Biomarkers in colorectal cancer

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Chapter 4

Are pathological high risk features in locally advanced rectal cancer a useful selection tool for adjuvant chemotherapy?

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ABSTRACT

BACKGROUND

Several histological high risk factors are used as an indication for adjuvant therapy in stage II colon cancer. Those and other factors, including lymphatic invasion, perineural invasion, venous invasion and tumour budding are associated with decreased outcome. In this study, we evaluated the prognostic and predictive value of these biomarkers in a cohort of rectal cancer patients.

MATERIALS AND METHODS

The trial-based cohort consisted of 221npTNM stage II-III rectal cancer patients, included in the PROCTOR/SCRIPT trial, a multicentre randomized phase III trial. Patients treated with neoadjuvant radiotherapy and TME surgery, were randomized between adjuvant chemotherapy or observation. Lymphatic invasion, perineural invasion, extramural venous invasion, intramural venous invasion and tumour budding was determined in standard tissue slides.

RESULTS

The presence of perineural invasion (HR 3.36; 95%CI 1.82-6.21), extramural vascular invasion (HR 1.93; 95%CI 1.17-3.19), and tumour budding (HR 1.83, 95%CI 1.11-3.03) was associated with a significant worse overall survival. The presence of $\geq$ 2 adverse biomarkers resulted in a stronger prediction of adverse outcome in terms of overall survival (HR 2.82; 95%CI 1.66-4.79), disease-free survival (HR 2.27; 95%CI 1.47-3.48) and distant recurrence (HR 2.51; 95%CI 1.56-4.02). None of these markers alone or combined predicted a beneficial effect of adjuvant chemotherapy.

DISCUSSION

We confirmed that several stage independent biomarkers were significantly associated with a decreased outcome in rectal cancer patients. More importantly, these markers did not have predictive value, and are thus no useful to select for adjuvant therapy in rectal cancer.

INTRODUCTION

Treatment regimens in patients with rectal cancer are primarily influenced by the tumour, node and metastasis (TNM) classification and the circumferential resection margin, which provide an estimation of the patient's prognosis [1]. Pathological staging is essential for planning the most appropriate treatment in patients with rectal cancer, however outcome among patients with the same tumour stage differs significantly [2]. Consequently, it could be stated that conventional classification does not provide adequate individualized assessment.

For patients with stage III or high risk stage II colon tumours, adjuvant chemotherapy is indicated after surgery [3,4]. The high risk stage II colon tumour is mainly defined by histopathologic characteristics such as the presence of a T4 tumour, extramural vascular invasion (EMVI), poor differentiation, less than 10 harvested lymph nodes or patients who have had obstruction or perforation [3-5]. In order to optimize the delivery of adjuvant chemotherapy in rectal cancer, additional histological risk factors should be explored. Those factors, include lymphatic invasion, perineural invasion (PNI), EMVI, intramural venous invasion (IMVI) and tumour budding which are all associated with decreased clinical outcome [6-12]. In the seventh edition of the TNM, these items were included as accessory markers because of their relevance [1]. It has been proposed that these biomarkers may guide treatment decisions, particular the use of adjuvant chemotherapy [10,13,14]. Thus, in contrast with colon cancer, the benefit of adjuvant chemotherapy in rectal cancer has not been demonstrated [15,16]. In the future improvements of the patient selection might reveal high risk rectal cancer patients who do benefit from adjuvant chemotherapy. Therefore, the proposed prognostic [10,13,14] and predictive value of the above mentioned biomarkers was evaluated on standard tissue slides, of patients with stage II-III locally advanced rectal cancer included in the PROCTOR-SCRIPT trail.

MATERIALS AND METHODS

PATIENT SELECTION

Data were derived from patients included in the PROCTOR-SCRIPT trial (ISRCTN; 36266738), a multicentre randomized phase III trial, that included patients with (y)pTNM stage II-III rectal cancer treated with neoadjuvant (chemo)radiotherapy and TME surgery, randomly assigned to adjuvant chemotherapy or observation. The results of the primary and secondary endpoints have been published previously [15]. Informed consent for

participation and retrospective use of samples was obtained from all patients. Formalin-fixed paraffin-embedded (FFPE) tumour samples of the included Dutch patients were collected. Only patients treated with neoadjuvant radiotherapy (5x5Gy) were included in this analysis. Patients treated with neoadjuvant chemoradiotherapy were excluded in order to establish a cohort with similar neoadjuvant treatment regimes.

PATHOLOGICAL ASSESSMENT

Standardized pathological examination according to Quirke *et al.* was performed in the laboratories of the referring hospitals [17]. FFPR tumour tissue sections of 4 µm were stained with Haematoxylin and Eosin. All tumour sections were reviewed by a single pathologist (I.D.N.) for the presence or absence of lymphatic invasion, PNI, EMVI, IMVI, and tumour budding. Lymphatic invasion was defined as the presence of tumour cells within an endothelial-lined lymphatic channel. PNI was defined as, tumour cells growing around, within and through any of the three nerve layers and should surround more than 33% of the nerve circumference [11]. Venous invasion was defined as tumour cells within an area lined by endothelial and smooth muscle cells or elastic fibres. Venous invasion was divided in IMVI and EMVI, whereas EMVI was venous invasion located outside the muscularis propria within the surrounding mesorectal fat [18]. Thereby, the presence of an adjacent arterial structure was required. Tumour budding was evaluated as positive when small clusters of tumour cells, fewer than five undifferentiated tumour cells, were observed at the invasive front [19]. The impact of these factors on outcome was analysed separately for each factor and in combination, where patients with none or one biomarker present were compared to patients with two or more biomarkers present.

STATISTICAL ANALYSIS

Statistical analyses were performed using the statistical package SPSS (version 20.0 for Windows; SPSS Inc.). The Student T-test and Chi-square test were used to evaluate association between the biomarkers, and combinations thereof, and clinico-pathological parameters.

Overall survival (OS) was defined as time since randomization until death. Disease-free survival (DFS) was defined as time since randomization until local recurrence, distant recurrence or death, whichever came first. Time to distant recurrence (DR) was defined as time to distant metastasis, or end of follow-up, deaths were censored in this analysis. For survival probabilities the Kaplan-Meier method was used and for comparison of survival curves the Log-Rank test was used. Univariate and multivariate Cox regression analyses were performed to evaluate the differences in OS, DFS and DR. Covariates entered in the multivariate model were age, gender, stage and circumferential resection margin. For all tests a p-value of <0.05 was considered as statistically significant.

RESULTS

PATIENT CHARACTERISTICS

In total 470 patients were enrolled in the PROCTOR-SCRIPT trial (146 Swedish and 324 Dutch patients). Only tumour tissue of Dutch patients was available for this study, and was successfully obtained for 262 patients, of whom 11 were ineligible. In order to establish a homogenous cohort, patients treated with neoadjuvant chemoradiotherapy were excluded (n=30). This resulted in a total study cohort of 221 patients with locally advanced rectal cancer, treated with neoadjuvant short-course radiotherapy (5x5Gy) Figure 1. Of the eligible patients, 104 patients were randomized assigned to adjuvant chemotherapy and 117 patients to observation, with a median follow-up of 5.4 years for the total cohort. Patients characteristics of the study cohort are summarized in Table 1. As shown in this table the presence of ≥ 2 biomarkers was associated with a higher disease stage.

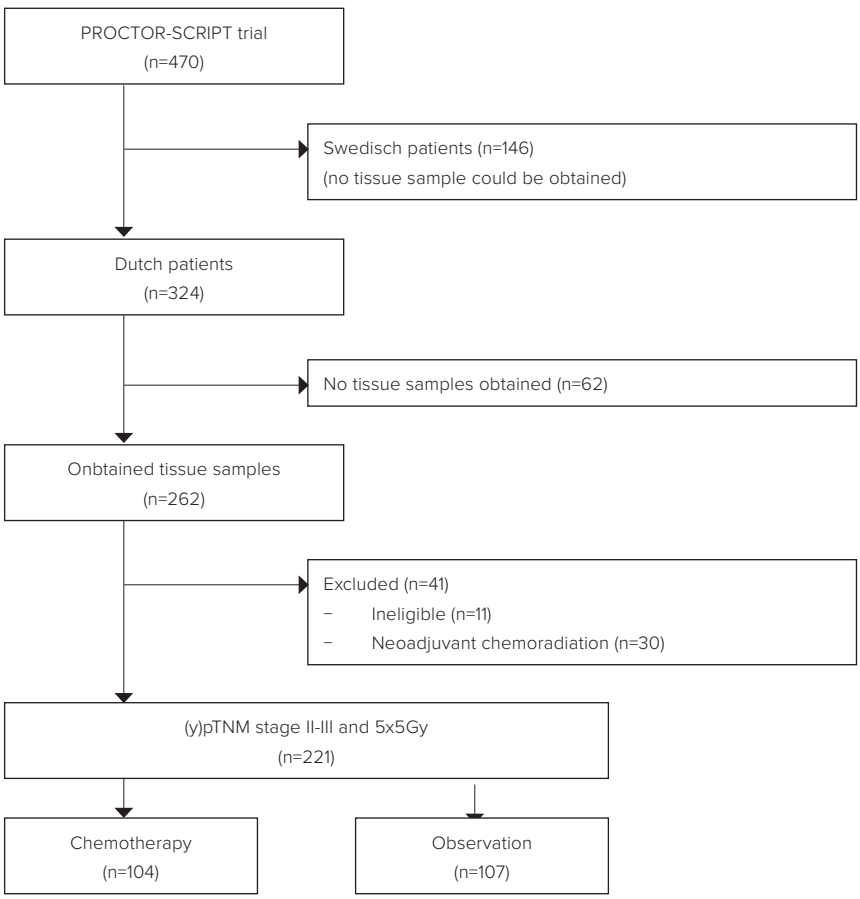

Figure 1: Patient selection.

Table 1: Patient characteristics of the total study cohort and stratified for < 2 and ≥ 2 adverse biomarkers.

			Adverse biomarkers				
	Total population		< 2 biomarkers		≥ 2 biomarkers		
	n=221	(%)	n=118	(%)	n=103	(%)	P-value
Age median	59.8	(±9.7)	58.7	(±9.4)	61.0	(±9.9)	0.10
Gender							
Male	136	(61.5)	72	(61.0)	64	(62.1)	0.86
Female	85	(38.5)	46	(39.0)	39	(37.9)	
Tumour location from the anal verge							
< 5 cm	61	(27.6)	29	(24.6)	32	(31.1)	0.49
5-9.9 cm	69	(31.2)	40	(33.9)	29	(28.2)	
>10 cm	85	(38.5)	46	(39.0)	39	(37.9)	
Unknown	6	(2.7)	3	(2.5)	3	(2.9)	
(y)pTNM							
II	29	(13.1)	21	(17.8)	8	(7.8)	0.03
III	192	(86.9)	97	(82.2)	95	(92.2)	
Differentiation							
Well	5	(2.3)	4	(3.4)	1	(1.0)	0.05
Moderate	196	(88.7)	107	(90.7)	89	(86.4)	
Poor	18	(8.1)	5	(4.2)	13	(12.6)	
Unknown	2	(0.9)	2	(1.7)	0	(0.0)	
CRM							
Negative	208	(94.1)	110	(93.2)	98	(95.1)	0.55
Positive	13	(5.9)	8	(6.8)	5	(4.9)	
Adjuvant treatment							
Chemotherapy	104	(47.1)	58	(49.2)	46	(44.7)	0.50
Observation	117	(52.9)	60	(50.8)	57	(55.3)	

SINGLE BIOMARKERS IN RELATION TO PATIENT OUTCOME

As shown in Figure 2, the presence of PNI (HR 3.36; 95%CI 1.82-6.21), EMVI (HR 1.93; 95%CI 1.17-3.19), and tumour budding (HR 1.83; 95%CI 1.11-3.03) was significantly associated with a decreased overall survival in the univariate analysis. For lymphatic invasion (HR 1.61; 95%CI 0.96-2.70) and IMVI (HR 1.30; 95%CI 0.71-2.40) trends towards a worse prognosis could be observed. In the multivariate analysis, corrected for age, gender, stage and, circumferential resection margin status, the effects remained significant for PNI (HR 2.68; 95%CI 1.41-5.11), EMVI (HR 2.08; 95%CI 1.26-3.46) and tumour budding (HR 1.54; 95%CI 1.09-3.03). As shown in Table 2, significantly worse DFS and a higher distant recurrence rates were observed in patients with PNI, EMVI, IMVI, and, tumour budding.

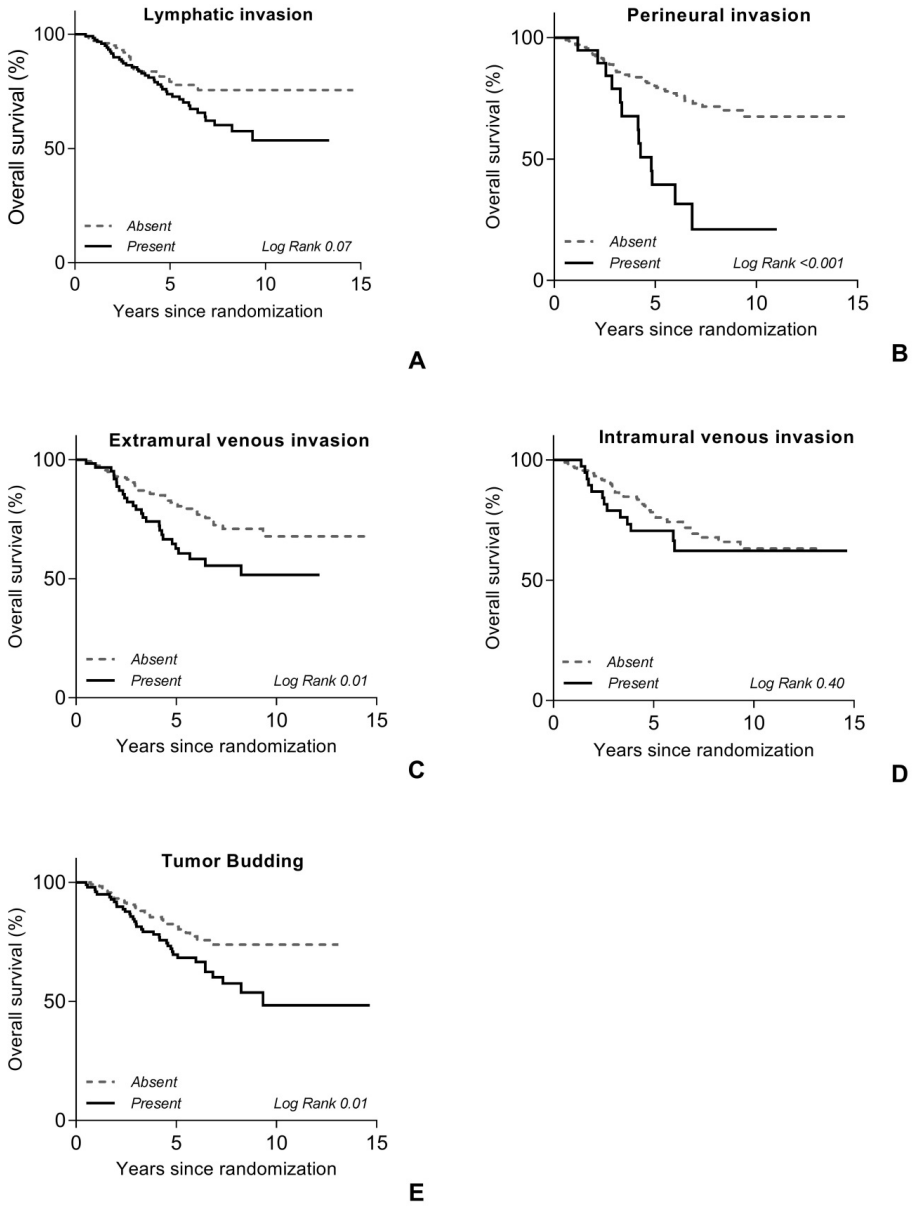

Figure 2: Kaplan-Meier analysis for overall survival in 221 stage II-III rectal cancer patients according to the status of the different tumour parameters, dichotomized as present or absent. The p-value represents the Log-Rank test. (A) Lymphatic invasion. (B) Perineural invasion. (C) Extramural vascular invasion. (D) Intramural vascular invasion. (E) Tumour budding.

Table 2. Univariate and multivariate survival analysis for disease-free survival and time to distant recurrence according to different pathological factors. Covariates entered in the multivariate model were age, gender, stage and circumferential resection margin status.

		Disease-free Survival				Time to distant recurrence			
		Univariate		Multivariate		Univariate		Multivariate	
	Patients (%)	HR (95%CI)	P-value	HR (95%CI)	P-value	HR (95%CI)	P-value	HR (95%CI)	P-value
Lymphatic invasion									
Absent	100 (45)	1.00	0.19	1.00	0.24	1.00	0.31	1.00	0.36
Present	120 (55)	1.33 (0.87-2.05)		1.30 (0.84-2.04)		1.27 (0.80-2.01)		1.25 (0.78-2.00)	
Perineural invasion									
Absent	202 (91)	1.00	0.001	1.00	0.007	1.00	<0.001	1.00	0.001
Present	19 (9)	2.63 (1.46-4.76)		2.33 (1.26-4.31)		3.09 (1.69-5.64)		2.91 (1.56-5.45)	
Extramural vascular invasion									
Absent	159 (72)	1.00	0.04	1.00	0.03	1.00	0.03	1.00	0.02
Present	62 (28)	1.59 (1.03-2.45)		1.64 (1.06-2.55)		1.66 (1.04-2.66)		1.76 (1.10-2.83)	
Intramural vascular invasion									
Absent	182 (83)	1.00	0.02	1.00	0.07	1.00	0.008	1.00	0.03
Present	38 (17)	1.79 (1.09-2.92)		1.60 (0.97-2.65)		2.01(1.20-3.35)		1.82 (1.08-3.08)	
Tumor Budding									
Absent	119 (55)	1.00	0.05	1.00	0.05	1.00	0.05	1.00	0.05
Present	99 (45)	1.52 (0.99-2.32)		1.54 (1.00-2.37)		1.59 (1.00-2.52)		1.60 (1.00-2.57)	

COMBINED ANALYSIS OF BIOMARKERS IN RELATION TO PATIENT OUTCOME

To analyse the effect of multiple biomarkers on patient outcome, all single biomarkers (lymphatic invasion, PNI, EMVI, IMVI, tumour budding) were combined, and stratified in two groups. The first group consisted of patients with none or just one biomarker was observed to be present in the tumour tissue section. The second group consists of patients with the presence of ≥ 2 biomarkers present. As shown in Figure 3, patients with ≥2 adverse biomarkers had a significant worse OS (p<0.001) and DFS (p<0.001). The cumulative incidence for distant recurrence was 23% in patients with <2 adverse biomarkers 47% in patients with ≥2 adverse biomarkers (p<0.001). In the multivariate analysis, corrected for age, gender, stage and circumferential resection margin, a significant worse OS (HR 2.73; 95%CI 1.58-4.71), DFS (HR 2.30; 95%CI 1.48-3.59) and DR (HR 2.59; 95%CI 1.60-4.22) was observed in patients with ≥2 adverse biomarkers compared to the group of patients with <2 adverse biomarkers.

EFFECT OF ADJUVANT CHEMOTHERAPY IN PATIENTS WITH ADVERSE PROGNOSTIC BIOMARKERS

No benefit of adjuvant chemotherapy was shown for any biomarker-based subgroup regarding OS (Figure 4). Similar results were obtained for DFS and DR (data not shown). When patients with <2 and ≥ 2 adverse biomarkers were evaluated for the effect of adjuvant chemotherapy, no statistically significant beneficial effect for the use of adjuvant chemotherapy could have been observed, (HR 1.46; 95%CI 0.59-3.56) and (HR 1.08; 95%CI 0.59-1.97) respectively (Figure 4). Thus, in patients with a significant worse overall (≥ 2 adverse biomarkers), no beneficial effect of adjuvant chemotherapy was observed.

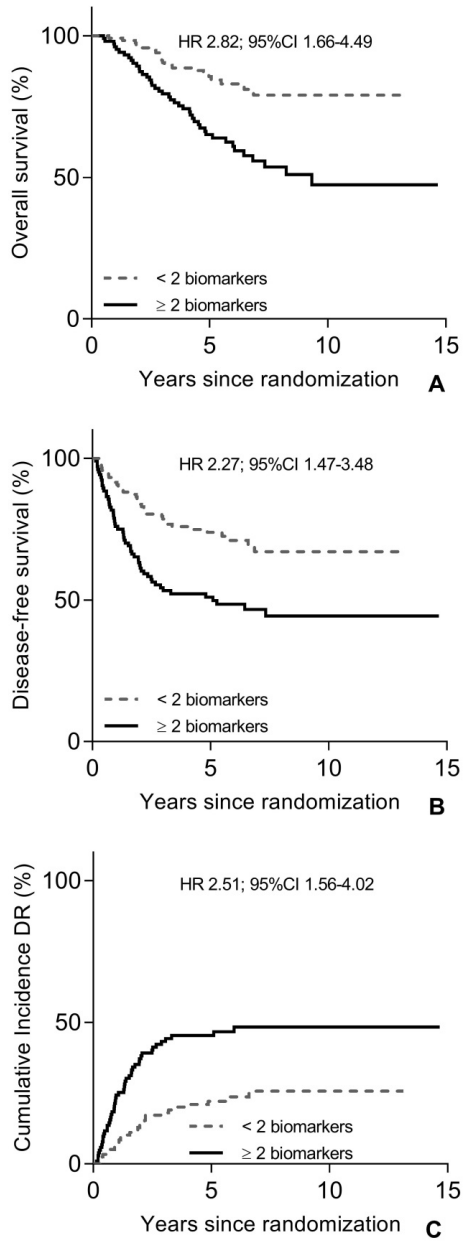

Figure 3: Survival curves for (A) overall survival, (B) disease-free survival and (C) cumulative incidence of distant recurrence, according to the presence of < 2 or ≥ 2 adverse biomarkers. Biomarkers include; lymphatic invasion, perineural invasion, extramural vascular invasion, intramural vascular invasion or tumour budding. Hazard ratio's (HR) and 95% confidence interval (95%CI) in the graph represents the univariate analysis.

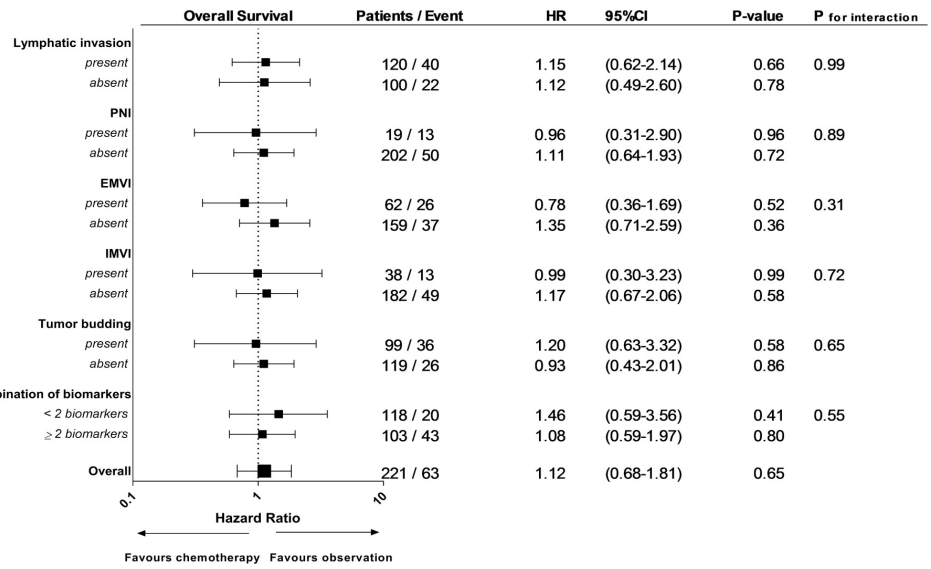

Figure 4. Overall survival for all patients and by patients subgroups, comparing observation and adjuvant chemotherapy.

DISCUSSION

Standardized histopathological staging for rectal cancer is currently the cornerstone for prognostic assessment and highly influences rectal cancer treatment. In the current study, additional stage independent pathological markers were investigated. This study confirmed that, stage-independent pathological markers, including PNI, EMVI, IMVI, and tumour budding, were powerful tools for prognostication. Especially when all biomarkers were combined. The strong prognostic effect observed in rectal cancer patients of which the tumour showed the presence of ≥ 2 adverse biomarkers, could be explained by the access of multiple routes for metastatic spread. These tumour cells can disseminate through more than one route, blood, lymph channels or along nerves, and consequently could result in more extensive metastasis, as has been suggested before. [20]

In earlier studies, it was hypothesized that the investigated biomarkers were considered as good indicators for the use of adjuvant chemotherapy [7,10,21], since adjuvant chemotherapy might eliminate micrometastases and circulating tumour cells, preventing distant metastasis. However, when comparing the different rectal cancer patient subgroups based on these biomarkers, no beneficial effect of adjuvant

chemotherapy could be demonstrated in this randomised cohort comparing adjuvant chemotherapy with observation. More interestingly, no beneficial effect of adjuvant chemotherapy was observed in the patient group with the poorest prognosis, as indicated by the presence of two or more biomarkers. These findings were in line with the previously published overall results of the PROCTOR-SCRIPT trial and a more recently published meta-analysis, both demonstrating no beneficial effect of adjuvant chemotherapy in patients with locally advanced rectal cancer treated with neoadjuvant (chemo)radiotherapy and TME surgery [15,16].

Overall, the reported incidence of the investigated biomarkers varies in literature, most likely caused by the different criteria used for the detection. For example, in the present study 9.5% of the patients had PNI, which is lower than the recently reported incidence of 20.8% in rectal cancer [9]. However, neoadjuvant treatment is associated with less frequent PNI [9,22]. In the current study, EMVI was observed in 28% and IMVI was observed in 17.2% of the patient cohort, which is comparable to the incidence reported elsewhere [7]. IMVI was not significantly associated with survival. However, significantly more distant recurrences did occur in patients with IMVI. This is in line with the current literature, demonstrating a less clear relation of IMVI with survival compared to EMVI [6,18].

Although our findings are interesting, we acknowledge that the performed study has some limitations. First, our sample size for analysis performed in some subgroups were moderately sized. Secondly, standard tissue slides were used in this study. For more detailed evaluation additional immunohistochemical staining could be used for the investigated biomarkers. These limitations are exceeded by the strengths of this study, the large trial-based cohort, with prospectively collected patient data and the random allocation to observation or adjuvant chemotherapy. Furthermore, to the best of our knowledge this is the first study evaluating these pathological biomarkers in a rectal cancer cohort receiving a 5x5Gy as pre-operative treatment, in contrast with the study performed by Nikberg *et al* where 53% of the patients received neoadjuvant radiotherapy (17% neoadjuvant chemoradiotherapy and 20% no pre-operative therapy) [10]. Moreover, our study shows that, in rectal cancer, prognostic factors cannot as yet be used as predictive factors for adjuvant therapy. Therefore, we must be cautious with nomograms that are currently advocated as tools for selection of patients for adjuvant therapy [23].

In addition to the histological biomarkers investigated in this study, molecular biomarkers such as microsatellite instability (MSI) and *RAS/RAF* mutational status are entering the clinic. However, the implications of these molecular biomarkers in rectal

cancer are as yet undefined. Large cohort studies are warranted as the next step for more personalized treatment in rectal cancer.

In conclusion, in the current study we confirmed that stage independent biomarkers in locally advanced rectal cancer are significantly associated with adverse survival, especially when two or more biomarkers were present. More importantly, these factors do not have predictive value, and do not warrant an indication for adjuvant therapy in rectal cancer patients treated with neoadjuvant short course radiotherapy and TME surgery.

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