 (17.5)	13 (23.2)		44 (17.7)	9 (25.7)		30 (17.0)	23 (21.3)	
TRG 2	62 (27.2)	19 (33.9)		71 (28.5)	10 (28.6)		51 (29.0)	30 (27.8)	
TRG 3	82 (36.0)	14 (25.0)		86 (34.5)	10 (28.6)		61 (34.7)	35 (32.4)	
TRG 4	44 (19.3)	10 (17.9)		48 (19.3)	6 (17.1)		34 (19.3)	20 (18.5)	
R1 resection	15 (6.6)	2 (3.6)	0.59	15 (6.0)	2 (5.7)	1.00	12 (6.8)	5 (4.6)	0.62

Patients with residual tumour (n = 231), presence of histopathological factors in the resection specimen (<1% versus ≥1%)

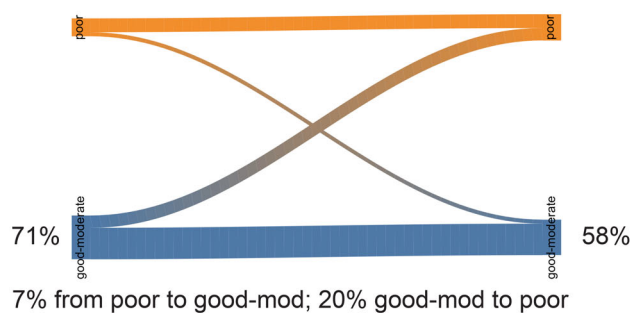|	Extracellular mucin			Signet-ring cells			Poorly cohesive cells		
	<1% n = 153	≥1% n = 78	P	<1% n = 201	≥1% n = 30	P	<1% n = 142	≥1% n = 89	P*
Age	65 (58–70)	63 (57–69)	0.37	65 (57–70)	63 (57–67)	0.48	65 (58–70)	64 (56–70)	0.47
Male	129 (84.3)	68 (87.2)	0.70	170 (84.6)	27 (90.0)	0.61	**130 (91.5)**	**67 (75.3)**	**0.001**
Poor differentiation (biopsy) (versus good–moderate)	**37 (24.2)**	**30 (38.5)**	**0.04**	**50 (24.9)**	**17 (56.7)**	**0.001**	**32 (22.5)**	**35 (39.3)**	**0.01**
Poor differentiation (res.) (versus good–moderate)	60 (39.2)	38 (48.7)	0.22	**75 (37.3)**	**23 (76.7)**	**<0.001**	**52 (36.6)**	**46 (51.7)**	**0.03**
cT3–4a (versus cT1–2)	113 (73.9)	67 (85.9)	0.06	157 (78.1)	23 (76.7)	1.00	110 (77.5)	70 (78.7)	0.96
cN+ (versus cN0)	93 (60.8)	50 (64.1)	0.72	124 (61.7)	19 (63.3)	1.00	90 (63.4)	53 (59.6)	0.66
prepT3–4 (versus prepT1–2)	119 (77.8)	65 (83.3)	0.41	**155 (77.1)**	**29 (96.7)**	**0.03**	109 (76.8)	75 (84.3)	0.23
prepN+ (versus prepN0)	85 (55.9)	49 (62.8)	0.39	**109 (54.5)**	**25 (83.3)**	**0.005**	76 (53.9)	58 (65.2)	0.12
TRG			0.41			0.17			**0.01**
TRG 2	57 (37.3)	24 (30.8)		73 (36.3)	8 (26.7)		**59 (41.5)**	**22 (24.7)**	
TRG 3	64 (41.8)	32 (41.0)		85 (42.3)	11 (36.7)		**57 (40.1)**	**39 (43.8)**	
TRG 4	32 (20.9)	22 (28.2)		43 (21.4)	11 (36.7)		**26 (18.3)**	**28 (31.5)**	
R1 resection	7 (4.6)	10 (12.8)	0.05	13 (6.5)	4 (13.3)	0.33	**6 (4.2)**	**11 (12.4)**	**0.04**

Data are n (%), or median (IQR).
*Statistical significance at p < 0.05 (shown in bold font).

the paper of Corsini *et al*, a clear distinction between SRCs and PCCs is not described. Moreover, this distinction is rarely mentioned in regular pathology reports since they are included in the differentiation grade: in biopsies foci with the worst grade determine the overall tumour differentiation grade [14]. In other studies with contradicting findings, the use of mixed neoadjuvant treatment regimens and extraction of available pathology data from reports might have contributed to conflicting findings with the present results [23,24].
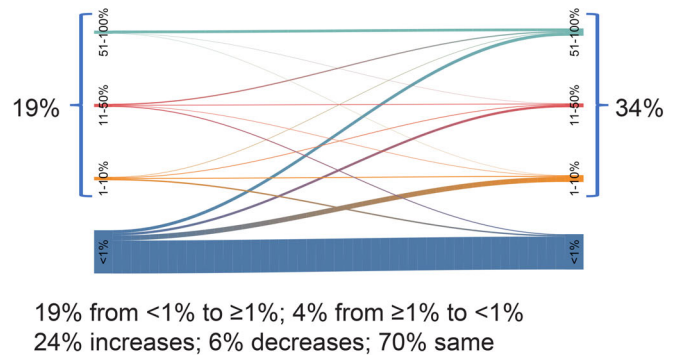
In the present study, the pCR rate did not differ between patients with SRCs versus patients with regular type adenocarcinoma (20% versus 16% respectively). This finding is in line with a study based on the National Cancer Database from the United States on adenocarcinoma patients treated between 2004 and 2015 with nCRT including mixed regimens (21% versus 19% respectively) [25]. However, significantly different pCR rates between patients with and without SRCs have been reported in the literature (6–13% versus 23–26% respectively) [8,12,26,27]. This discrepancy is not easily explained, but might be due to differences in sample size, patient selection, and methodology for scoring. The present study confirmed a
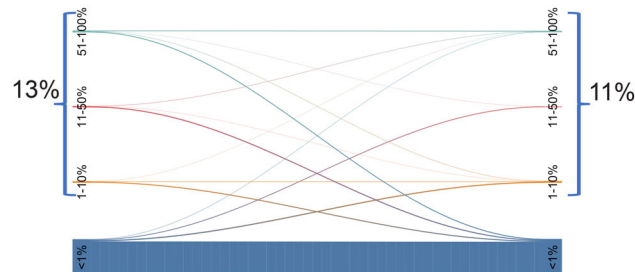
A Differentiation grade

7% from poor to good-mod; 20% good-mod to poor

B Extracellular mucin

19% from <1% to ≥1%; 4% from ≥1% to <1%
24% increases; 6% decreases; 70% same

C Signet-ring cells

5% from <1% to ≥1%; 7% from ≥1% to <1%
6% increases; 9% decreases; 85% same

D Poorly cohesive cells

12% from <1% to ≥1%; 19% from ≥1% to <1%
20% increases; 23% decreases; 58% same
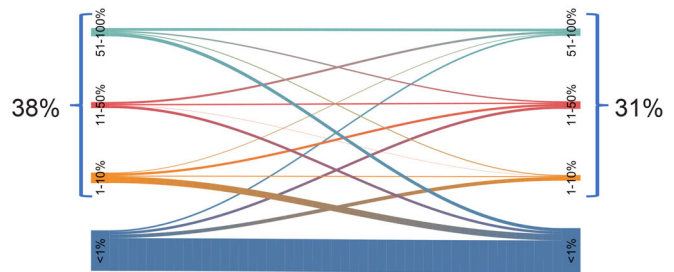
Figure 3. River plot showing the presence of histopathological factors as scored in different categories (<1%; 1–10%; 11–50%; 51–100%) on the left side in every figure for pre-treatment biopsies, and on the right side for matched post-treatment resection specimens. (A and B) Histopathological factors in patients who underwent resection and had residual tumour after chemoradiotherapy (TRG 2–3–4; n = 231). (C and D) histopathological factors in all patients who underwent resection (TRG 1–2–3–4; n = 284). Patients with TRG 1 (0% residual tumour) were assigned to the <1% category of the histopathological factor.

previously reported positive association between pre-treatment poor tumour differentiation and pCR [8]. The authors of that Dutch nationwide cohort study have suggested that poorly differentiated tumours are more susceptible to DNA damage and thus these tumours have a higher chance of reaching pCR after nCRT. The pCR rate of all adenocarcinomas, which was 16% in the present study, and for comparison, 19% in the cohort study [8], is lower than the pCR rate of 23% as published in the CROSS trial [2]. In line with current guidelines, over the last years larger parts or even complete inclusion of the tumour bed have been submitted for histopathological analysis, which might explain the lower pCR rate of the present study to some extent [19]. Additionally, over time more advanced tumours might have been treated with CROSS. For example, the present cohort included

patients with cT4a stage, whereas in the CROSS trial inclusion criteria were limited to cT1N1 or cT2–3 cN0/cN+ stage [2].

One of the strengths of the present study is that all patients were treated with the same neoadjuvant treatment regimen. Moreover, patients who did not proceed to resection after nCRT were included in order to evaluate prognosis in all patients with an initial indication for nCRT and resection. Another strength, as already touched upon, is the extensive review of pathology slides performed by experienced GI pathologists, instead of using pathology reports for data extraction. Still, some inter-observer variability cannot be ruled out, though minimised via the repeated consensus meetings. The histopathological factors were separately assessed, which allowed for example that tumour differentiation grade was scored independently of other

Table 3. Association between histopathological factors in biopsies pre-treatment and presence of substantial residual tumour after chemoradiotherapy (TRG 3–4)

		OR (95% CI), adjusted clinical variables*	P	OR (95% CI), adj. clinical variables* + pre-treatment tumour differentiation grade	P
The effect of differentiation grade as a confounder					
Differentiation grade	poor (versus good-moderate)	0.69 (0.42–1.15)	0.16	–	–
Primary analyses					
Extracellular mucin	≥1% (versus <1%)	0.61 (0.34–1.10)	0.10	0.67 (0.36–1.23)	0.20
	>10% (versus 0–10%)	0.54 (0.27–1.07)	0.08	0.60 (0.29–1.25)	0.18
Signet-ring cells	≥1% (versus <1%)	0.81 (0.40–1.63)	0.55	1.01 (0.46–2.23)	0.97
	>10% (versus 0–10%)	0.66 (0.28–1.51)	0.33	0.81 (0.32–1.99)	0.64
Poorly cohesive cells	≥1% (versus <1%)	0.89 (0.55–1.45)	0.64	1.08 (0.62–1.92)	0.78
	>10% (versus 0–10%)	1.00 (0.56–1.76)	0.99	1.40 (0.70–2.85)	0.34
Exploratory analyses					
Signet-ring cells + poorly cohesive cells	≥1% (versus <1%)	0.83 (0.51–1.35)	0.46	0.99 (0.56–1.75)	0.98
	>10% (versus 0–10%)	0.78 (0.45–1.33)	0.36	0.97 (0.49–1.92)	0.93
Highest category of all factors	≥1% (versus <1%)	0.85 (0.53–1.37)	0.50	1.00 (0.58–1.73)	1.00
	>10% (versus 0–10%)	0.65 (0.38–1.10)	0.11	0.73 (0.38–1.42)	0.36

*Adjusted for age, sex, cT stage (cT3–4 versus cT1–2), and cN stage (cN+ versus cN0).

Table 4. Association between pre- and post-treatment histopathological factors and survival

All patients (n = 325), histopathological factors in pre-treatment biopsies

		OS		DFS	
		Adjusted* HR (95% CI)	P	Adjusted* HR (95% CI)	P[†]
Primary analyses					
Extracellular mucin	≥1% (versus <1%)	1.06 (0.72–1.56)	0.76	1.03 (0.72–1.48)	0.88
	>10% (versus 0–10%)	0.90 (0.57–1.41)	0.63	0.94 (0.61–1.43)	0.77
Signet-ring cells	≥1% (versus <1%)	0.94 (0.57–1.53)	0.79	1.08 (0.69–1.70)	0.73
	>10% (versus 0–10%)	0.90 (0.50–1.61)	0.72	1.10 (0.65–1.87)	0.72
Poorly cohesive cells	≥1% (versus <1%)	1.17 (0.82–1.67)	0.37	1.34 (0.96–1.86)	0.08
	>10% (versus 0–10%)	1.49 (0.99–2.24)	0.06	**1.73 (1.19–2.53)**	**0.005**
Exploratory analyses					
Signet-ring cells + poorly cohesive cells	≥1% (versus <1%)	1.20 (0.85–1.71)	0.30	1.35 (0.97–1.88)	0.07
	>10% (versus 0–10%)	1.53 (1.02–2.29)	0.04	**1.71 (1.17–2.48)**	**0.005**
Highest category of all factors	≥1% (versus <1%)	1.25 (0.89–1.75)	0.21	1.36 (0.99–1.87)	0.06
	>10% (versus 0–10%)	1.38 (0.93–2.06)	0.11	**1.54 (1.06–2.24)**	**0.02**
Patients with residual tumour (n = 231), histopathological factors in the resection specimen					
		OS		DFS	
		Adjusted[†] HR (95% CI)	P	Adjusted[†] HR (95% CI)	P[†]
Primary analyses					
Extracellular mucin	≥1% (versus <1%)	1.07 (0.74–1.56)	0.72	1.10 (0.77–1.57)	0.61
	>10% (versus 0–10%)	0.87 (0.55–1.37)	0.55	0.94 (0.61–1.44)	0.77
Signet-ring cells	≥1% (versus <1%)	**1.81 (1.10–2.99)**	**0.02**	**1.74 (1.07–2.83)**	**0.02**
	>10% (versus 0–10%)	**2.55 (1.30–5.04)**	**0.007**	**2.64 (1.38–5.07)**	**0.004**
Poorly cohesive cells	≥1% (versus <1%)	0.97 (0.66–1.45)	0.90	1.09 (0.75–1.58)	0.65
	>10% (versus 0–10%)	0.87 (0.56–1.34)	0.52	1.01 (0.67–1.51)	0.98
Exploratory analyses					
Signet-ring cells + poorly cohesive cells	≥1% (versus <1%)	1.11 (0.76–1.64)	0.59	1.24 (0.86–1.80)	0.24
	>10% (versus 0–10%)	1.16 (0.77–1.74)	0.49	1.31 (0.89–1.92)	0.17
Highest category of all factors	≥1% (versus <1%)	1.09 (0.75–1.57)	0.66	1.19 (0.83–1.69)	0.35
	>10% (versus 0–10%)	0.97 (0.66–1.42)	0.87	1.12 (0.78–1.60)	0.55

*Adjusted for: age, sex, cT stage (cT3–4 versus cT1–2), cN stage (cN+ versus cN0), and pre-treatment tumour differentiation grade.
[†]Statistical significance at p < 0.025 (shown in bold font).
[†]Adjusted for age, sex, prepT stage (prepT3–4 versus prepT1–2), prepN stage (prepN+ versus prepN0), ypT stage (ypT3–4 versus ypT1–2), ypN stage (ypN+ versus ypN0), TRG (TRG 3–4 versus TRG 2), resection margin (R1 versus R0), and pre-treatment tumour differentiation grade.

A Poorly cohesive cells in pre-treatment biopsies
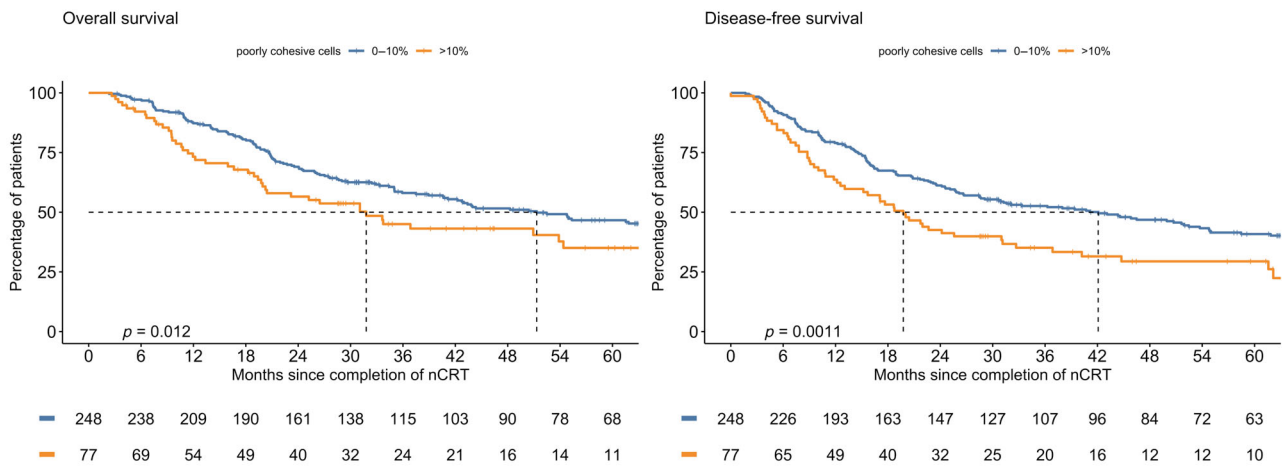
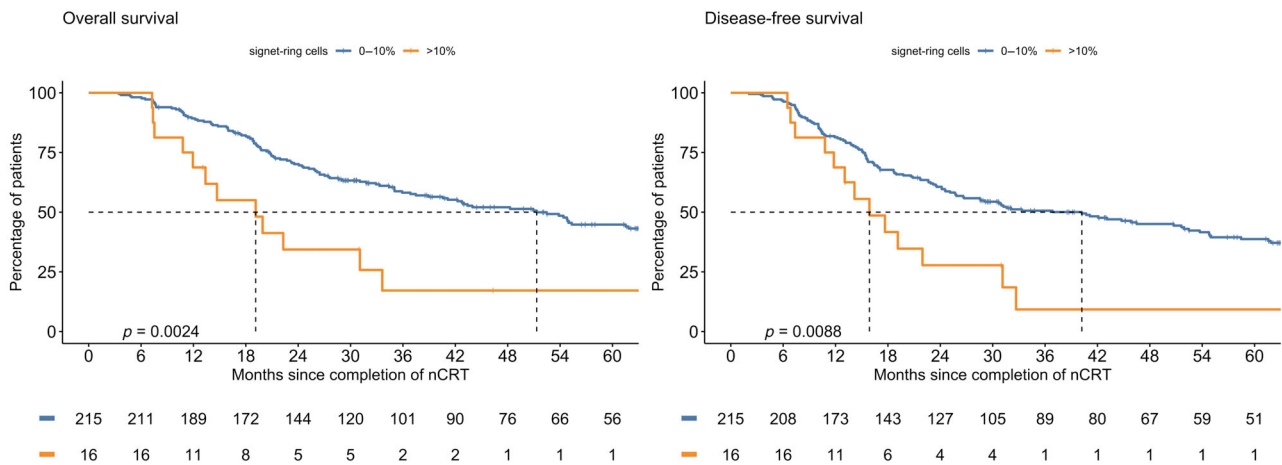## B Signet-ring cells in post-treatment resection specimens

Figure 4. OS and DFS in (A) patients with and without >10% PCCs in pre-treatment biopsies and (B) patients with and without >10% SRCs in the resection specimens after nCRT.

factors such as SRCs. However, careful assessment of well-formed glands in the resection specimen may have been somewhat compromised by the effects of nCRT.

An important limitation of the study is its retrospective design with the inherent issue of selection bias. Patients were included when both biopsy and resection specimen slides (the latter only if resection was performed) were available at the participating centres. This resulted in exclusion of a substantial number of patients, limiting the sample size. Absolute numbers of patients with presence of a histopathological factor were therefore much smaller than for example in the study of Corsini et al [12]. This increased the risk of a false-negative result (type 2 error). Furthermore, histopathological factors were adjusted in multivariable analysis, but not all potentially relevant variables, such as performance status, were available. Sampling error in biopsies is another issue that has been described previously [12,28,29]. A discordance between SRCs in the resection specimen but no SRCs in pre-treatment biopsies was seen in 5% of patients, which might be due to sampling error (Figure 3C). At the same time, these matched pre-treatment and post-treatment samples might represent a treatment effect to some extent, such as with the increase in extracellular mucin (Figure 3B). Previously, acellular mucin pools in patients with pCR have been associated with better survival [7]. Unfortunately, our study could not repeat this particular subgroup analysis since mucin pools were only scored relative to the tumour area, i.e. in patients without pCR after nCRT.

The results of the present study suggest heterogeneity in SRCs in oesophageal tumours. Even though the numbers are small, a novel finding is that patients who converted from having SRCs in biopsies to none in the resection specimen had similar prognosis compared with patients with regular type adenocarcinoma (supplementary material, Figure S1). In contrast, patients with persistent SRCs in the resection specimen had worse prognosis in multivariable analysis compared with patients with residual tumour but without SRCs after nCRT. Exploratory examination of the resection specimens of patients with SRCs after nCRT revealed a Barrett segment in 20%. This suggests that the tumour in these patients showing clear evidence of a Barrett segment, albeit based on a small number of patients, originates from the oesophagus rather than the proximal stomach. SRCs in a tumour originating from the oesophagus might be a different entity with a different prognosis, than SRCs in a tumour from the stomach. However, establishing the origin in all patients is problematic. The tumour origin cannot be reliably confirmed in the pre-treatment work-up and endoscopic landmarks are difficult to assess [30,31]. In future, the presence of specific markers at the genomic or molecular level could be investigated to define responders with SRCs from non-responders.

Why a substantial (>10%) degree of pre-treatment PCCs is associated with worse DFS in this study is unclear. In gastric cancer, PCCs have been proposed to be classified within different categories of percentages SRCs and/or PCCs [32]. This classification might facilitate future studies on prognostication. In the present study, the sum of SRCs and PCCs was explored in line with this concept. However, the prognostic relevance of the summation was not of added prognostic value compared with PCCs as a parameter alone.

Importantly, the finding of inferior prognosis with >10% PCCs before nCRT, and any percentage of SRCs after nCRT, should be further confirmed in independent prospective cohorts with sufficiently large sample sizes. Only after validation, these parameters might be included as separate fields in standard pathology reports and be used in future research on therapeutic strategies.

Conclusion

Extracellular mucin, SRCs, and/or PCCs in biopsies at diagnosis are not associated with substantial residual tumour after nCRT. Their presence should not be a reason to refrain from nCRT. A considerable percentage of PCCs pre-treatment and any percentage of SRCs after nCRT seem indicators of inferior prognosis independent of tumour differentiation grade, but this should be confirmed in independent larger cohorts.

Author contributions statement

All authors contributed to the conception and design of the study. Data were collected by MJV, AMV, RSvdP, AHAGO, LO and MD. Statistical analysis was performed by MJV. All authors interpreted the results, revised the manuscript and approved the final version.

Data availability statement

The datasets generated during and/or analysed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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SUPPLEMENTARY MATERIAL ONLINE

Figure S1. SRCs before and after nCRT as one variable per patient versus survival

Figure S2. Survival curves for pre-treatment histopathological factors

Figure S3. Survival curves for post-treatment histopathological factors

Table S1. The scoring of the different categories of histopathological factors

Table S2. Histopathological factors (0–10% versus >10%) versus clinicopathological characteristics

Table S3. Pre-treatment histopathological factors versus survival and the effect of tumour differentiation grade as a confounder

Table S4. Post-treatment histopathological factors versus survival and the effect of tumour differentiation grade as a confounder

Table S5. Histopathological factors versus survival, unadjusted for covariates