Applications of the tumor-stroma ratio: Towards clinical implementation

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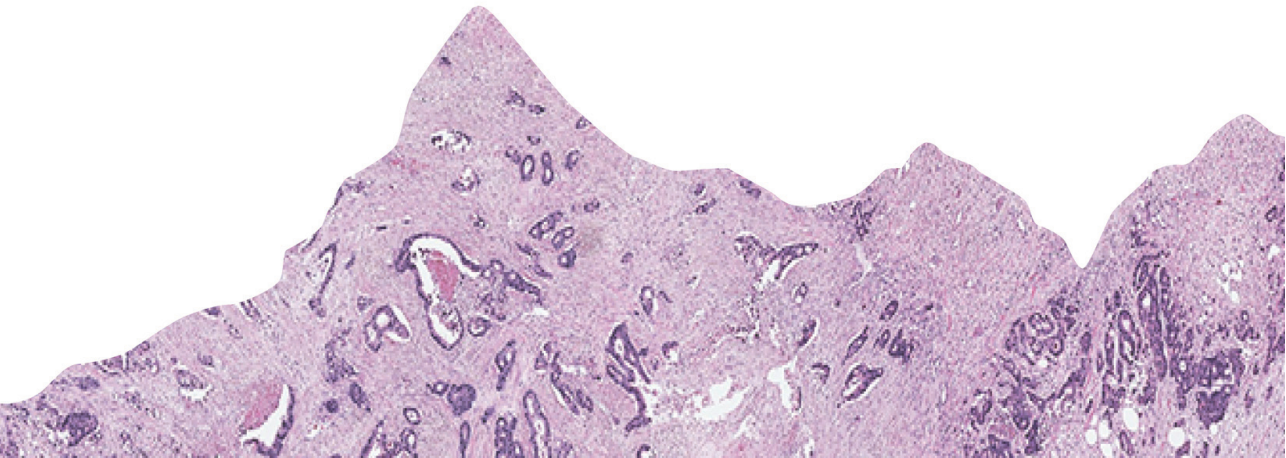

CHAPTER 7

Tumor-stroma ratio as predictor of response to neoadjuvant chemoradiotherapy in rectal cancer

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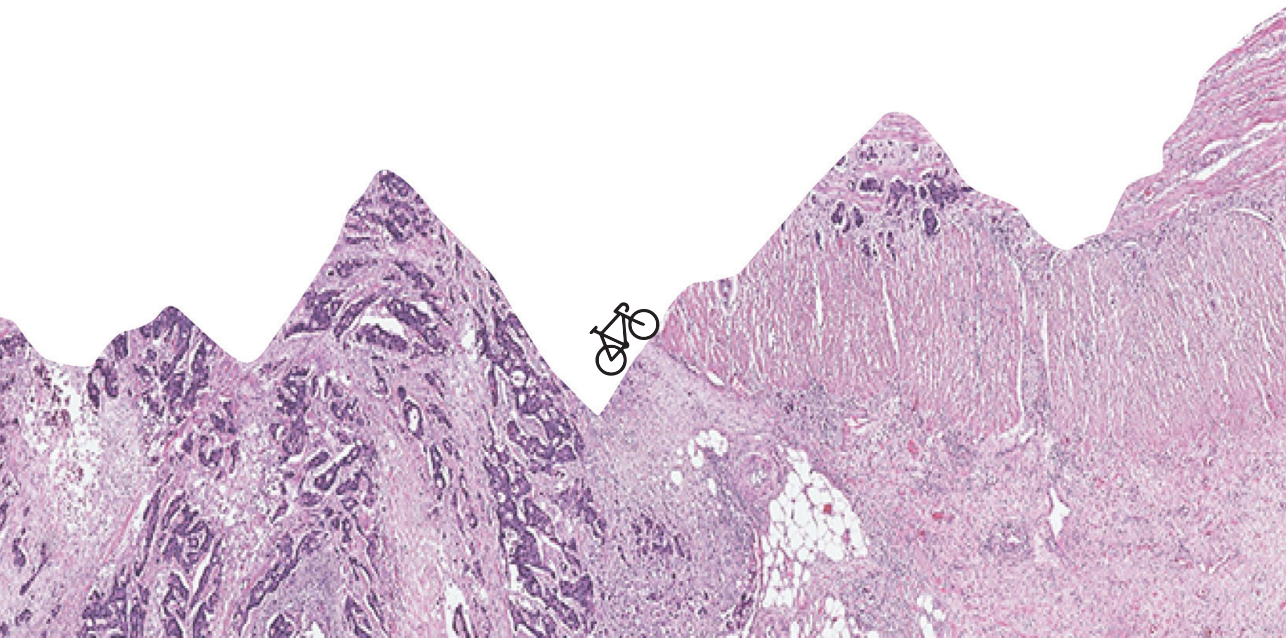

Abstract

Purpose There are no predictive markers for response to neoadjuvant chemoradiotherapy (nCRT) in rectal cancer patients. The tumor-stroma ratio (TSR) has proven to be a prognostic marker in several types of cancer, but its value in predicting pathologic complete response (pCR) in rectal cancer patients treated with nCRT remains unknown.

Methods The study cohort consisted of patients with rectal adenocarcinoma who received nCRT followed by surgery. Hematoxylin and eosin (H&E) stained sections of diagnostic biopsies were digitally assessed for TSR by two independent investigators. Patients were categorized in stroma-low (TSR ^{3} 50%) and stroma-high (TSR < 50%) groups for further analyses. The tumor regression grade (TRG) was assessed on H&E stained sections of the resected primary tumor specimens to determine pathologic response.

Results A total of 76 patients were included in this study, of which 37 patients (49%) were categorized as stroma-low and 39 (51%) as stroma-high. Eighteen patients (24%) had a pCR (TRG 1) to capecitabine-based chemoradiotherapy. pCR was numerically higher in stroma-low patients (32%, 95%CI 19%-50%) as compared to stroma-high tumors (15%, 95%CI 6%-31%; odds ratio 2.61, P = 0.09). At 6 years follow-up, relapse-free survival rate was 83% (95%CI 71%-96%) in stroma-low patients and 53% (95%CI 29%-97%) in stroma-high (hazard ratio 0.46, P = 0.10).

Conclusion TSR may help predict pCR and long-term relapse rate in rectal cancer patients receiving standard nCRT, with stroma-high patients presenting poor outcomes. The digital pathology assessment of TSR will facilitate validation studies and implementation in daily practice.

Keywords

Biopsy, neoadjuvant chemoradiotherapy, pathologic response, prediction, rectal cancer, tumor-stroma ratio

Introduction

In Europe, approximately 30% of patients diagnosed with colorectal cancer (CRC) in 2018 suffered from invasive rectal adenocarcinoma, with a mortality rate of 40% [1]. The incidence of rectal cancer is increasing, particularly in the younger population [2]. Currently, the recommended treatment for patients with high-risk locally advanced rectal carcinoma is neoadjuvant chemoradiotherapy (nCRT) [3]. In approximately 20% of the patients, nCRT leads to a complete pathological response (pCR), which is associated with better long-term outcomes [4, 5]. It is debatable whether these patients need resection of the primary tumor or can be offered an active wait-and-see approach [6-8]. In contrast, non-responders to nCRT will have higher risk of local and systemic relapse [4, 5] while retaining all potential side effects of the treatment. Hence, the importance to define biomarkers that predict whether or not a patient with rectal cancer will achieve pCR with standard nCRT.

Clinical factors, including carcinoembryonic antigen (CEA) levels at diagnosis, tumor size, clinical T- and N-stage, distance of the tumor from the anal margin, and the time interval from nCRT to surgery, are associated with response to nCRT in rectal cancer. In addition, some pathological features have been shown to predict poor response to nCRT, like tumor differentiation, absence of circumferential tumor margin involvement, mucinous type and the presence of macroscopic ulceration [9-12].

Imaging modalities such as ^{18}F-labelled 3'-deoxy-3'-fluorothymidine (FLT) positron emission tomography (PET) and ^{18}F-fluorodeoxyglucose (FDG)-PET combined with computed tomography (CT) have limited value in predicting response in patients with rectal cancer treated with nCRT [13, 14]. Microarray studies showed promising results in different cohorts, but implementation in routine clinical practice is difficult [15, 16]. Furthermore, radiomics and transcriptomics markers have substantial costs.

In the last decade it has been recognized that tumor growth patterns and inflammatory response are strong determinants of prognosis in CRC [17]. Huang et al. showed that tumor microenvironment features may also play a role in predicting tumor response to nCRT [18]. They evaluated both local tumor microenvironment (tumor-infiltrating lymphocytes (TILs), intratumor budding (ITB)) as well as the systemic inflammatory environment (neutrophil-to-lymphocyte ratio, C-reactive protein) and found that the combination of CD8+ intraepithelial TILS and ITB was an independent predictive factor for the pathological response to nCRT in rectal cancer patients.

A simple method to assess the tumor microenvironment on routine hematoxylin and eosin (H&E) stained sections of biopsies (or primary tumors), is the tumor-stroma ratio (TSR). It has prognostic value in multiple types of epithelial cancers like colon, breast and gastric adenocarcinomas [19-23]. A high stroma component (low TSR) is related to worse patient outcomes after curative treatment. In the current study we investigated whether TSR, determined in biopsy specimens before nCRT, could aid in predicting therapy response in rectal cancer patients.

Material and Methods

Patients

In a prospective patient cohort of 82 consecutive patients with rectal cancer from the Vall d'Hebron University Hospital, Barcelona, Spain, we retrospectively analyzed the impact of TSR on clinical outcomes. All patients were diagnosed with clinical stage II-III rectal carcinoma between 2011 and 2018 and were treated according to standard-of-care protocols (neoadjuvant chemoradiotherapy followed by surgery). Radiotherapy consisted of a total dose of 50.4 Gy, given in 28 fractions of 1.8 Gy, 5 fractions per week. Concurrent chemotherapy consisted of capecitabine alone or in combination with oxaliplatin. A minimum follow-up of 12 months from surgery to last follow-up in patients alive was required.

This research has been approved by the local ethics committee of the Vall d'Hebron University Hospital. All samples were handled in a coded de-identified fashion, according to national data privacy regulations.

Tumor-stroma ratio (TSR)

The TSR was determined on digital H&E biopsy sections using NanoZoomer Digital Pathology (NDP.view 2, Hamamatsu, Hamamatsu City, Shizuoka, Japan). The area with the highest amount of stroma was selected, using a circular annotation of 3.46 mm^2. This annotation mimics the microscopical scoring with a 10x objective on most commonly used microscopes. The amount of stroma present in the selected area was visually estimated in increments of 10%. Tumor cells were to be present at the four borders of the selected area. Identifying one single area with high stroma content was decisive for a final stroma classification. Patients were categorized in two groups, i.e. stroma-low (TSR ^{3} 50%) and stroma-high (TSR < 50%) (Fig. 1). A detailed TSR scoring protocol has been published previously [24]. All sections were independently scored by two observers (GP, SZ), blinded for any clinical information.

The response to nCRT was assessed on the resection specimens by experienced gastrointestinal pathologists using the tumor regression grade (TRG) defined by Mandard [25]. This classification is defined by 5 categories. TRG 1 is defined as complete regression with no residual cancer but only fibrosis through all layers of the rectum wall and is called pathologic complete response (pCR); TRG 2 is characterized by scattered residual cancer cells or groups of cells within the fibrosis; TRG 3 shows an increase of residual cancer cells but fibrosis predominates; TRG 4 is characterized by residual cancer outgrowing the fibrosis, and TRG 5 is defined by absence of any regressive changes.

Statistical analysis

Statistical analysis was performed using IBM SPSS software version 25 (Armonk, New York, USA) and R statistical software version 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria). Differences in categorical variables between patient, tumor and treatment characteristics for the TSR groups were analyzed using the Fisher's exact test. For comparison of continuous variables, the Mann-Whitney U test was used. Inter-observer variability was analyzed using the Cohen's kappa coefficient. For the predictive correlative endpoint of TRG versus stroma content, we performed univariable logistic regression and TRG variable was dichotomized in two groups, TRG 1 (complete response, pCR) versus TRG 2-5 (non-complete response). Our target population was a sample size of 80 evaluable patients, which would give 80% power to detect an increase in pCR from < 15% in stroma-high group to > 45% in stroma-low group, assuming a 50%/50% prevalence of stroma-high/-low. Univariable survival analyses were conducted with Cox's proportional hazards regression. Kaplan-Meier survival curves were compared with the log-rank test. Six-year relapse-free survival (RFS, considering relapse or death from any cause as events and censoring in the case of no event within six years) was used as endpoint. Multivariable models were not constructed given the small sample of this exploratory cohort. Final *P* values <0.05 were considered statistically significant.

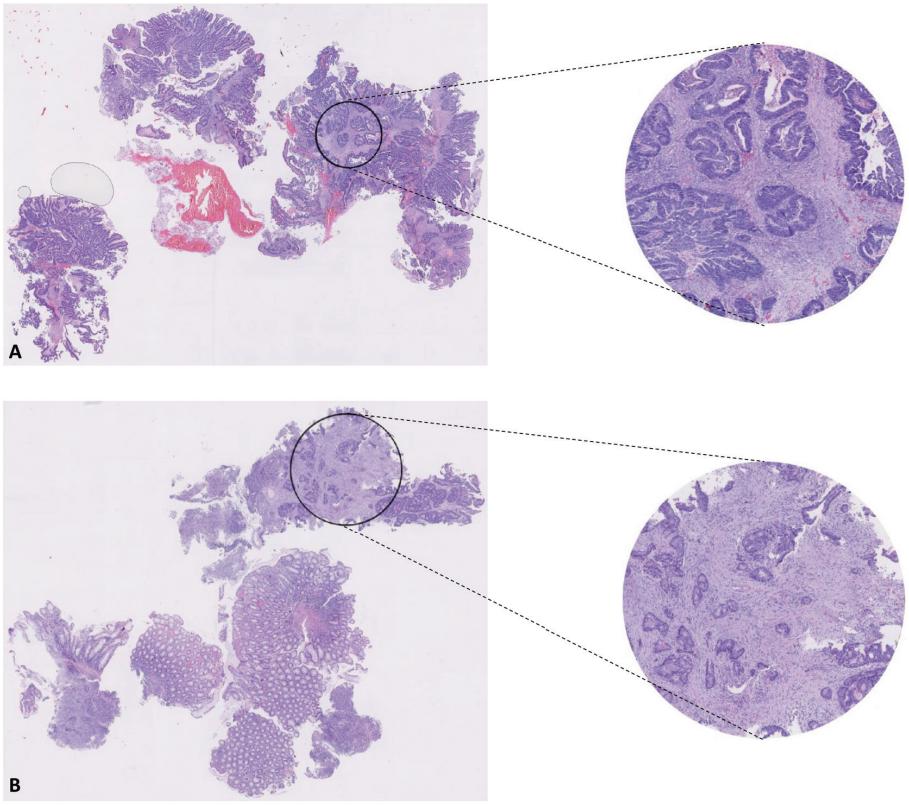

Figure. 1 Examples of H&E stained biopsy sections of rectal carcinoma, with on the left an overview of the biopsy with the annotated area magnified on the right. A) stroma-low, B) stroma-high. In both A) and B) the annotated area is 3.46 mm² in size.

Results

Patients

The initial cohort consisted of 82 stage II-III rectal cancer patients. Six patients were excluded because of diagnosis of metastatic disease during neoadjuvant therapy or participation in clinical trials with novel chemotherapy regimens, leaving 76 patients for downstream analyses. Median age was 69 years (range 46-87) at the start of nCRT, 58% (*N* = 44) were men and most patients (87%, *N* = 66) had clinical stage III disease. All patients completed radiotherapy as intended, however 3 patients (4%) stopped chemotherapy early because of toxicity. Median time between nCRT and surgery was 2.4 months (range 0.6-4.1). Clinico-pathological data of patients are shown in table 1. There were no significant differences between the two TSR groups.

Table 1. Patient, tumor and treatment characteristics, stratified by TSR.

	Total ***N*** **= 76 (%)**	**Stroma-low** ***N*** **= 37 (%)**	**Stroma-high** ***N*** **= 39 (%)**	***P*-value**
Gender				
Male	44 (58)	25 (68)	19 (49)	0.11
Female	32 (42)	12 (32)	20 (51)	
Median age years [range]	68.6 [45.5-86.8]	68 [48-86]	67 [45-87]	0.37
Median length tumor[a] (cm) [range]	2.0 [0.2-16.0]	2.2 [0.3-4.0]	2.0 [0.2-16.0]	0.52
Histology[b]				
Adenocarcinoma	52 (68)	22 (59)	30 (77)	0.21
Mucinous adenocarcinoma	6 (8)	3 (8)	3 (8)	
No tumor	18 (24)	12 (32)	6 (15)	
cT status				
cT2	15 (20)	10 (27)	5 (13)	0.24
cT3	47 (62)	22 (59)	25 (64)	
cT4	14 (18)	5 (14)	9 (23)	
cN status				
cN0	9 (12)	6 (16)	3 (8)	0.55
cN1	36 (47)	17 (46)	19 (49)	
cN2	29 (38)	13 (35)	16 (41)	
cN3	1 (1)	1 (3)	0 (0)	
Missing	1 (1)	0 (0)	1 (3)	
cTNM				
II	9 (12)	6 (16)	3 (8)	0.37
III	66 (87)	31 (84)	35 (90)	
Missing	1 (1)	0 (0)	1 (3)	
Differentiation grade[b]				
Well	24 (32)	9 (24)	15 (38)	0.25
Moderate	27 (36)	14 (38)	13 (33)	
Poor	6 (8)	2 (5)	4 (10)	
Not applicable	18 (24)	12 (32)	6 (15)	
Missing	1 (1)	0 (0)	1 (3)	
Therapy				
RT + Capecitabine/Oxaliplatin	7 (9)	2 (5)	5 (13)	0.43
RT + Capecitabine	69 (91)	35 (95)	34 (87)	
Pre CEA				
≤ 3.5 ng/ml	45 (59)	20 (54)	25 (64)	0.48
> 3.5 ng/ml	31 (41)	17 (46)	14 (36)	
Post CEA				
≤ 3.5 ng/ml	64 (84)	30 (81)	34 (87)	0.75
> 3.5 ng/ml	11 (15)	6 (16)	5 (13)	
Missing	1 (1)	1 (3)	0 (0)	
Median time between nCRT and surgery (months)[range]	2.4 [0.6-4.1]	2.6 [1.4-4.1]	2.3 [0.6-3.7]	0.12

[a]Tumor length was determined by MRI after neoadjuvant treatment.

[b]Differentiation grade and histology were determined on surgical resection specimen.

Abbreviations: TSR tumor-stroma ratio, TNM tumor-node-metastasis, RT radiotherapy, CEA carcinoembryonic antigen, nCRT neoadjuvant chemoradiotherapy.

Tumor regression grade

The pathological response assessment revealed 18 patients (24%) to have achieved pCR (TRG 1), whereas 12 (16%) cases reached almost complete response (TRG 2). The remaining 46 cases showed less than substantial to no tumor regression (TRG 3, N = 35; TRG 4, N = 8 and TRG 5, N = 3, respectively)(Table 2). Out of 18 patients who achieved a pCR (TRG 1), 2 (11%) had disease recurrence, as compared to 16 (28%) out of 58 patients without pCR (TRG2-5).

Table 2. Distribution of TRG categories versus TSR categories.

	Pathologic complete responders	**Non-responders**					
	TRG 1	TRG 2	TRG 3	TRG 4	TRG 5	**TRG 2-5**	Total
Stroma-low	**12 (32%)**	5	17	3	0	**25 (68%)**	37
Stroma-high	**6 (15%)**	7	18	5	3	**33 (85%)**	39
Total	18 (24%)	12	35	8	3	58 (76%)	76

Abbreviations: TRG tumor regression grade, TSR tumor-stroma ratio.

Tumor-stroma ratio

Out of 76 biopsies analyzed, 39 (51%) were categorized as stroma-high and 37 (49%) as stroma-low. A substantial inter-observer agreement was found for the assessment of the TSR (83% agreement, $\kappa = 0.67$). Discordant cases were re-assessed by the observers together and consensus was reached.

Predictive value of tumor-stroma ratio

In univariable logistic regression cT status was found to be a critical determinant for reaching pCR, whereas age was borderline significant. From 39 patients with a stroma-high biopsy, 6 (15%, 95%CI 6%-31%) had a pCR compared to 12 out of 37 (32%, 95%CI 19%-50%) of the stroma-low group (Fig. 2A). A non-significant difference was found for higher pCR numbers in stroma-low as compared to stroma-high group (OR 2.61, 95%CI 0.77-9.71, $P = 0.09$). None of the other variables were found to be of (potential) predictive value for pCR (Table 3).

Prognostic value of tumor-stroma ratio

Median follow-up of the entire cohort was 63 months (95%CI 59.8-67.2) and median RFS was not reached. Six-year RFS rate was 82.9% (95%CI 71.3% - 96.3%) in stroma-low patients and 52.9% (28.9% - 96.6%) in stroma-high population (HR = 0.46, 95%CI 0.17-2.24, $P = 0.10$)(Fig. 2B).

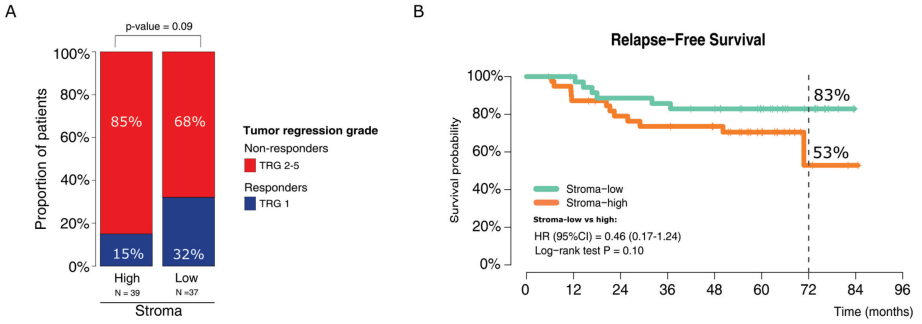

Figure. 2 A) The distribution of pathologic major responders within the stroma categories. The percentage of responders (in blue) versus non-responders (in red) within stroma-low and stroma-high categories, respectively. B) Kaplan-Meier survival curve for relapse free survival for stroma-low (green line) versus stroma-high (orange line).

Table 3. Univariable logistic regression analyses for having a complete response (TRG 1).

	Univariable analysis		
	OR	**95% CI**	***P*-value**
Gender			
Female	Ref		
Male	0.88	0.30-2.62	0.81
Age (years)	0.95	0.90-1.00	0.06
Length tumor (cm)	1.14	0.58-1.59	0.48
Pre-nCRT histology			
Adenocarcinoma	Ref		
Mucinous adenocarcinoma	0.91	0.13-4.24	0.91
cT status			
cT2	Ref		
cT3	0.21	0.06-0.71	0.01
cT4	0.07	0.00-0.47	0.02
cN status			
cN0	Ref		
cN1	0.57	0.11-3.17	0.49
cN2	0.52	0.10-3.02	0.44
cN3	0	0 -> 1000	0.99
cTNM			
II	Ref		
III	0.54	0.12-2.80	0.42
Therapy			
RT + Capecitabine/Oxaliplatin	Ref		
RT + Capecitabine	0.76	0.11-8.68	0.67
Pre CEA			
≤ 3.5 ng/ml	Ref		
> 3.5 ng/ml	0.33	0.07-1.23	0.99

Table 3. Continued

	Univariable analysis		
	OR	**95% CI**	***P*-value**
Post CEA			
≤ 3.5 ng/ml	Ref		
> 3.5 ng/ml	0.28	0.00-2.25	0.28
Tumor-stroma ratio			
Stroma-high	Ref		
Stroma-low	2.61	0.77-9.71	0.09

Abbreviations: TRG tumor regression grade, nCRT neoadjuvant chemoradiotherapy, TNM tumor-node-metastasis, RT radiotherapy, CEA carcinoembryonic antigen, OR odds ratio, CI confidence interval.

Discussion

Our results suggest that patients with high stroma tumors seem to be less likely to respond to nCRT compared to patients with tumors harboring low stroma content, which is linked to higher relapse rates with long-term follow-up. Eighty-five percent of the stroma-high patients did not have a response on nCRT, suggesting that novel treatment approaches are needed. Tumors with high stroma content might represent a group of lesions with an environment that is well armed against chemoradiation, or can even become resistant to therapy. The tumor stroma influences the aggressive behavior of cancer cells not only through cell-cell contact and auto- and paracrine signaling but also through mechanical pressure. Due to the abundant extracellular matrix and the high number of cancer-associated fibroblasts (CAFs), the tumor stroma forms a physical barrier around the tumor that increases the interstitial pressure and hypoxia in the tumor. Cancer cells respond to hypoxic conditions by up-regulating hypoxia-inducible factor 1α (HIF1α), a master transcription factor that activates a whole range of genes involved in angiogenesis, migration, metabolism, tumor invasion and metastasis [26]. Moreover, Lotti et al. showed chemotherapy-treated CAFs promoted tumor-initiating cells and tumor growth *in vivo* [27]. Similar results were found in endothelial cells able to induce chemo-resistance in CRC cells [28].

Different treatment strategies may have to be considered for stroma-high patients in order to achieve pCR. For instance, these tumors could be future candidates for therapies targeting activated oncogenic pathways (e.g. the TGF-β or PDGFR pathways), matrix remodeling, angiogenesis or CAFs [29-31].

In recent years, efforts have been made to find biomarkers which can predict the response to neoadjuvant therapy for rectal cancer patients. Multiple studies found pathological features and microenvironment signatures to be predictive of pCR [18, 32-36], whereas one study found no clinical and pathological variables to significantly associate with response to nCRT [37]. Furthermore, Van Stiphout et al. identified post-nCRT maximal radioactive isotype uptake (SUVmax) and relative change of SUVmax to be strong determinants of response [35, 36]. However, these are rather complex and expensive parameters, whereas the TSR is easy to assess with low costs and high reproducibility. In addition, microenvironmental features recently have been shown to be most critical determinants of patient outcome [17, 18].

Limitations of this study are the retrospective nature and the small sample size, underpowered for conclusive statistical analyses, which means that results should be interpreted with caution and validated in larger cohorts.

Another issue is the potential impact of the time-lag between the last nCRT dose and surgery influencing the rate of regression found in the surgical specimen. One might hypothesize that near-complete responders will further regress towards a pCR when surgery is delayed for 2-4 weeks more weeks [38].

While these results require validation in larger series, this study has shown that stromal infiltration could be an important marker to take into consideration when developing predictive models of response to neoadjuvant chemoradiotherapy in rectal cancer patients, besides stage at diagnosis and imaging techniques.

Conflict of interest

RD reported receiving honoraria for speaker activities and participation in advisory boards from Roche, Boehringer-Ingelheim, Ipsen, Amgen, Sanofi, Servier, Merck-Sharp Dome, and research grants from Merck and Pierre-Fabre.

RPL reported receiving research grant from AstraZeneca.

JC reported scientific consultancy role for Novartis, Pfizer, Ipsen, Bayer, Eisai, Sanofi, Advanced Accelerator Applications, Exelixis and Merck Serono.

All other authors declare no conflicts of interest.

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